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Sand accumulation in the digestive tract of rabbits (*Oryctolagus cuniculus*) and guinea pigs (*Cavia porcellus*): The role of the appendix

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Abstract

We determined location and amount of accumulated sand in the gastrointestinal tract (GIT) of rabbits (*Oryctolagus cuniculus*) and guinea pigs (*Cavia porcellus*) fed diets containing external (silicate) abrasives. Computed tomographic abdominal images of rabbits (n = 44) and guinea pigs (n = 16) that each received varying numbers (4–7) of different diets for 14 days each (total n = 311 computed tomographs), and radiographs of dissected GIT and presence of silica in GIT content (n = 46 animals) were evaluated. In rabbits, the majority of accumulated sand was located in the caecal appendix, an elongated, intestinal structure in the left side of the abdomen. The 'wash-back' colonic separation mechanism in rabbits may be partly responsible for a retrograde transport of sand back to the caecum, where dense, small particles accumulate in the appendix. The appendix likely acted as a reservoir of these particles, leading to significant effects not only of the momentary but also of the previous diet on recorded sand volumes in the rabbits. Guinea pigs have no caecal appendix and a colonic separation mechanism not based on a 'wash-back'. Less sand accumulation was found in their GIT without a specific location pattern, and there were less previous diet effects in this species. None of the rabbits or guinea pigs developed clinical signs of obstruction during the study, and the recorded sand volumes represented 1.0 ± 1.2% of the 14-d sand intake in rabbits and 0.2 ± 0.2% in guinea pigs. Accumulation of sand in volumes up to 10 cm^3 in the GIT of rabbits does not seem to cause clinical health impairment. Large inter-individual differences in rabbits indicate inter-individual variation in proneness to sand accumulation. The reason for the presence of a sand-trapping caecal appendix in animals that are, due to their burrowing lifestyle and feeding close to the ground, predestined for accidental sand ingestion, remains to be unveiled.

KEYWORDS
abrasives, diet, gastrointestinal tract, Lagomorpha, Rodentia, sedimentation trap

1 | INTRODUCTION

When feeding in a natural setting, herbivorous animals unavoidably also ingest external inorganic material like sand, soil or dust (Beyer et al., 1994; Hummel et al., 2011; Turner et al., 2013). Therefore, one would assume that animals have evolved to cope with a certain amount of sand in their gastrointestinal tract (GIT), and sand or soil ingestion need not primarily be considered problematic. On the one hand, soil ingestion can have crucial functions in some species, for example for iron uptake, with negative effects on newborn domestic animals that do not have access to natural soil (Brommer & van Oldruitenborgh-Oosterbaan, 2001; Walker & Taylor, 1965). On the other hand, individual reports exist that animals withstand substantial ingestion of sand or stones without apparent clinical problems (Davies et al., 2001; Dirksen, 2002).

Nevertheless, sand ingestion is mainly represented as a health risk in the veterinary literature, and the fate of sand in the GIT of mammals remains largely unexplored. The main exception is ruminating foregut fermenters (taxonomic ruminants and camelids). Due to the density and fluid-dependent sorting mechanism in their forestomachs, sand is washed off the digesta prior to regurgitation for rumination and accumulates without clinical consequences in a part of the stomach complex before being excreted (Hatt et al., 2019; Hatt et al., 2020; Hatt et al., 2021). In non-ruminant foregut fermenters like hippopotamus, sloths or peccaries, the accumulation of sand in certain, sometimes dead-end structures of the forestomach, without apparent clinical problems, has also been reported (Schwarm et al., 2010; Schwarm et al., 2013; Wings et al., 2008). For non-ruminant hindgut fermenters; however, reports are mainly limited to the evaluation of the risk for, and description of clinical cases of, sand impaction in horses (Hassel et al., 2020; Husted et al., 2005; Kendall et al., 2008; Siwińska et al., 2019). In horses, the fact that sand impaction occurs only in some individuals of populations that are exposed to common conditions has been interpreted as indication for individual predisposition (of hitherto unknown factors) for the development of clinical signs (Niinistö et al., 2019).

Rabbits (*Oryctolagus cuniculus*) are non-ruminant hindgut fermenters that naturally forage close to the ground and live in burrows. This lifestyle makes accidental ingestion of substantial amounts of soil or sand likely. Arthur and Gates (1988) reported a soil intake of a magnitude of more than 6% of dry matter in free-ranging black-tailed jackrabbits (*Lepus californicus*), and Rödel (2005) described the presence of sand in the faeces of European rabbits. The lagomorph GIT anatomy is characterised by an elongated caecum that ends in a voluminous appendix (Snipes, 1979), which is situated on the left side of the abdomen (Nicoletti et al., 2018; Rees Davies & Rees Davies, 2003). The lagomorph GIT thus comprises a distinct dead-end structure that might be particularly prone to the accumulation of soil. This could be considered all the more likely because the lagomorph GIT physiology is characterised by a 'wash-back' colonic separation mechanism (Björnhag & Snipes, 1999; Cork et al., 1999; Snipes et al., 1982), which directs fluids backwards from the proximal colon into the caecum. Its main purpose is considered the retention of fine particulate matter

such as microbes, which are subsequently excreted as 'soft faeces' or 'caecotrophs' and re-ingested by the animal. This mechanism might also direct a certain proportion of ingested soil or sand particles towards the caecum and appendix. Other small herbivores, such as hystricomorph rodents (including guinea pigs *Cavia porcellus*), do not only have no caecal appendix (Snipes, 1982), but also have a different kind of colonic separation mechanism that relies more on mucous trapping of microbes than on backwards washing (Björnhag & Snipes, 1999; Cork et al., 1999). We predicted that this could lead to a lesser accumulation of sand in their caecum.

We used computed tomography (CT) images to test our prediction on differences in sand accumulation in the GIT of rabbits and guinea pigs. CT scans of heads and teeth had been generated in several studies evaluating the effect of different siliceous abrasives on tooth wear in rabbits and guinea pigs (Martin, Ackermans, Richter, et al., 2021; Martin, Ackermans, Tollefson, et al., 2021; Müller et al., 2014; Müller et al., 2015). In those same experiments, CT scans of the abdomen had also been acquired to document the deposition of the radiopaque abrasives in the GIT. Here, we evaluate these CTs to test the above predictions on a difference in sand accumulation in the GIT between rabbits and guinea pigs.

2 | MATERIAL AND METHODS

The data generated in the present study derived from four different feeding experiments designed to explore mechanisms of tooth wear in rabbits and guinea pigs. The experiments are here designated as A–C, with a subscript denoting the species (A_r–C_r for rabbits, C_{gp} for guinea pigs). Details on the husbandry, diets and tooth wear have been previously published: Experiment A_r was designed to test the effect of diet consistency on tooth wear in rabbits (Martin, Ackermans, Tollefson, et al., 2021); experiment B_r to test the effect of additions of different concentrations and sizes of quartz silica on tooth wear in rabbits (Martin, Ackermans, Richter, et al., 2021); experiment C to test effects of different levels of phytoliths and the addition of sand on tooth wear in rabbits (Cr; Müller et al., 2014) and guinea pigs (Cgp; Müller et al., 2015).

2.1 | Animals and husbandry

The experiments were approved by the Cantonal Veterinary Office in Zurich, Switzerland (licence numbers ZH135/16, ZH010/16 and ZH080/2012). A total of 44 Rabbits (A_r: n = 6 males, n = 8 females, 6 months old, 3.0 ± 0.15 kg; B_r: n = 7 males, n = 7 females, 3 months old, 2.52 ± 0.4 kg; C_r: 16 females, 7 months old, 2.75 ± 0.16 kg) and 16 guinea pigs (C_{gp} all female, 2.5 months old, 0.48 ± 0.02 kg) were part of the experiments. The animals were kept individually in hutches (0.75 m^2) on woodchip bedding with hides but no other gnawing opportunities except their diet. The enclosures were split by sex and were exposed to a 12-h light/dark cycle. Olfactory and visual contact with neighbouring animals was provided through small holes at

different height levels in separating walls. Water was provided for *ad libitum* consumption from open bowls and changed daily. For the guinea pigs, water was supplemented with 200–400 mg/L Vitamin C (Redoxon, Bayer AG, Zurich, Switzerland). During an adaptation period of 7 days, the animals were slowly switched from the breeder's diet to the pelleted experimental control diet. The animals underwent a weekly clinical examination including body weights measurement. During the second week of each feeding period, all excreted faeces were collected for each animal during three consecutive days. Feed and faeces were dried to constant weight to determine their dry matter concentration.

2.2 | Experimental diets

The experimental diets were fed at known quantities for *ad libitum* consumption, and the food leftovers were weighed daily and replaced with fresh food. Group A_r received a timothy grass meal-based diet in either pelleted or extruded form, with or without the addition of 5% fine sand (mean particle size 130 μm, METTET AF100, SCR-Sibelco N.V., Belgium) to the basal mix prior to pelleting/extruding (4 diets; 8 weeks of experiment in total). The pelleted diets of group B_r were based on lucerne and contained either no abrasives (control), 4% or 8% of added quartz abrasives of a different size as fine silt (mean particle size 4 μm, SIRCON® M500, SCR-Sibelco N.V., Belgium), coarse silt (mean particle size 50 μm, MICROSIL® M4, SCR-Sibelco N.V., Belgium) or fine sand (mean particle size 130 μm, METTET AF100, SCR-Sibelco N.V., Belgium) (seven diets; 14 weeks of experiment in total). Group C animals received either grass hay fed as whole forage (H), or pelleted diets of increasing abrasiveness from lucerne pellets (L), grass pellets (G), grass and rice hull pellets (GR) and grass and rice hull pellets with an addition of 5% coarse sand (GRS; mean particle size 230 μm, sand for playgrounds, REDSUN garden products B.V., Heijen, Denmark; 5 diets; 10 weeks of experiment in total). In general, the inclusion of abrasives did not affect the acceptance of diets.

2.3 | Experimental procedures

In the beginning of each experiment, an animal was randomly assigned to one experimental diet for a feeding period of 14 days. At the end of each feeding period, an abdominal CT scan was performed before randomly switching the animal to the next experimental diet of its respective study. In order to perform the CT scans, an animal was placed under general anaesthesia with isoflurane (Attane®, Provet AG, Lyssach, Switzerland) administered to effect in oxygen via facemask after sedation with 0.5–1.5 mg/kg midazolam (Dormicum®, Roche AG, Reinach, Switzerland) intramuscularly. Images were acquired with a helical, multi-slice Siemens scanner (Siemens Medical Solutions, Erlangen, Germany) at 120 kV tube voltage and 150 mA with a slice thickness of 600 μm or a 16 slice, spiral CT-scanner (Philips Brilliance 16, Philips Healthcare, Zurich, Switzerland) at 120 KV, 117 mA, and 10 cm FOV with a slice thickness of 1 mm. The animal was positioned in ventral recumbency

on the CT table in order to obtain transverse slices. At the end of the respective experiment, the animals were euthanized in accordance with AVMA guidelines (Leary et al., 2013) shortly before performing the last CT scan. Subsequently, the GIT was dissected, freed of mesenteries and adhering adipose tissue, and placed on a flat panel detector for digital radiography. Afterwards, the contents of the stomach, caecum, appendix (in rabbits) and proximal colon were collected and dried to constant weight; in six rabbits, the stomach contained visually distinguishable caecotrophs, which were sampled separately.

Thus, each animal received all diets of its respective experiment, so that data on intake, faecal excretion and an abdominal CT was available for each diet. By contrast, the additional GIT radiograph and GIT content analyses were only available for the last diet an animal received in its respective experiment (14 rabbits each of A_r and B_r, and 15 rabbits from C_r where the GIT of one animal was lost). For the guinea pigs, only three GIT had been stored after the experiment.

2.4 | Diet, faeces and gastrointestinal content analyses

Representative samples of the diets, each individual's faeces on a specific diet, and GIT content (representing the last experimental diet for each individual) were submitted to acid detergent insoluble ash (ADIA) measurement (Hummel et al., 2011) as a measure for silica. Using the ADIA concentration of diet and faeces, and the quantified dry matter intake and faecal excretion, ADIA intake, excretion and their difference (i.e., ADIA retention) were calculated for each individual on each of its respective diets. Cumulative ADIA intake was calculated as the sum of the ADIA intake of a specific feeding period and the ADIA intake of all previous feeding periods of the respective animal. Due to differences in the total experimental periods, the total cumulative intake varied between studies.

The density of the added abrasives was determined by filling Eppendorf containers of determined volume (8.8 ml) with the respective abrasive and weighing the mass; this was replicated five times per abrasive. Due to the difficulty of condensing the dry material in the containers, these measurements should be considered with caution. The densities thus determined were 1.02 ± 0.02 g/ml for the fine silt, 1.51 ± 0.03 g/ml for the coarse silt, and 1.30 ± 0.03 g/ml for the fine sand. Ingested ADIA in cm^3/day was calculated for each animal and each of its respective feeding periods, using these densities and the respective mass of ADIA intake.

2.5 | Quantification of accumulated silica

The original CT data were reconstructed with a soft tissue algorithm and were reviewed using an abdominal soft tissue window setting (window width = 350 HU, window level = 40 HU). Abdominal CT images were investigated using Horos software v3.3.3 (Horos Project 2015). Sand accumulations (visible as radiopaque matter) were measured with the help of a closed polygon, a function appropriate to measure curved structures. Respective volumes (cm^3) were calculated by manually defining regions of interest (ROIs) on every other slice

and automated generation of missing ROIs. Not connected sand volumes were measured independently of one another, which often resulted in several different sand volumes per CT scan. Of every sand volume, the position in the abdomen was noted including the side of the abdomen (left, middle, right) and the position relative to special orientation points such as stomach, lumbar vertebrae (rabbits L1–L7, guinea pigs L1–L6) and sacrum. Each animal was given, for each of its diets, a side score ranging from 1 to 3 (where 1 denotes sand at only one, and 3 sand at all three of the three possible side locations), and a position score that indicated over how many lumbar vertebrae the localisation of the sand was spread.

2.6 | Statistical analysis

Statistical analyses were performed in R (R Core Team, 2017). Linear mixed effect models were applied using packages tidyverse (Wickham et al., 2019), lmerTest (Kuznetsova et al., 2017), MuMIn (Bartoń, 2013), car (Fox & Weisberg, 2019), moments (Komsta & Novomestky, 2015). Data were analysed for each group (A_r, B_r, C_r and C_{gp}) separately, with individual as random factor. 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. Results were considered significant at $p < .05$.

3 | RESULTS

None of the 60 experimental animals ever showed clinical signs of GIT pathology; neither the general composure and posture nor the food intake was ever disturbed in any animal at any time.

3.1 | ADIA intake, excretion and retention

Dry matter intake and faecal excretion only differed between diets for group C animals (Table S1). The ADIA intake and faecal ADIA output; however, was different between diets for all experimental groups as planned in the experimental design (Table S1, Figure S1 and S2). The discrepancies between ADIA intake and output led to a retention of ADIA, which again differed between diets (supplementary online material, Table S1, Figure S3; for group B_r the difference due to diet was only close to significant at a p value of .052). Integrating the previous diet into the statistical model, the diet before the respective feeding period had a significant effect on the faecal ADIA and ADIA retained in the C rabbits, with more faecal ADIA excreted and less ADIA retained if diet GRS was the 'before' diet; GRS did not have such an effect in guinea pigs (supplementary online material, Table S2). Looking at how many feeding periods had passed since the experiment C animals were fed the 5% coarse sand (GRS), there was a significant negative effect on faecal ADIA and a significant positive effect on ADIA retained for the rabbits but not the guinea pigs (supplementary online material, Table S3).

3.2 | Location

The largest radiodense structures in the rabbit GITs were usually found in a tubular, intestinal structure on the left side of the abdominal cavity (Figure 1), and this location was uniform across animals (Figure 2). Notably, the tip of the structure pointed sometimes to the cranial, and sometimes to caudal part of the animal (Figure 3). The structure was tentatively interpreted as the caecal appendix. In specimens in which the connection of this structure with the caecum could be traced on the CT scan, it originated from the structure interpreted
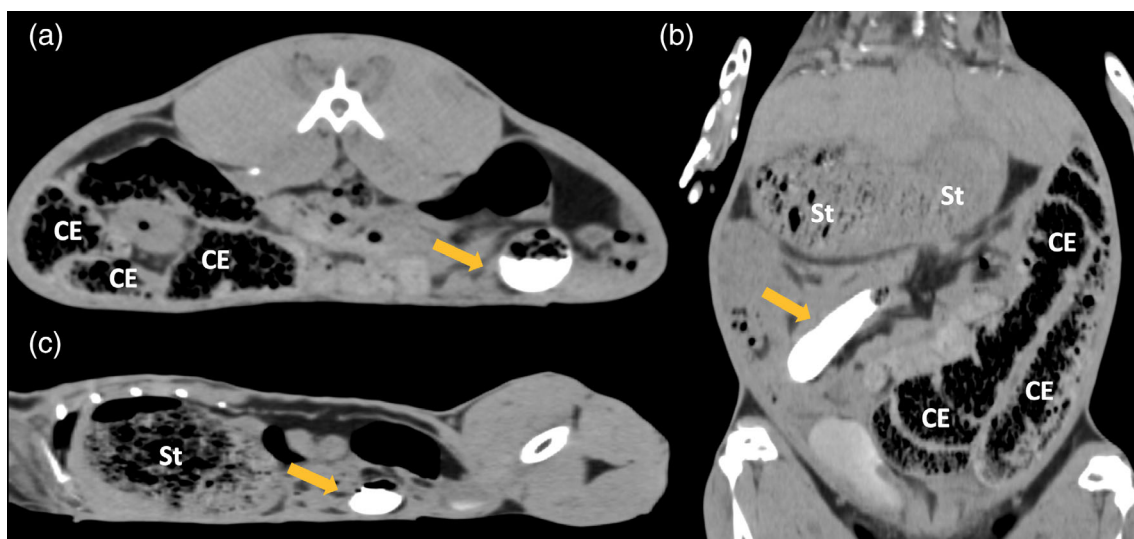

FIGURE 1 In situ transverse, dorsoventral and sagittal computed tomographic images in a soft tissue window of rabbits fed different amounts of sand as part of a serial feeding experiment. Sand accumulation in the gastrointestinal tract is mainly focused in one particular structure. Accumulation of sand is visible as strong radiodense (white) matter (indicated by yellow arrows). Images represent (a) transversal craniocaudal view, (b) dorsoventral view and (c) sagittal view. Ce, caecum; St, stomach
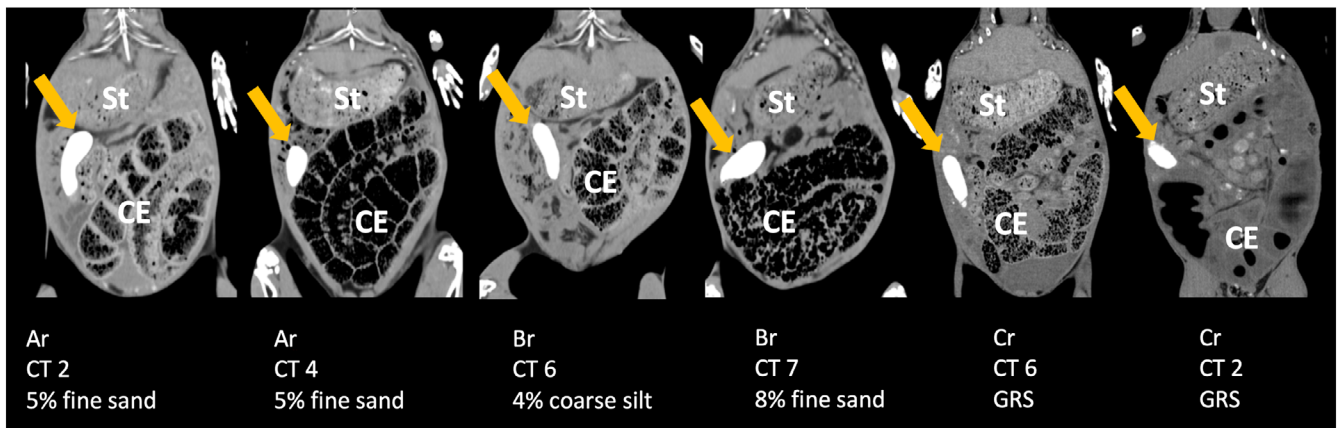
FIGURE 2 Serial dorsoventral computed tomographic images in a soft tissue window of rabbits fed different amounts of sand as part of a serial feeding experiment. Given images are from different individuals in order to create an impression of the reproducibility of the sand's position in the abdomen. Sand accumulation is visible as strong radiodense (white) matter (indicated by yellow arrows) lying always on the left side of the abdomen next to the main caecal body. Ce, caecum; St, stomach
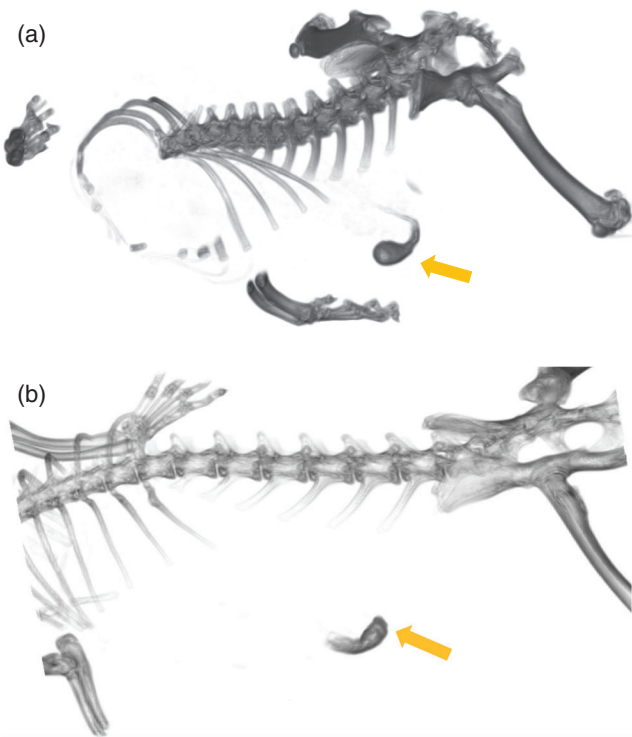

FIGURE 3 Three-dimensional computed tomography reconstruction of radiodense structures in two rabbits (*Oryctolagus cuniculus*). Note the tip of the appendix filled with radiodense material (yellow arrow), pointing (a) cranially and (b) caudally

as the caecum tip on the right side of the abdominal cavity and extended as an elongated tubular structure on top of the caecal coil to the left (Figure 4). By contrast, guinea pigs generally did not show a particular accumulation of radiodense material in their GIT (Figure 5). The position of radiodense material in the left side of the abdomen was uniform across experiments in rabbits, whereas radiodense

material was evenly distributed across the sides in guinea pigs (Figure 6a). In rabbits, the location was mainly focussed on the area of lumbar vertebrae 2–4, whereas no similar accretion area was evident in the guinea pigs (Figure 6b). Radiographs of dissected GIT consistently showed that accumulation of radiodense material occurred in the appendix of rabbits and did not indicate a specific area of accumulation in guinea pigs (Figure 7). This corresponded to the distribution of ADIA in the different GIT compartments, where concentrations were extremely high in the rabbit appendix but otherwise showed no remarkable difference between stomach, caecum and colon (Figure 8). In the six animals in which caecotrophs were detected in the stomach, the ADIA concentration of this material did not differ from that of the caecum of the same animals (data not shown, but can be found in the original data supplement).

3.3 | Volume measurements and correlations

The volume of the radiodense material depended significantly on the individual rabbit but not on the individual guinea pig (supplementary online material, Table S4). The volume varied significantly with diet, daily ADIA intake and intake volume (supplementary online material, Table S4, Figure S4). It correlated positively with both, the side score and the location score, indicating that larger volumes were more likely to be spread across more than one side and several reference vertebrae (supplementary online material, Table S5).

In rabbits but not in guinea pigs, there were additional effects: In B_r and C_r, the cumulative ADIA intake (over the course of the whole experiment) and the CT number (representing the number of diets already fed) were also positively correlated to the volume (supplementary online material, Table S4). Correspondingly, tracing the radiodense volume in individual rabbits over time indicated an overall trend of increase over the course of the experiment in some but also
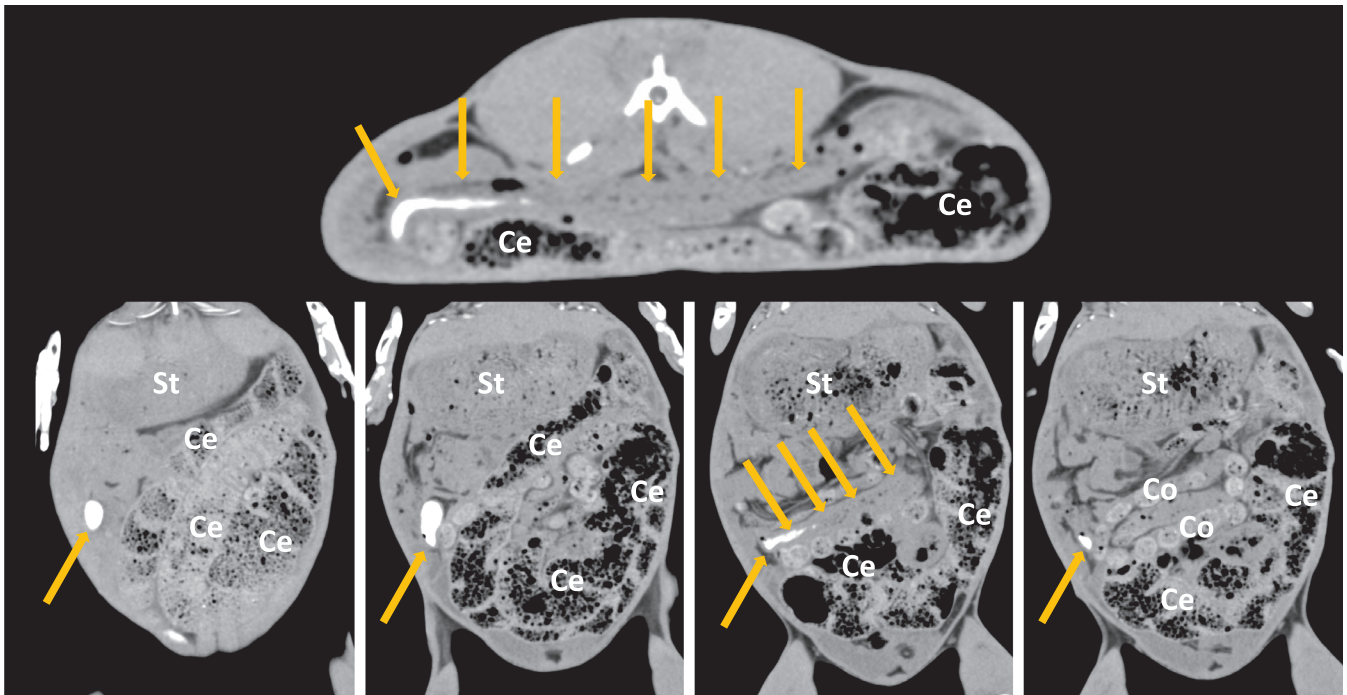
FIGURE 4 Transversal caudocranial computed tomographic (CT) image and serial dorsoventral CT images of a rabbit (*Oryctolagus cuniculus*) indicating the topographic anatomy of the caecum and appendix. Left and right are as in the natural position; the dorsoventral views (lower row) are from the most ventral (left) to the most dorsal (right). The caecal coil is the most ventral structure, making a clockwise turn, with the caecum tip in the right half of the abdomen. From there, the appendix is directed to the left side, on top of the caecal coil, nearly perpendicular to the body axis, so that the appendix tip lies in the left half of the abdomen. When filled with dense material, as in this case, the appendix tip is pulled ventrally on the same level as the caecum. The yellow arrows indicate the appendix. Ce, caecum; Co, colon; St, stomach
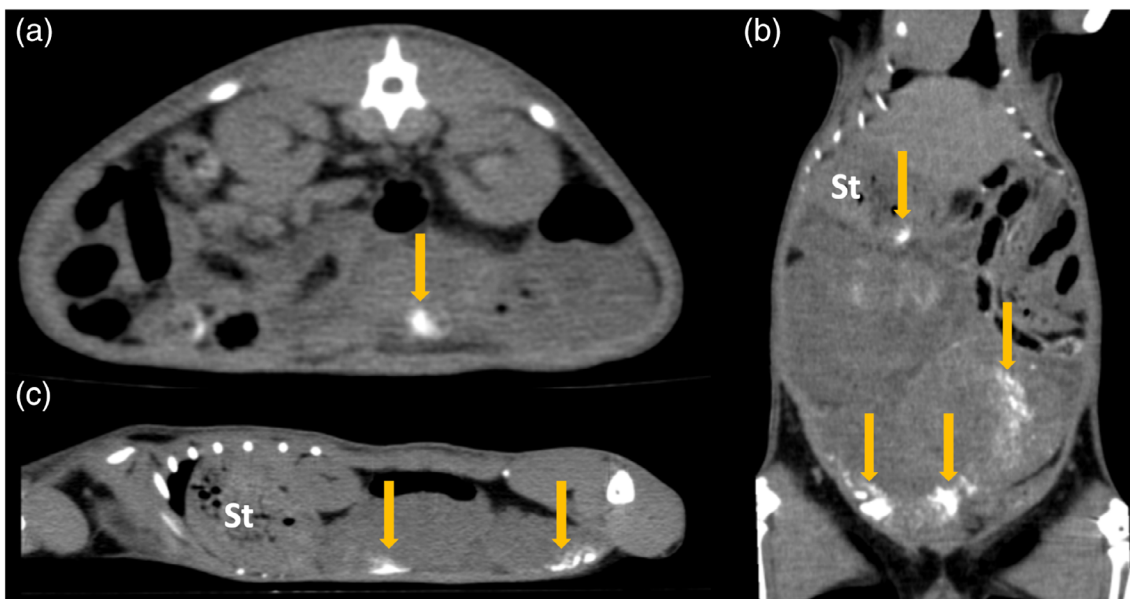
FIGURE 5 In situ computed tomography with dorsoventral, transverse and sagittal view of a guinea pig (*Cavia porcellus*) on an experimental diet with added quartz sand. Accumulation of sand is visible as strong radiodense (white) matter (indicated by yellow arrows). Images represent (a) transversal craniocaudal view, (b) dorsoventral view and (c) sagittal view. St, stomach
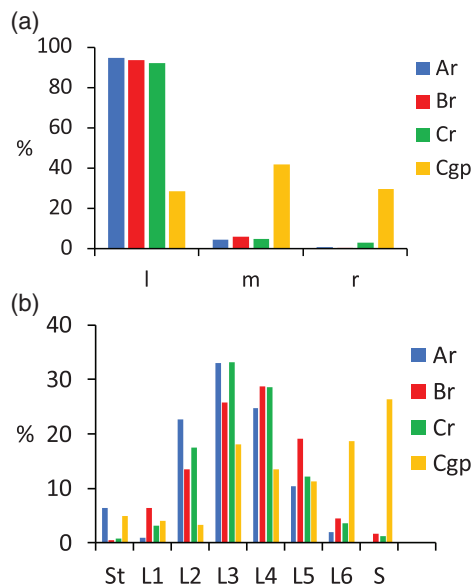
FIGURE 6 Distribution of radiodense material (sand) volume across (a) the sides of the abdomen (left l, middle m or right r) and (b) across the stomach (St), the different sections marked by lumbar vertebrae (L1–L6) and the sacrum (S) in rabbits (r, *Oryctolagus cuniculus*) and guinea pigs (gp, *Cavia porcellus*) in three different studies (A, B, C). Note the difference in sand position between the species with respect to both lateral placement (a) and longitudinal placement (b) of the sand accumulation
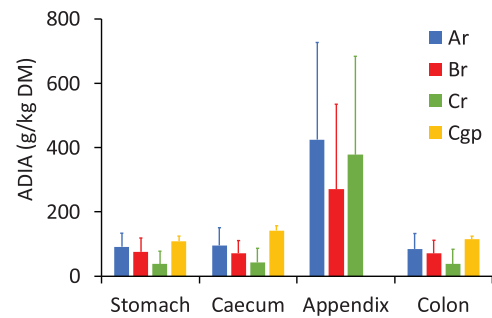

FIGURE 8 Acid detergent insoluble ash (ADIA, a measure for silica, in g/kg dry matter) concentrations in the contents of the stomach, caecum, caecal appendix (rabbits only) and proximal colon in rabbits (r, *Oryctolagus cuniculus*) and guinea pigs (gp, *Cavia porcellus*) in three different studies (A, B, C). Note the distinct outlier position of the appendix with very high accumulations of silica

reductions of volume in other individuals, and even the absence of accumulation at any time in yet other individuals (Figure 9).

In the rabbits, the sand volumes recorded on CTs corresponded to 14 ± 17% (range 0%–120%) of the daily ingested ADIA volume; the value exceeded the daily intake in only a single animal on a single diet. In the guinea pigs this value was 3 ± 3% (range 0%–10%). Compared to the total ADIA volume ingested over the 14 days of a diet treatment, these percentages were 1.0 ± 1.2% (range 0%–8.6%) in the rabbits and 0.2 ± 0.2% (range 0%–0.7%) in the guinea pigs.

4 | DISCUSSION

This study documents that ingested sand, added to diets at different particle sizes and concentrations, is mostly excreted via faeces by rabbits and guinea pigs; over the course of various periods, ranging from 2 weeks in experiment C to a potential maximum of 12 weeks in
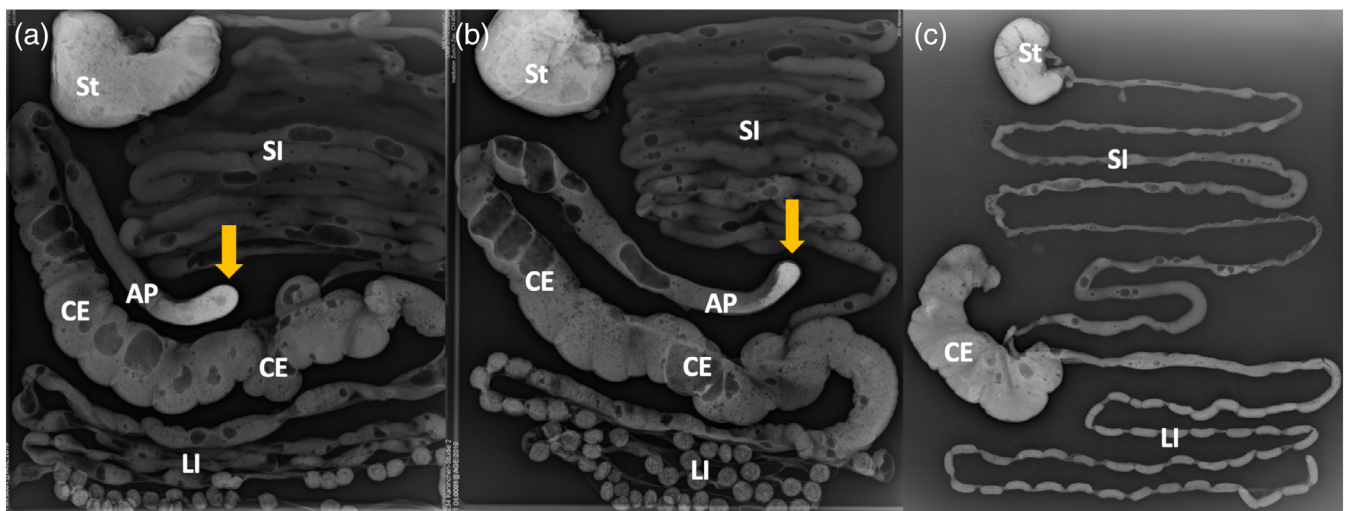

FIGURE 7 Radiographs of dissected gastrointestinal tracts of two rabbits (*Oryctolagus cuniculus*) and one guinea pig (*Cavia porcellus*) fed different amounts of sand as part of a serial feeding experiment. (a) and (b) a distinct accumulation of sand (radiodense matter) is visible in the caecal appendix on the radiograph of two different rabbits. (c) Radiograph of a gastrointestinal tract of a guinea pig. Note that guinea pigs do not possess a caecal appendix and that the radiograph lacks a visible accumulation of radiodense matter at a specific location. In all animals, stomach contents are diffusely denser than most other parts of the intestinal tract. Ap, appendix (in rabbits); Ce, caecum; LI, large intestine; SI small intestine; St, stomach
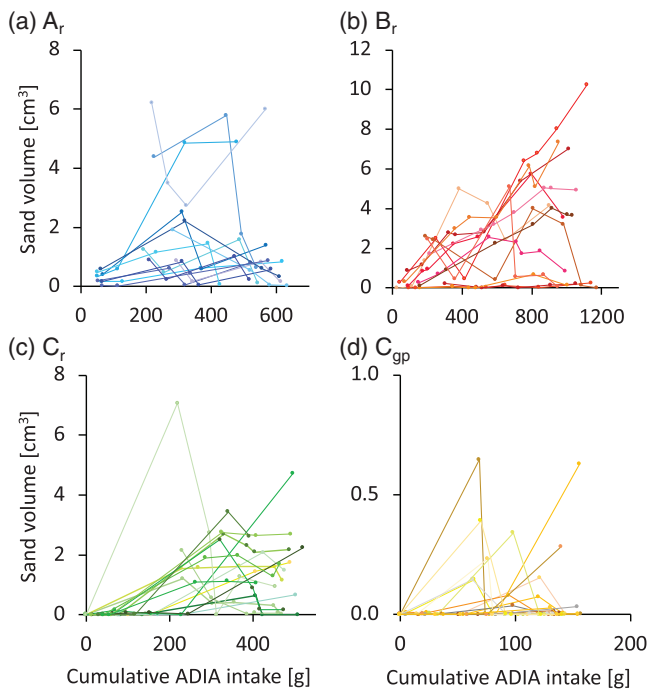
FIGURE 9 Relationship between the sand volume measured in computed tomographic images with the cumulative silica (measured as acid detergent insoluble ash, ADIA) intake over the course of all feeding experiments in rabbits (r, *Oryctolagus cuniculus*) and guinea pigs (gp, *Cavia porcellus*) in three different studies (A, B, C). Each individual is marked by a line that links the different diets within a particular study. (a) A_r, (b) B_r, (c) C_r, (d) C_{gp}. Note that in the two longer studies (with more diets) (b, c), some rabbits show a pattern of sand accumulation over the course of the study, or a particular propensity for sand accumulation, whereas others do not. There is no evident accumulating effect in the guinea pigs (d). For statistics, see Table S4

experiment B, the daily ingestion of sand at levels of ≥4% of dry matter did not lead to clinical signs. While long-term studies would be required to prove that a more chronic intake of sand does not lead to health problems, these findings, together with reported soil intake in free-ranging lagomorphs (Arthur & Gates, 1988; Rödel, 2005), and the knowledge of the burrowing lifestyle of many lagomorphs, suggest that the rabbit's GIT can cope with substantial sand ingestion. Also, in comparison with guinea pigs, the present study indicates that the rabbit's caecal appendix is a predilection site for sand accumulation. In addition to its dead-end structure, the wash-back colonic separation mechanism of lagomorphs (Björnhag & Snipes, 1999; Cork et al., 1999; Snipes et al., 1982) makes a particular accumulation of a part of the ingested silica especially likely.

The extent to which soil particles are retained in the caecal appendix of free-ranging lagomorphs has, to our knowledge, not been investigated so far. Reported faecal concentrations of sand in free-ranging rabbits reach magnitudes of 8 ± 2% of faecal dry matter (Rödel, 2005), which is well in the range of the present study. Whether the interaction of the appendix with ingested non-dietary components represents an adaptive feature of lagomorph physiology

is similarly unknown. Apart from secreting bicarbonate that acts as a buffer for the carbohydrate-fermenting microbiome in the caecum (Williams et al., 1961), the caecal appendix is mainly considered a lymphatic organ with a largely undefined immunological function. Resection of the appendix in neonate rabbits leads to a reduced immunological response, both in the gut-associated immune system and in the serum (Dasso & Howell, 1997).

Whether exposure of lymphatic tissue to soil can prepare the immune system for wounds inflicted by male and female rabbits on each other with their soil-contaminated claws is at this stage purely speculative. While this scenario appears plausible, it should be noted that burrowing hystricomorph rodents exist, like the plains viscacha (*Lagostomus maximus*), that resemble the guinea pig in the macroscopic structure of its digestive tract without a caecal appendix (Hagen et al., 2015), and that numerous other burrowing rodents or insectivores also do not have an appendix (Langer, 2017). This does not necessarily invalidate the speculative explanation—it would just mean that this is a feature peculiar for lagomorphs and not convergent across burrowing small mammals in general. More detailed studies on the immunological reactions in the lymphoid tissue of the appendix are required to address this hypothesis.

Because sand accumulated in the appendix, this organ most likely served as a reservoir from which sand was excreted continuously even after a sand-containing diet was discontinued in the present investigation. This effect was evident in several different findings: faecal sand excretion, sand retention, and the volume of sand detected on CTs did not only depend on the momentary diet, but also on the previous diets in rabbits within the two experiments (B and C) that ran for a longer period due to the evaluation of more than four diets. By contrast, no such effect of the previous diet was detected in the guinea pigs. The—admittedly limited—observation that caecotrophs did not contain ADIA levels that were higher than caecum contents suggest that content of the appendix plays no role in the formation of caecotrophs.

With respect to using rabbits in digestion studies, these results caution against the use of acid insoluble ash as a digestibility marker (Papadomichelakis & Fegeros, 2020) in animals that had previous access to soil or soil-contaminated diets, or against diets that contain substances that might be sequestered by the 'wash-back' colonic separation mechanism. Nevertheless, the observation that the vast majority of the 14-day silica intake (app. 99%) in our study was not retained could explain why even a finely powdered particulate marker like titanium dioxide, with a density above 4 g/ml, can produce digestibility measures similar to those measured by total faecal collection when fed consistently for 2 weeks (Safwat et al., 2015).

The variety of positions of the caecal appendix on CT scans (Figures 2, 3) raises the question about its topographic position in living animals. The appendix is typically depicted as a straight tubular organ that is an elongation of the caecum tip in anatomical drawings (Langer, 2017; Rees Davies & Rees Davies, 2003; Snipes, 1979). These depictions obliterate the observation that the appendix crosses the abdominal cavity from the right to the left side, so that its tip can typically be detected via ultrasound in the left abdomen (Nicoletti et al., 2018). We provide an interpretative graphic depiction of the

FIGURE 10 Schematic representation of the topography of the appendix in rabbits (*Oryctolagus cuniculus*) produced from the photograph of (a) a plastinate (dorsal view) in which the appendix is not positioned in its correct topographic position; (b) ventral view and (c) dorsal view. (b and c) manipulated in photoshop to present the correct position, with the indication of the possible different direction of the appendix tip caudally indicated in stippled lines. The scale shows 1 cm-steps, and is always placed on the side representing the right side of the animal. Plastinate courtesy of the Institute of Veterinary Anatomy of the Vetsuisse Faculty, Zurich
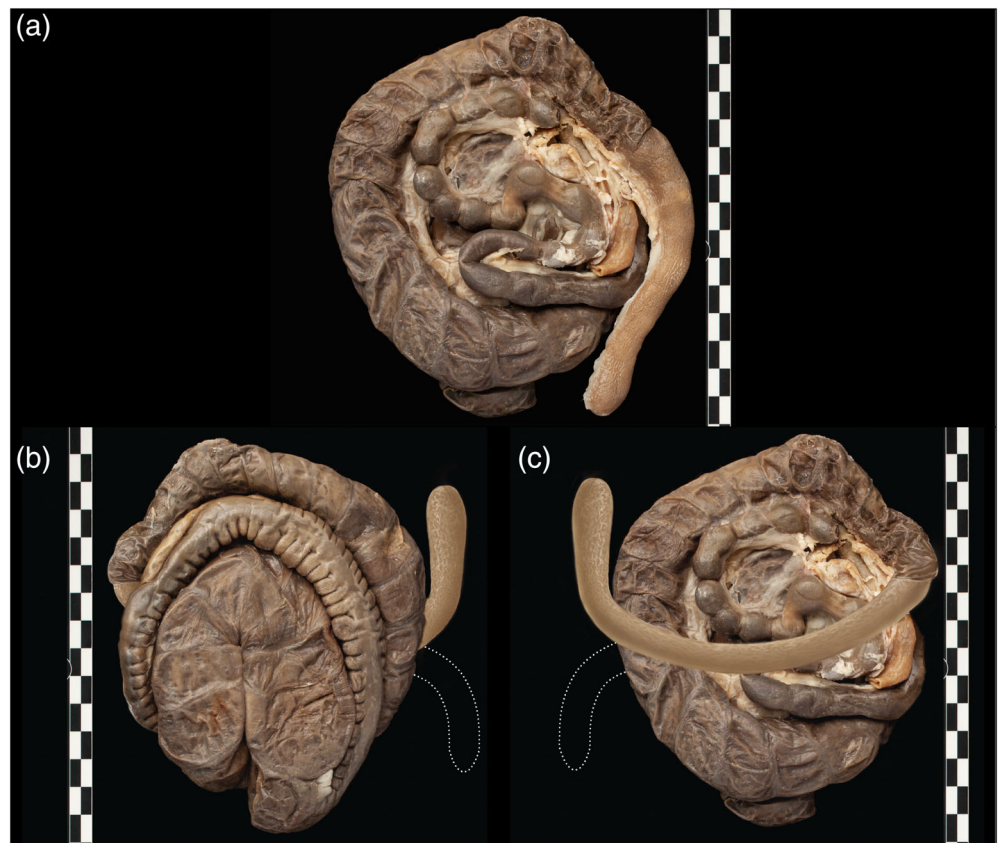
rabbit caecum and appendix, to better explain their *in situ* topography (Figure 10). Given that the body of the appendix passes towards the right side dorsally to the caecal centrifugal gyrus, it will mainly remain unattainable for ultrasound diagnostics, in contrast to the appendix tip that may be close to the abdominal wall. Our observations suggest that whether the tip points cranially or caudally is a matter of chance. As a side note, in one of 40 domestic rabbits in which Nicoletti et al. (2018) visualised the appendix tip via ultrasound, the content of the appendix created an acoustic shadow that prevented the view of the appendix' distal wall; possibly, this was radiodense sand. To which extent such appendix sand occurs in rabbits kept as pets, and in free-ranging animals, remains to be investigated.

5 | CONCLUSION

The caecal appendix of rabbits has a special topography, transversing the abdominal cavity more or less horizontally from the end of the caecum on the right to the appendix tip on the left side of the body. The caecal appendix of rabbits, and by inference of lagomorphs in general, may act as a sedimentation trap for fine, dense particulate material such as the sand in the present study. Whether soil accumulation at this side occurs in free-ranging specimens, and whether this is an adaptive feature of lagomorph morphophysiology that facilitates exposure of this material to the immune system, or just represents an

accidental side-effect of an organ evolved for other reasons, remains to be investigated.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

AUTHOR CONTRIBUTIONS

Nicole Winter: Investigation (lead); methodology (equal); project administration (equal); visualization (equal); writing – original draft (equal). **Marcus Clauss:** Conceptualization (equal); data curation (equal); formal analysis (equal); funding acquisition (equal); methodology (equal); project administration (equal); supervision (equal); visualization (equal); writing – original draft (equal). **Daryl Codron:** Formal analysis (equal); writing – review and editing (equal). **Jürgen Hummel:** Methodology (equal); resources (equal); writing – review and editing (equal). **Jaqueline Müller:** Investigation (equal); writing – review and

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REFERENCES

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*, 162–166.

Bartoń K. 2013. MuMIn: Multi-model inference. R package version *1*. 5.

Beyer, W. N., Connor, E. E., & Gerould, S. (1994). Estimates of soil ingestion by wildlife. *Journal of Wildlife Management*, *58*, 375–382.

Björnhag, G., & Snipes, R. L. (1999). Colonic spearation mechanism in lagomorph and rodent species–a comparison. *Zoosystematics and Evolution*, *75*, 275–281.

Brommer, H., & van Oldruitenborgh-Oosterbaan, M. M. S. (2001). Iron deficiency in stabled Dutch warmblood foals. *Journal of Veterinary Internal Medicine*, *15*, 482–485.

Cork, S. J., Hume, I. D., & Faichney, G. J. (1999). Digestive strategies of nonruminant herbivores: The role of the hindgut. In H. J. G. Jung & G. C. Fahey (Eds.), *Nutritional ecology of herbivores* (pp. 210–260). American Society of Animal Science.

Dasso, J. F., & Howell, M. D. (1997). Neonatal appendectomy impairs mucosal immunity in rabbits. *Cellular Immunology*, *182*, 29–37.

Davies, Z. E., Guise, H. J., Penny, R. H. C., & Sibly, R. M. (2001). Effects of stone chewing by outdoor sows on their teeth and stomachs. *The Veterinary Record*, *149*, 9–11.

Dirksen, G. (2002). Magen-Darmversandung. In G. Dirksen, H. D. Gründer, & M. Stöber (Eds.), *Innere medizin und chirurgie des rindes* (pp. 510–512). Parey.

Fox, J., & Weisberg, W. (2019). *An {R} companion to applied regression* (3rd ed.). Sage.

Hagen, K. B., Besselmann, D., Cyrus-Eulenberger, U., Vendl, C., Ortmann, S., Zingg, R., Kienzle, E., Kreuzer, M., Hatt, J.-M., & Clauss, M. (2015). Digestive physiology of the plains viscacha (*Lagostomus maximus*), a large herbivorous hystricomorph rodent. *Zoo Biology*, *34*, 345–359.

Hassel, D. M., Curley, T., & Hoaglund, E. L. (2020). Evaluation of fecal sand clearance in horses with naturally acquired colonic sand accumulation with a product containing probiotics, prebiotics, and psyllium. *Journal of Equine Veterinary Science*, *90*, 102970.

Hatt, J.-M., Codron, C., Richter, H., Kircher, P. R., Hummel, J., & Clauss, M. (2021). Preliminary evidence for a forestomach washing mechanism in llamas (*Lama glama*). *Mammalian Biology*. https://doi.org/10.1007/s42991-021-00142-1

Hatt, J.-M., Codron, D., Ackermans, N. L., Martin, L. F., Richter, H., Kircher, P. R., Gerspach, C., Hummel, J., & Clauss, M. (2020). Differences in the rumen washing mechanism in sheep fed diets with abrasives of various concentrations and sizes. *Palaeogeography Palaeoclimatology Palaeoecology*, *550*, 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.

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*, *278*(1712), 1742–1747.

Husted, L., Andersen, M. S., Borggaard, O. K., Houe, H., & Olsen, S. N. (2005). Risk factors for faecal sand excretion in Icelandic horses. *Equine Veterinary Journal*, *37*, 208–221.

Kendall, A., Ley, C., Egenvall, A., & Bröjer, J. (2008). Radiographic parameters for diagnosing sand colic in horses. *Acta Veterinaria Scandinavica*, *50*, 1–6.

Komsta L, Novomestky F. 2015. Moments: Moments, cumulants, skewness, kurtosis and related tests. R package version 014 https://CRANR-projectorg/package=moments.

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). lmerTest package: Tests in linear mixed effects models. *Journal of Statistical Software*, *82*, 1–26.

Langer, P. (2017). *Comparative anatomy of the gastrointestinal tract in Eutheria: Taxonomy, biogeography and food. Vol I: Afrotheria, xenarthra and euarchontoglires. Vol II: Laurasiatheria, general discussion*. De Gruyter.

Leary, S., Underwood, W., Anthony, R., Cartner, S., Corey, D., Grandin, T., Greenacre, C. B., Gwaltney-Bran, S., McCrackin, M. A., Meyer, R., Miller, D., Shearer, J., & Yanong, R. (2013). *AVMA guidelines for the euthanasia of animals*. American Veterinary Medical Association.

Martin, L. F., Ackermans, N. L., Richter, H., Kircher, P. R., Hummel, J., Codron, D., Clauss, M., & Hatt, J.-M. (2021). Macrowear effects of external quartz abrasives of different size and concentration in rabbits (*Oryctolagus cuniculus*). *Journal of Experimental Zoology B* (in review).

Martin, L. F., Ackermans, N. L., Tollefson, T. N., Kircher, P. R., Richter, H., Hummel, J., Codron, C., Hatt, J.-M., & Clauss, M. (2021). Tooth wear, growth, and height in rabbits (*Oryctolagus cuniculus*) fed pelleted or extruded diets with added sand. *Journal of Animal Physiology and Animal Nutrition*. https://doi.org/10.1111/jpn.13565

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 A*, *321*, 283–298.

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*, 591–604.

Nicoletti, A., Di Girolamo, N., Zeyen, U., Selleri, P., Masi, M., & Fonti, P. (2018). Ultrasound morphology of cecal appendix in pet rabbits. *Journal of Ultrasound*, *21*, 287–291.

Niinistö, K. E., Määttä, M. A., Ruohoniemi, M. O., Paulaniemi, M., & Raekallio, M. R. (2019). Owner-reported clinical signs and management-related factors in horses radiographed for intestinal sand accumulation. *Journal of Equine Veterinary Science*, *80*, 10–15.

Papadomichelakis, G., & Fegeros, K. (2020). Reliability of acid-insoluble ash as internal marker for the measurement of digestibility in rabbits. *World Rabbit Science, 28*, 1–12.

R Core Team. (2017). *R: A language and environment for statistical computing. Version 3.4.1.* R Foundation for Statistical Computing http://www.R-project.org/

Rees Davies, R., & Rees Davies, J. A. E. (2003). Rabbit gastrointestinal physiology. *Veterinary Clinics of North America: Exotic Animal Practice, 6*, 139–153.

Rödel, H. G. (2005). Winter feeding behaviour of European rabbits in a temperate zone habitat. *Mammalian Biology, 70*, 300–306.

Safwat, A. M., Sarmiento-Franco, L., Santos-Ricalde, R. H., Nieves, D., & Sandoval-Castro, C. A. (2015). Estimating apparent nutrient digestibility of diets containing *Leucaena leucocephala* or *Moringa oleifera* leaf meals for growing rabbits by two methods. *Asian-Australasian Journal of Animal Sciences, 28*, 1155–1162.

Schwarm, A., Ortmann, S., Fritz, J., Rietschel, W., Flach, E. J., & Clauss, M. (2013). No distinct stratification of ingesta particles and no distinct moisture gradient in the forestomach of nonruminants: The wallaby, peccary, hippopotamus, and sloth. *Mammalian Biology, 78*, 412–421.

Schwarm, A., Ortmann, S., Rietschel, W., Kühne, R., Wibbelt, G., & Clauss, M. (2010). Function, size and form of the gastrointestinal tract of the collared *Pecari tajacu* and white-lipped peccary *Tayassu pecari*. *European Journal of Wildlife Research, 56*, 569–576.

Siwińska, N., Łuczka, A., Żak, A., Słowikowska, M., & Niedźwiedź, A. (2019). Assessment of sand accumulation in the gastrointestinal tract and its excretion with stool in silesian foals. *Polish Journal of Veterinary Sciences, 22*, 337–343.

Snipes, R. (1982). Anatomy of the Guinea pig cecum. *Anatomy and Embryology, 165*, 97–111.

Snipes, R. L. (1979). Anatomy of the rabbit cecum. *Anatomy and Embryology, 155*, 57–80.

Snipes, R. L., Clauss, W., Weber, A., & Hörnicke, H. (1982). Structural and functional differences in various divisions of the rabbit colon. *Cell and Tissue Research, 225*, 331–346.

Turner, W. C., Imologhome, P., Havarua, Z., Kaaya, G. P., Mfune, J. K. E., Mpofu, I. D. T., & Getz, W. M. (2013). Soil ingestion, nutrition and the seasonality of anthrax in herbivores of Etosha National Park. *Ecosphere, 4*(1), art13.

Walker, E. A., & Taylor, J. H. (1965). The effect of parenterally administered iron upon outdoor-reared piglets. *Animal Science, 7*, 1–6.

Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L. D. A., François, R., Grolemund, G., Hayes, A., Henry, L., Hester, J., & Kuhn, M. (2019). Welcome to the Tidyverse. *Journal of Open Source Software, 4*, 1686.

Williams, J. A., Griffen, W. O., Sharma, A., & Wangensteen, O. H. (1961). Composition and source of secretion from lymphoid aggregations in the rabbit gut. *British Journal of Experimental Pathology, 42*, 153–157.

Wings, O., Hatt, J.-M., Schwarm, A., & Clauss, M. (2008). Gastroliths in a pygmy hippopotamus (*Hexaprotodon liberiensis*). *Senckenbergiana Biologica, 88*, 345–348.

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