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Increasing food intake affects digesta retention, digestibility and gut fill but not chewing efficiency in domestic rabbits (*Oryctolagus cuniculus*)

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Abstract

In ruminants, the level of food intake affects net chewing efficiency and hence faecal particle size. For nonruminants, corresponding data are lacking. Here, we report the effect of an increased food intake of a mixed diet in four domestic rabbit does due to lactation, and assess changes in particle size (as determined by wet sieving analysis) along the rabbit digestive tract. During lactation, rabbits achieved a distinctively higher dry matter intake than at maintenance, with a concomitant reduction in mean retention times of solute and particle markers, an increase in dry matter gut fill, a reduction in apparent digestibility of dry matter, and an overall increase in digestible dry matter intake. By contrast, there was no change in faecal mean particle size (mean ± SD: 0.58 ± 0.02 vs. 0.56 ± 0.01 mm). A comparison of diet, stomach content and faecal mean particle size suggested that 98% of particle size reduction occurred due to ingestive mastication and 2% due to digestive processes. Very fine particles passing the finest sieve, putatively not only of dietary but mainly of microbial origin, were particularly concentrated in caecum contents, which corresponds to retention of microbes via a 'wash-back' colonic separation mechanism, to concentrate them in caecotrophs that are re-ingested. This study gives rise to the hypothesis that chewing efficiency on a consistent diet is not impaired by intake level in nonruminant mammals.

KEYWORDS

digestion, herbivore, lactation, lagomorph, mastication, microbes

1 | INTRODUCTION

Reducing food particle size by chewing is a prominent characteristic of mammals (Reilly et al., 2001). Apart from making ingestion physically feasible, particle size reduction enhances the rate of digestion (Bjorndal et al., 1990; Hummel et al., 2020), and a compromise of chewing efficiency is among the limiting factors for mammalian survival and reproductive success (King et al., 2005; Kojola et al., 1998; Skogland, 1988). Accordingly, preventing an impairment of chewing efficiency is important for mammals.

Different factors can influence particle size reduction of food. The diet itself is among them, as different diets may be chewed to different sizes (Hummel et al., 2008; Jalali et al., 2015; Kljak et al., 2019). The factors producing these effects are not well understood, likely because the interplay of dental morphology, chewing movements and various physical characteristics of food is complex and not easily measured beyond the resulting particle size. In terrestrial herbivores, mastication is the main factor contributing to particle size reduction, with microbial fermentation and enzymatic digestion playing only a minor role (McLeod & Minson, 1988; Poppi et al., 1980; Spalinger & Robbins, 1992). Therefore, faecal particle size is used as a proxy for chewing efficiency (Fritz et al., 2009). Nevertheless, particle size reduction along the gastrointestinal tract does occur to a minor degree, and this effect is suggested to be more relevant in small herbivores (reviewed in Naumova et al., 2021).

The time an animal allocates to chewing is most likely another important factor for chewing efficiency. On the one hand, oral processing via chewing is considered the main factor responsible for the 'functional response' observation that instantaneous food intake does not increase monotonously with offered food density, but reaches a plateau (Hummel & Clauss, 2011; Yearsley et al., 2001). On the other hand, chewing intensity might decrease, due to time constraints, at increasing levels of food intake. Theoretically, there could be a trade-off between the amount of food ingested, and the intensity with which it is masticated. For domestic ruminants, such a trade-off has been shown experimentally, where higher levels of intake of a consistent diet led to reduced chewing intensity (Coulon et al., 1987) and larger particles in the faeces (Kaske & Groth, 1997; Kovács et al., 1997; Shaver et al., 1988). To minimize this effect, animals might increase time spent chewing at the cost of other activities, as shown in lactating cattle (Coulon et al., 1987) or mountain goats (*Oreamnos americanus*) (Hamel & Côté, 2008) and koalas (*Phascolarctos cinereus*) (Logan & Sanson, 2003), or increase their chewing frequency to compensate for the effect, as shown in lactating bighorn sheep (*Ovis canadensis*) (Blanchard, 2005).

Corresponding studies for nonruminants are scarce. Horses did not show significant variation in faecal particle size across a very large range of intake levels (31–93 g $kg^{-0.75}$ d^{-1}; Clauss et al., 2014); however, this range was achieved by varying degrees of food restriction below ad libitum intake at maintenance energy requirements. Therefore, in that study, the expectation had been that the low intake level might lead, due to hunger, to more hastily food intake and hence less thorough mastication, as observed in ruminants (Luginbuhl et al., 1989). Investigations with nonruminants under conditions of increased intake, such as during lactation, are lacking to our knowledge. The aim of this study was to address this gap and test whether the increased intake during lactation would affect chewing efficiency in rabbits.

2 | MATERIALS AND METHODS

Experiments were performed under Animal Experiment Licence 56-2 of the Official Veterinary Office, Bonn, Germany. Four female domestic rabbits *(Oryctolagus cuniculus)* of the Czech Spot breed, of unknown relatedness, aged 8 months at the beginning of the study, were used at three different time points: at maintenance and in primiparous lactation for the assessment of intake on digesta retention, digestibility, gut fill and faecal particle size, and later again at maintenance for the assessment of particle size in different sections of the digestive tract. Rabbits were adult (non-lactating = 8 months; lactating = 11 and 14 months) and without obvious dental problems. They were fed a diet with a constant proportion of 50% chopped grass hay and 50% concentrate (Table 1) at two food intake levels representative for maintenance and lactation (Table 2), based on intake levels for does in the literature (Gidenne & Lebas, 2006; Lebas et al., 1975). The concentrate was fed twice daily at 08:00 and 16:00; the hay was given in several smaller portions across the day. The diet was always consumed completely. Animals had ad libitum access to water. During the collection periods, they were kept in cages allowing separation of individuals and total collection of faeces. The litter (4–6 young) was kept in a separate nest box. Suckling was allowed twice daily at 08:30 and 16:30, and the mothers were weighed before and after nursing to confirm milk production and the corresponding higher energy and nutrient requirements during lactation.

TABLE 1 Feeds used in the present study (means with standard deviation; *n* = 3)

		Grass hay	Pelleted diet[a]	Total diet
Total ash	g kg^{-1} DM	105 ± 24	95 ± 1	100 ± 12
Crude protein	g kg^{-1} DM	111 ± 22	190 ± 4	150 ± 13
Ether extracts	g kg^{-1} DM	25 ± 7	29 ± 0	27 ± 3
Neutral detergent fibre	g kg^{-1} DM	496 ± 84	367 ± 10	431 ± 47
Acid detergent fibre	g kg^{-1} DM	311 ± 45	206 ± 6	259 ± 20
Acid detergent lignin	g kg^{-1} DM	51 ± 1	58 ± 5	54 ± 3
Mean particle size	mm	21.13	0.58	10.85

[a] Ingredients in % of dry matter (DM): lucerne meal (38.00), wheat middlings (18.70), soybean meal (12.00), sunflower meal (10.00), barley grain (8.00), oats huskmeal (6.25), molasses (4.75), soybean oil (0.45), feeding lime (0.45), monocalcium phosphate (0.15), mineral-vitamin-mix (1.25).

TABLE 2 Body mass and measures of digestive physiology (means with standard deviation) in four rabbits (*Oryctolagus cuniculus*) fed at maintenance or during lactation

Measure	Unit	Maintenance	Lactation	p[a]
Body mass	kg	3.61 ± 0.06	4.30 ± 0.61	0.106
Dry matter intake	g d^{-1}	110	220	–
Relative dry matter intake	g kg$^{-0.75}$ d^{-1}	42 ± 0	75 ± 8	0.004
	g kg$^{-0.67}$ d^{-1}	47 ± 0	84 ± 8	0.003
Mean retention time	h			
MRT$_{solute}$		76 ± 6	53 ± 4	0.001
MRT$_{particle}$		26 ± 2	17 ± 4	0.008
MRT$_{particle}$/MRT$_{solute}$		0.34 ± 0.04	0.33 ± 0.06	0.595
Faecal excretion	g DM d^{-1}	24 ± 2	78 ± 12	0.004
Dry matter GIT fill	g	72 ± 5	107 ± 19	0.024
	g kg^{-1} BM	20 ± 1	25 ± 4	0.047
Apparent digestibility	%			
Dry matter		78 ± 2	65 ± 5	0.028
Organic matter		78 ± 1	64 ± 5	0.023
Crude protein		82 ± 2	76 ± 4	0.135
Neutral detergent fibre		67 ± 2	38 ± 10	0.018
Relative digestible dry matter intake	g kg$^{-0.67}$ d^{-1}	37 ± 1	54 ± 7	0.019
Mean faecal particle size	mm	0.56 ± 0.01	0.58 ± 0.02	0.546
Very fine faecal particles	% all particles	20.4 ± 4.0	25.2 ± 10.2	0.437

Abbreviation: MRT, mean retention time.

[a] Paired *t* test.

The length of the experimental period at maintenance was 22 days, consisting of a 14-day period for adaptation (during which the diet was fed at the designated amount) and an 8-day period for collecting samples. The length of the period during lactation was, adapted to the peak of lactation curve, 19 days including 14 days adaptation and a 5-day period for collecting samples, starting 3 days postpartum. Samples of feedstuffs were taken daily during the trial and were pooled. Faeces were collected quantitatively at intervals necessary for determination of digesta mean retention time (MRT). Two different markers were ingested by the animals on day 15 with a small proportion of morning concentrate. The animals were dosed with 2.7 g chromium (Cr)-mordanted fibre (based on 1–2 mm particles from grass hay) and 0.27 g cobalt(III)ethylene diamine tetraacetate (Co-EDTA; solutes) (Udén et al., 1980). To ensure total consumption, Co-EDTA was dissolved in water, mixed with the concentrate and the Cr-mordanted fibre, and dried again before feeding (60°C, 6 h). Note that passage markers need to be basically indigestible and that their excretion patterns have to be interpreted correspondingly, and that depending on the study objective and the degree to which marker migration is relevant, ytterbium-labelled particles or Cr-mordanted fibre might be considered the more suitable particle marker in rabbits (Gidenne, 1988). Faecal samples were collected at time intervals of increasing length (Day 1–2: 4 h; Day 3–5: 6 h; Day 6–7: 8 h; Day 7–8: 12 h). One part was dried at 60°C for 24 h and after that at 100°C for another 24 h, and then milled and stored for marker analysis; another part was pooled over the sampling period and stored frozen for wet-sieving and chemical analysis.

After the litters had been weaned and the rabbits were on maintenance intake level, they were euthanized within 1.5 h after a morning meal and dissected. The total contents of stomach, caecum, and colon were taken and stored frozen. A representative part of the sample was used for wet sieving.

Chemical analysis was done according to VDLUFA (2012) for dry matter (DM) (method 3.1; drying at 103°C), ash (method 8.1; combustion at 550°C), crude protein (method 4.1.2; Dumas method; instrument FP-328; LecoEnterprise) and starch (enzymatically; method 7.2.3). Ether extract was analyzed after acid hydrolysis using an ANKOM Extractor (Ankom Technology) according to AOCS and Firestone (2009) (Am 5-04 official method). Neutral detergent fibre (NDFom; not assayed with a heat stable amylase), and, in feeds, acid detergent fibre (ADFom) and acid detergent lignin were analysed following Van Soest and Robertson (1985); all values are given without residual ash. Analysis of faecal samples for retention

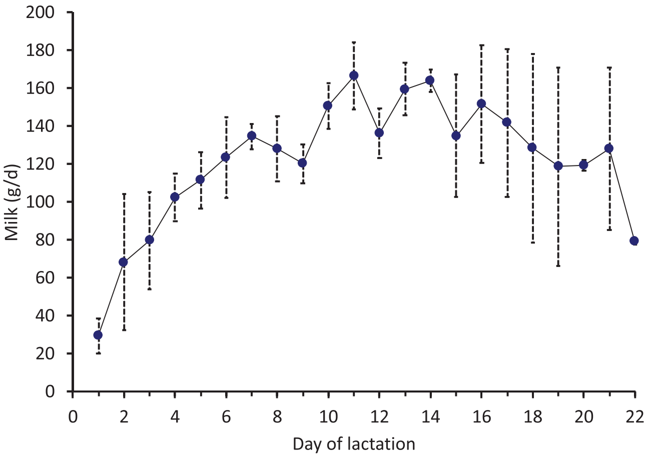

FIGURE 1 Mean (±standard deviation) daily milk production in the four rabbits (*Oryctolagus cuniculus*) of the present study [Color figure can be viewed at wileyonlinelibrary.com]

markers followed the procedure of Behrend et al. (2004) and Hummel et al. (2005): Approximately 0.3 g dry faeces were mixed in test tubes with 5 ml 72% H_2SO_4 and placed on a shaker overnight. The following day, the samples were filtrated into fresh tubes. Co and Cr were directly measured in this solution by atomic absorption spectroscopy (Perkin-Elmer 1100 B).

The faeces and feeds were also subjected to a wet-sieving procedure (sieves of 16, 8, 4, 2, 1, 0.5, 0.25, 0.125, and 0.063 mm mesh size). Known amounts of samples were soaked in water before sieving to separate all cohering particles (hay for 10 min, concentrate for 30 min, faeces overnight in a refrigerator). Wet sieving was done for 10 min with a water flow of 2 l/min sprayed on the top sieve using a Vibrotronic Type VE 1 (Retsch Technology, Haan, Germany). The amplitude of the sieve shaker was adjusted at 2 mm.

Relative dry matter intake was expressed per $BM^{0.75}$ and also per $BM^{0.67}$ as suggested for small herbivores (Müller et al., 2013). Digestibility was calculated as the amount (of nutrient) not eliminated as faeces in percent of the amount ingested. The calculation of MRT from faecal marker concentrations was done according to Thielemans et al. (1978), as

$$\mathrm{MRT} = \frac{\Sigma\, t_i C_i dt_i}{\Sigma\, C_i dt_i}$$

with C_i = marker concentration in the faecal samples from the interval represented by time t_i (hour after marker administration, using the midpoint of the sampling interval) and dt_i = the interval (hour) of the respective sample

$$dt_i = \quad ((t_{i+1}-t_i) + (t_i-t_{i-1}))/2$$

Dry matter gut fill was estimated from dry matter intake, particle MRT, and dry matter digestibility using the linear equation of Holleman and White (1989). Mean particle size (MPS) of material retained on the sieves was calculated as dMean following Fritz et al. (2012). The difference between the amount of dry matter subjected to sieve analysis (as calculated from the amount of sample and the respective dry matter concentration) and the dry matter retained on all sieves was calculated to represent the very fine particle (<0.063 mm) fraction. Sieve analysis data for the gastrointestinal tract of five domestic rabbits from Fritz (2007) were subjected to the same calculations.

Statistical analyses were performed in R (R Core Team, 2017). Differences between the maintenance and lactation intake levels were assessed by paired *t* test. Differences between the GIT sections (stomach, caecum, colon) in the percentage of very fine particles and in MPS were assessed by mixed models using the "nlme" package (Pinheiro et al., 2016), where individual was a random factor (to account for repeated measures), with post hoc Tukey tests performed using the "emmeans" package (Lenth et al., 2018). The significance level was set to 0.05.

3 | RESULTS

As planned, the rabbits had a significantly higher food intake during lactation, both in absolute and relative terms (Table 1). Daily milk production, determined by weighing mothers before and after suckling, corresponded to published lactation information for domestic rabbits (Casado et al., 2006) (Figure 1). Maximal milk yield ranged between 171 and 182 g/day in the four does, with the peak occurring between the 11th and the 17th day of lactation.

Retention marker excretion curves resembled those reported earlier for rabbits (Franz et al., 2011) (Figure 2a), with a fast descent of the concentration of the particle marker but a slow, gradual descent of the solute marker that was interrupted more or less regularly by secondary peaks of this marker that indicate coprophagy. At the higher intake, $\mathrm{MRT}_{\mathrm{solute}}$ (Figure 2b) and $\mathrm{MRT}_{\mathrm{particle}}$ (Figure 2c) were significantly shorter; their ratio, however, remained unchanged (Table 2).

On the higher intake, the rabbits also had a higher faecal output, a higher dry matter gut fill, and lower apparent digestibility of dry matter, organic matter and NDF; the digestibility of crude protein, however, remained unchanged (Table 2). Regardless of the lower digestibility, overall digestible dry matter intake was higher at the increased intake (Table 2). There was no difference in the percentage of very fine particles or the MPS in the faeces between the two intake levels (Figure 3a, Table 2).

In the four rabbits of the present study, a distinct drop in particle size was evident when comparing sieve fractions of the diet and the stomach contents (Figure 3b). The percentage of very fine particles was highest in the caecum, and this was significantly different from both the stomach ($p < .001$) and the colon ($p = .001$), with no difference between stomach and colon ($p = .684$) (Figure 3b). The MPS was lowest in the caecum (0.41 ± 0.01 mm), intermediate in the colon (0.54 ± 0.03 mm) and highest in the stomach (0.77 ± 0.02 mm); pairwise differences were significant between the three sites (p always <.001). In the five rabbits from Fritz (2007), the percentage of very fine particles was also highest in the caecum, and this was

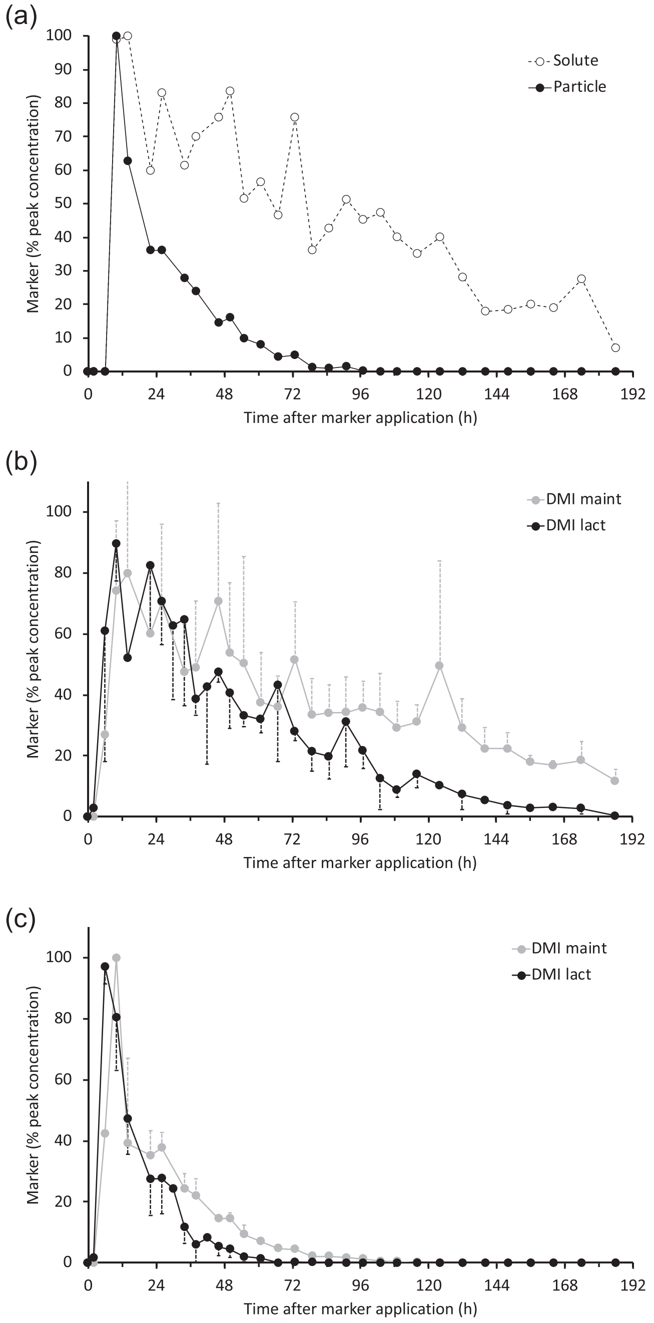

FIGURE 2 Marker excretion graphs (solute marker: Co-EDTA; small particle (1–2 mm) marker: Cr-mordanted fibre) in rabbits (*Oryctolagus cuniculus*) on a consistent diet; (a) example in a single rabbit on maintenance dry matter intake (DMI) level; (b) mean (with standard deviation) solute marker excretion of 4 rabbits on maintenance (maint) and lactation (lact) DMI; (c) mean (with standard deviation) particle marker excretion of four rabbits on maintenance and lactation DMI. Note the secondary excretion peaks for the solute marker indicating coprophagy, and the faster marker excretion on the lactation intake level

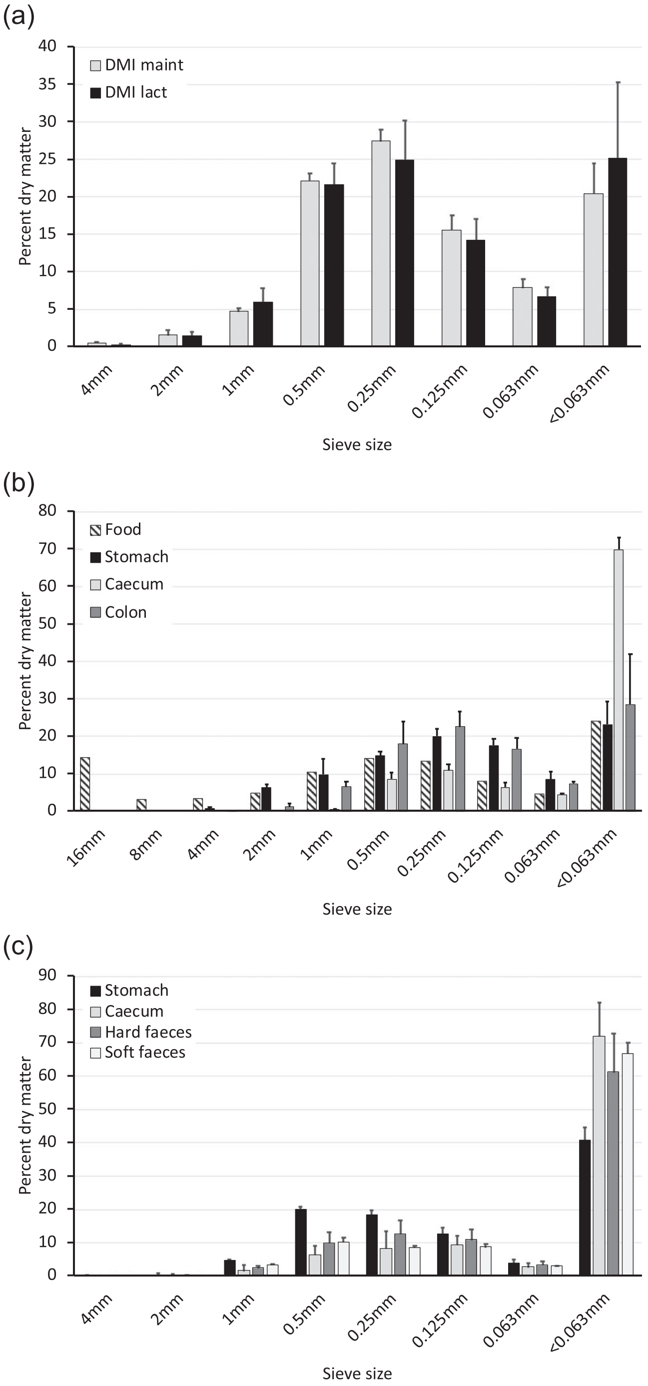

FIGURE 3 Mean (with standard deviation) percentage of dry matter of faeces and gastrointestinal contents of rabbits (*Oryctolagus cuniculus*) submitted to wet sieve analysis; (a) particle size distribution of the faeces of four rabbits on a consistent diet at maintenance (maint) or lactation (lact) dry matter intake level; (b) particle size distribution of gastrotinestinal contents of the same four rabbits after slaughter in comparison to the particle size distribution of the diet;
(c) gastrointestinal contents of five rabbits and two samples of soft faeces from the study of Fritz (2007)

significantly different from the stomach ($p < .001$) but not from the colon ($p = .105$), with a significant difference between stomach and colon ($p = .005$) (Figure 3c). The MPS was lowest in the caecum (0.45 ± 0.04 mm), intermediate in the colon (0.47 ± 0.03 mm) and highest in the stomach (0.55 ± 0.06 mm); differences were significant between stomach and caecum ($p = .015$), stomach and colon ($p = .040$), but not between caecum and colon ($p = .786$). Additionally, two samples of "soft faeces" or "caecotrophs" had a MPS of 0.53 mm; in terms of the proportion of very fine particles, they were numerically between the caecum and the colon contents (Figure 3c).

4 | DISCUSSION

Our study indicates that different intake levels, while affecting digestive physiology in ways corresponding to previous reports, do not affect chewing efficiency as measured by faecal particle size in rabbits. Additionally, they indicate that very fine particles accumulate particularly in the caecum of rabbits, and that particle size reduction does not only occur at ingestion via chewing, but also during passage through the gastrointestinal tract.

Evidently, an important constraint of the present study was the low number of animals at $n = 4$. Initially, it had been planned to use six rabbits, but two failed to deliver a litter successfully in the time available for the project. Nevertheless, given the nature of our results, with either clear or no differences, our findings can be considered reliable. The rabbits used were primiparous, and had comparatively small litter sizes (4–6 young); therefore, the lactation dry matter intake was, at 66–82 g $\text{kg}^{-0.75}$ d^{-1}, not as high as some values reported in the literature for lactating rabbit does with larger litters of ≥8 young on a comparable diet (e.g., 106 g $\text{kg}^{-0.75}$ d^{-1} in Pascual et al., 1999). The use of primiparous animals in the present study most likely does not represent an important constraint, given that the milk production was as expected for rabbits. In cattle, an ontogenetic decrease of chewing intensity has been demonstrated (Grandl et al., 2016). For example, primiparous cows have a higher chewing intensity, that is, they chew more per dry matter intake, than multiparous cows (Beauchemin & Rode, 1994). This is an effect of the fact that cattle are bred and give birth at an age where the molar teeth have not yet erupted completely. As available chewing surface determines chewing intensity (Pérez-Barbería & Gordon, 1998), chewing intensity decreases in parallel with molar eruption (Grandl et al., 2018). In rabbits, the permanent dentition has completely erupted within the first month after birth (Bertonnier-Brouty et al., 2020; Michaeli et al., 1980), so that the animals used in the present study, with an initial age of 8 months, had their fully functional dentition.

We fed the rabbits a mixed diet, rather than a forage-only diet that would on the one hand resemble more closely the natural diet of rabbits, and on the other hand require a distinctively longer intake (Müller et al., 2014; Schröder, 2000) and hence might more probably exert a time constraint. In the future, it might be interesting to repeat this assessment on a forage-only diet that requires distinctively more time for ingestion than the mixed diet of our study (Zumbrock, 2002). Another limitation was that we did not have the equipment to document the chewing behaviour of the rabbits, and therefore could not determine chewing intensity (in chews per gram dry matter intake).

The response of herbivores that increase intake of a consistent diet can generally react along a continuum of two extremes (Hume, 2005): given spare gut capacity, they can increase their gut fill and retain their original digesta retention time, or their gut fill remains constant, and digesta retention time decreases markedly. Typically, herbivores will show both effects, at varying degrees depending on their digestive anatomy and physiology (Clauss et al., 2007; Findeisen et al., 2021; Munn et al., 2015). With both an increase in gut fill and a decrease in retention time, the rabbits of the present study were no exception. Shorter retention times at higher intakes have been reported previously in rabbits (Bellier & Gidenne, 1996; Gidenne & Feugier, 2009). While intake generally affects both the retention of particle and solute markers, their ratio typically remains constant (reviewed in Clauss et al., 2014), as in the present study, suggesting that a specific degree of "digesta washing" (i.e., movement of liquid in relation to the movement of particulate matter) is a distinct feature of species-specific digestive physiology (Müller et al., 2011).

Because, in contrast to auto-enzymatic digestion of non-fibre substances, microbial digestion and fermentation of fibre is distinctively time-dependent (Hummel et al., 2006), one would intuitively expect that at shorter retention times, digestibility, and in particular fibre digestibility, is reduced. This was also evident in the present study, where an absolute increase (measured as g d^{-1}) in dry matter intake of 100%, representing an about 80% increase in relative dry matter intake (from 47 to 84 g $\text{kg}^{-0.67}$ d^{-1}), only led to a 48% increase in relative digestible dry matter intake (from 37 to 54 g $\text{kg}^{-0.67}$ d^{-1}, Table 2). It should be noted, however, that this does not mean that longer retention times, observed at ever-decreasing intakes, lead to ever-increasing digestibility: below a certain intake level, fibre digestibility is also reduced, most likely due to a shortage of nutrients necessary for microbial action (reviewed in Clauss et al., 2014).

Chewing efficiency as measured by faecal particle size is apparently not reduced by higher intake levels in rabbits. At least at the intake level of the present study, rabbits are not time-constrained to achieve the required food intake. Together with the finding of no effect of an intake reduction on chewing efficiency in horses (Clauss et al., 2014), this leads to the hypothesis that in nonruminants, a given diet is always chewed to a certain size before it is swallowed (Prinz & Lucas, 1997). The setpoints for this size or the related number of chews, and why this differs—in terms of the resulting particle size—between different diets also in nonruminants (Clauss et al., 2014; Naumova et al., 2021) remains to be clarified.

By contrast, in domestic ruminants, higher intakes influence faecal particle size (see Introduction). It could be speculated that this could be an effect of different diets used across experiments. However, a parallel experiment to the present one, in which domestic goats (*Capra hircus*) were fed a similar 50:50 mixture of grass hay and a concentrate feed at maintenance and lactation intake, also documented that a similar increase in faecal particle size with intake was evident as in other ruminant studies (Findeisen et al., 2021). The difference between ruminants and nonruminants might stem from the fact that in ruminants, ingestive mastication is less systematic and consistent, and hence possibly less 'fixed', than in nonruminants (Dittmann et al., 2017). The effect of intake on faecal particle size then stems from the fact that at high fill of the reticulorumen, larger particles may escape retention and re-mastication (Hummel et al., 2018), rather than a reduction in rumination chewing efficiency itself (Findeisen et al., 2021).

Similar to previous reports in several nonruminant herbivores (reviewed in Naumova et al., 2021), a reduction of particle size along the digestive tract was evident in both the rabbits of our study and those previously analysed by Fritz (2007) (Figure 3b,c). For ruminants, McLeod and Minson (1988) reported that 82% of large particle breakdown was due to mastication (ingestive as well as rumination), and 18% due to digestive processes and friction. Using the MPS results of the third part of the present study, a diet of an average MPS of 10.85 mm was reduced to a faecal particle size of 0.54 mm, that is, a total difference of 10.31 mm. Ignoring a possible effect of coprophagy, 10.08 mm of this size reduction (or 98%) occurred at the transition from the diet to the stomach, that is, by ingestive chewing, and only 0.23 mm (or 2%) during the passage from the stomach to the colon. Thus, the concept that mastication is mainly responsible for particle size reduction in terrestrial herbivores is supported. However, material in the stomach need not stem completely from ingested feed. Even though slaughtering of the animals was timed to occur after a morning meal, which ensured that freshly ingested and masticated feed was present in their stomachs, we cannot exclude that disintegrated "soft faeces" or "caecotrophs" constituted some part of the stomach contents. Even though caecotrophs are not chewed and are hence visible as distinct, round entities for a certain period of time (cf. the supplementary information of Schulz et al., 2013) before they disintegrate, they consist of particularly small particles (see below). Hence, the size reduction effect of ingestive mastication may be overestimated when comparing the particle size of diet and stomach contents. The fact that the reduction in digestibility with reduced retention times in the present study was not accompanied by a reduction in faecal particle size or the proportion of very fine particles in the faeces supports the notion that digestion itself has a comparatively minor effect on particle size reduction. Note that even in ruminants, with their distinctively longer particle retention than in rabbits (Müller et al., 2013), digestion effects only a minor part of the overall particle size reduction (McLeod & Minson, 1988).

The particles that pass through the finest sieve—in our study, 0.063 mm—represent unknown material. In contrast to Gidenne et al. (1989), who reported that this fraction was lower in rabbit hard faeces than particles between 0.315 and 0.05 mm, our results for hard faeces from several experiments consistently found it to be higher (Figure 3). As outlined in Naumova et al. (2021), this material should not only be considered of dietary, but also of microbial origin. Rabbits have a colonic separation mechanism of the "wash-back" type, which uses a retrograde fluid flow in the proximal colon to wash very fine particles, and especially microbes, back into the caecum (Björnhag & Snipes, 1999; Cork et al., 1999), so that detailed analyses can trace an increasing deprivation of gut contents of very fine particles along the length of the colon (Björnhag, 1972). Our findings of long solute marker retention times (Figure 2), and of a particularly high accumulation of very fine particles at this site (Fig. 3BC), correspond to these descriptions. These microbes then form a major component of the so-called "soft faeces" or "caecotrophs" that are excreted at certain times and directly reingested by the animal from the anus. With respect to MPS as derived from particles retained on sieves (i.e., without accounting for the very fine ones passing the lowest sieve), soft and hard faeces of rabbits are not different (Udén & Van Soest, 1982), which was confirmed in the few samples of the present study. However, one would expect a higher proportion of very fine particles in the soft faeces. The clear separation of caecum and colon contents in the proportion of very fine particles in the present study most likely stems from the clearly defined timepoint of euthanasia shortly after the morning meal, at a time when the colonic separation mechanism can be expected to be active and hard faeces fill the distal colon. Similar information was not available for the rabbits from Fritz (2007).

In conclusion, we did not find evidence of an intake constraint on chewing efficiency in rabbits fed a mixed diet at maintenance and during lactation. While the majority of particle size reduction occurred during ingestion (presumably due to mastication), a small additional particle size reduction occurred along the digestive tract (presumably due to digestion). Given that the only reports on an intake constraint on chewing efficiency available in the literature so far stem from ruminants, we hypothesize that this is due to the peculiar particle retention mechanism in ruminants that is linked to a relaxation of ingestive chewing consistency, and that in nonruminant mammals, chewing efficiency should remain constant for a given diet at varying levels of intake. This would mean that in nonruminant herbivores, energy and nutrient extraction efficiency on a given diet depend only on the gut capacity-modified interplay of intake level and digesta retention.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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