# Individual differences in digesta retention and their relation to chewing in cattle - A pilot investigation

**Journal Article**

**Author(s):**
Zhang, Xiaoyu; Li, Yang; Terranova, Melissa; Ortmann, Sylvia; Kreuzer, Michael; Hummel, Jürgen; Clauss, Marcus

**Publication date:**
2023-03

**Permanent link:**
https://doi.org/10.3929/ethz-b-000548444



**Originally published in:**
Journal of Animal Physiology and Animal Nutrition 107(2), https://doi.org/10.1111/jpn.13733

Received: 12 December 2021 | Accepted: 22 April 2022

DOI: 10.1111/jpn.13733

ORIGINAL ARTICLE

Ruminants

Journal of Animal Physiology and Animal Nutrition WILEY

# Individual differences in digesta retention and their relation to chewing in cattle—A pilot investigation

Xiaoyu Zhang[1] | Yang Li[2] | Melissa Terranova[3] | Sylvia Ortmann[4] | Michael Kreuzer[2] | Jürgen Hummel[1] | Marcus Clauss[5]

[1]Department of Animal Sciences, Ruminant Nutrition, University of Göttingen, Göttingen, Germany

[2]Institute of Agricultural Sciences, ETH Zurich, Lindau, Switzerland

[3]AgroVet-Strickhof, ETH Zurich, Lindau, Switzerland

[4]Leibniz Institute for Zoo and Wildlife Research (IZW), Berlin, Germany

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

**Correspondence**

Marcus Clauss, Clinic for Zoo Animals, Exotic Pets and Wildlife, Vetsuisse Faculty, University of Zurich, Winterthurerstrasse 260, Zurich 8057, Switzerland.
Email: mclauss@vetclinics.uzh.ch

## Abstract

While information on individual differences in digesta mean retention time (MRT) might be interesting when selecting phenotypes for digestive efficiency, MRT measurements are prohibitively labour-intensive for large-scale application. Therefore, more easily measured proxies of MRT might be helpful. We used the opportunity of an experiment applying saliva stimulant in cattle to investigate the effect of different individual chewing behaviour on fluid and particle MRT with a consistent diet. Four non-lactating cattle (670–850 kg body mass [BM]) were used in a 4 × 4 Latin square design, treated with the saliva stimulant pilocarpine in dosages of 0, 1, 2.5 and 5 mg/kg BM per day. The cattle were fed hay with dry matter intake (DMI) assigned according to their metabolic body weight. MRT in the whole gastrointestinal tract (GIT), the reticulorumen (RR) and the distal tract were measured using Co-EDTA, Cr-mordanted fibre and La-mordanted fibre as markers representing fluid, small particles (2 mm) and large particles (1 cm), respectively. The chewing behaviour was measured via noseband pressure sensor and expressed as chewing frequency (chews per time) and chewing intensity (chews per DMI), both for total chewing (ingestion plus rumination) and rumination chewing alone. The animals differed considerably in chewing behaviour and MRT measures. BM did not show a significant effect on chewing behaviour and MRT measures, though it tended to negatively correlated to total chewing intensity. Chewing intensity exerted a significant negative influence on MRT of fluid and particles in the RR, which was not the case for chewing frequency. Chewing frequency showed a significant relationship with MRT of large particles in the GIT. We suggest that chewing behaviour could influence MRT in two ways: (i) by affecting saliva production via the masticatory-salivary reflex and subsequently, the fluid inflow to the RR; (ii) by contributing to particle size reduction. Should the link between chewing behaviour and MRT be corroborated in larger studies, chewing measures, with their large interindividual variation, could emerge as an easy-to-measure proxy for MRT characteristics.

KEYWORDS

chewing halter, digesta kinetics, ingestion, passage, rumination

## 1 | INTRODUCTION

The mean retention time (MRT), that is, the time digesta require to pass through the digestive tract, is an important characteristic of the ruminant's digestive efficiency. It is mainly affected by the food intake level and characteristics of the diet of an experiment, but also by the rumen capacity of the individual (Lechner-Doll et al., 1991). Large differences in MRT between ruminant species have been demonstrated in many studies (Bartocci et al., 1997; Lechner-Doll et al., 1990; Przybyło et al., 2019). While most studies in domestic animals on this topic were designed to assess effects of diet and intake (e.g., Colucci et al., 1982), ontogenetic and reproductive life stages (e.g., Grandl et al., 2018; Linden et al., 2014), or climate (e.g., Kennedy & Milligan, 1978) on MRT, less attention has been paid to differences among individual animals. Nevertheless, such differences have been demonstrated, and it has been suggested that information on the phenotype-specific MRT could be useful for genetic selection: Digesta retention is generally positively related to digestibility and methane yield, and negatively to ruminal microbial yield (Goopy et al., 2014; Janssen, 2010; Pinares-Patiño et al., 2011), and could therefore theoretically be an important breeding target (Hegarty, 2004; Smuts et al., 1995; Thompson et al., 1989). However, measuring MRT is difficult and time-consuming; it normally takes 5–7 days of faeces collection after an adaptation period to the respective diet, and food intake must be measured in parallel to account for its distinct effect. Should differences in MRT between phenotypes be considered as an important factor for selective breeding, easier proxies to characterise the MRT phenotype on a large scale would be required.

One factor that theoretically should influence MRT but has, to our knowledge, not been investigated in detail so far in this respect (but see Gindri et al., 2021), is chewing characteristics: chewing intensity, that is, chews per dry matter intake (DMI); chewing frequency, that is, chews per time; and chewing time per DMI; chewing refers to both ingestive and rumination mastication. It is known that individual cattle differ in chewing characteristics (Dado & Allen, 1994). Assuming similarity in dental anatomy, a higher chewing intensity in phenotypes of a species should affect MRT in at least two ways (López-Paredes et al., 2020; Watt et al., 2015): (i) Increased chewing activity both during eating and ruminating should lead to more saliva production and inflow into the reticulorumen (RR), which should decrease the MRT of fluid. (ii) Increased chewing activity should achieve a faster particle size reduction, which should decrease particle MRT, as large particles are reduced faster to below the critical size threshold for leaving the RR.

We used the opportunity of an experiment performed to investigate the effect of a pharmacological saliva stimulant, pilocarpine, on methane emissions; in this experiment, food intake was kept constant per metabolic body weight (MBW, $kg^{0.75}$), and the MRT of a solute and two different-sized particle markers as well as chewing characteristics were measured. Although the study was explorative, we expected that MRT should differ between individuals, and that this should be related to chewing intensity and chewing frequency, with shorter MRT in individuals with higher chewing intensity.

## 2 | METHODS

### 2.1 | Animals, treatment and management

The experiment lasted from August 2020 to January 2021 at the research station AgroVet-Strickhof (Eschikon, Lindau, Switzerland). Four multiparous cattle (two black Holstein, one red Holstein and one Brown Swiss, non-pregnant and non-lactating, body weight from 670 to 850 kg) were used in 4 × 4 Latin square design with four treatments, consisting of one placebo and three oral dosages of pilocarpine (Fagron GmbH&Co. KG) of 0, 1, 2.5 and 5 mg/kg body weight per day at 0600, 1400 and 2200 h. All animals were clinically healthy with no evident chewing problems; however, the state of their dentitions was not documented. With respect to the results reported here, each treatment round consisted of four weeks: during the first week, animals were kept as a group without treatment (10 × 5 $m^2$, half the area with straw bedding), fed with hay for *ad libitum* consumption. In the second week, animals were kept in the same place, fed 60 kg (as fed) of hay daily in total spread across the feed bins, and individually dosed for the respective pilocarpine treatment. In the third (used for determining chewing data) and fourth week (used to determine MRT), animals were kept individually (tie-stall barn, 2 × 1.33 $m^2$, rubber mat with chopped straw bedding). The amount of hay fed per animal was restricted according to their MBW in these 2 weeks, aimed to maintain the body weight, and reduce the effect of intake on the measurements. The total amount of hay allotted to each animal was distributed into three portions, offered after the three daily dosages of pilocarpine or placebo. As a physical vector to which the pilocarpine powder would stick, and for better acceptance, the pilocarpine was mixed with a small amount of maize-grass mixed silage (Table 1). The main part of the diet was made up of the hay; the daily silage DMI accounted for only 4.8 ± 1.7% of daily total DMI. The placebo treatment consisted of the application of a similar amount of silage without pilocarpine. The hay used in the experiment originated from a single batch (Table 1) during the whole experiment. The animals were given 100 g multivitamin

**TABLE 1** Nutrient composition (g/kg dry matter) of provided hay and maize-grass mixed silage during the experiment.

| | Grass hay | Silage |
|---|---|---|
| Organic matter | 917 | 910 |
| Crude protein | 150 | 129 |
| Ether extracts | 23 | 32 |
| Neutral detergent fibre | 595 | 411 |
| Acid detergent fibre | 316 | 273 |
| Crude fibre | 279 | 232 |

and mineral supplement (Künzle Farma AG) and 50 g salt (Schweizer Salinen AG) per day. Water was provided at *ad libitum* access during the whole experiment.

## 2.2 | Preparation and application of digesta passage marker

Markers for ingesta retention, Co-ethylenediaminetetraacetic acid (Co-EDTA; solute marker), Cr-mordanted fibre (2 mm particle marker) and La-mordanted fibre (1 cm particle marker) were prepared according to Udén et al. (1980). The grass hay was dried and cut in a cutting mill to pass a 2- and 4-mm screen separately. The milled hay particles were sequentially screened by shaking on a particle size separator to obtain two size fractions (2 mm and 1 cm; note that milling through a 4-mm screen results in a certain proportion of 1-cm particles). The particles were washed in a washing machine with washing powder for 2 h at room temperature and thoroughly rinsed with water and then incubated with the respective mordant for 24 h (76 g $LaCl_3 7H_2O$ per 100 g 1-cm particles based on dry matter at 37°C; 33 g $Na_2Cr_2O_7 \cdot 2H_2O$ per 100 g 2-mm particles based on dry matter at 100°C). The particles were washed after mordanting, and the Cr-mordanted particles were treated with ascorbic acid and washed again. All particles were then dried at 65°C to constant weight. Co-EDTA was prepared as described by Udén et al. (1980). The dosage provided to the animals was approximately 0.1 g of particle marker and 0.01 g of Co-EDTA per kg of body weight. The passage marker was given in the morning of the first day of Week 4. For that, Co-EDTA was dissolved with hot water and mixed with the particle markers and the morning pilocarpine dose in the silage.

## 2.3 | Data recording, sampling and analysis

The chewing activity was monitored using a noseband pressure sensor (MSR Electronics GmbH) as described by Braun et al. (2013); the sensor was mounted on the noseband of a halter for two separate days during the third week. A data logger recorded pressure signals (10 Hz) from an oil-filled tube that was integrated in the noseband. The recorded signals were first cut to 24 h using the software MSR Cutter V6.05.00 (MSR Electronics GmbH), then evaluated by the software Viewer2 V2.02.00 and differentiated into ingestion, rumination and other activities (such as drinking, scratching, etc.). Then the results were visually evaluated and corrected if needed using the software Editor V2.02.00. The chewing behaviour was expressed as total and rumination chewing frequency (number of chews per time) and total and rumination chewing intensity (number of chews per DMI), where total chewing included ingestion and rumination. BM was measured before and at the end of each 4-week-run using a vehicle scale (±20 kg accuracy). The amount of water consumed was recorded during weeks three and four by water flow metres (GWF MessSysteme AG), which were installed on each individual water pipe to the water trough. Representative samples of the hay, the silage used for treatment dosing, and the individual leftovers of each animal were taken daily in weeks three and four. After each run, these samples were pooled per cow, dried at 60°C overnight and milled through a 0.75 mm sieve for later analysis. They were analysed for the contents of dry matter, organic matter, crude protein, ether extract, crude fibre, neutral detergent fibre and acid detergent fibre according to the standard methods of the Association of German Agricultural Analysis and Research Centers (VDLUFA, 2006). Neutral detergent fibre was analysed after adding amylase, and all detergent fibre values are corrected for residual ash. Faecal samples for MRT determination were collected before marker application (three samples per animal for baseline marker values) and 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 28, 32, 36, 40, 44, 48, 54, 60, 66, 72, 80, 88, 96, 104, 112, 120, 128, 136, 144, 152, 160 and 168 h after marker application. These faecal samples were dried at 105°C to constant weight and ground through a 1-mm screen with a cutting mill. The passage marker concentrations in the faeces were analysed as described by Frei et al. (2015) using inductively coupled plasma optical emission spectrometry (Optima 8000, PerkinElmer) after wet ashing. The baseline concentrations in faecal samples measured before the marker application were used to correct for background levels.

## 2.4 | Calculations and statistics

DMI was calculated as the difference in food offered and leftover, both expressed as dry matter. The MRT in the whole digestive tract (gastrointestinal tract [GIT]) was calculated according to Thielemans et al. (1978) as

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

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

$$dt_i = \frac{(t_{i+1} - t_i) + (t_i - t_{i-1})}{2}.$$

The MRT of fluid in the RR was calculated following Grovum and Williams (1973), and the MRT of the distal GIT as the difference of MRT GIT – MRT RR of the fluid marker. The MRT RR of the particles was calculated as MRT RR particles = MRT GIT particles – MRT distal GIT. This approach is based on the assumption that there is no differential passage of fluid and particles in the distal GIT, an assumption empirically confirmed repeatedly (Huhtanen & Kukkonen, 1995; Mambrini & Peyraud, 1997; Wylie et al., 2000).

To investigate differences between individuals (as opposed to a traditional analysis for a difference between treatments), analysis of variance was conducted with a linear mixed model (LMM) using R version 3.5.2 with experiment rounds and treatments as random factor and animals as fixed factor. The Shapiro–Wilk test was used to

assess residual normality; if needed, variables were ln-transformed (indicated in the results tables). Tukey's procedure was used for multiple comparisons when the LMM was significant ($p < 0.05$). Results are presented as numeric means and standard deviations.

The relationships between chewing intensity and chewing time per DMI were investigated by LMM as

$$\text{Chewing intensity} = \mu + \text{Chewing time per DMI} + \text{Treatment}_i + \text{Animal}_j + \text{Round}_k + e;$$

Chewing measures as well as MRT measures as dependent variables were fitted into LMM to investigate the effect of BM as

$$\text{Chewing or MRT measures} = \mu + \text{BM} + \text{Treatment}_i + \text{Animal}_j + \text{Round}_k + e;$$

MRT measures as dependent variables into LMM to investigate the effect of water intake as

$$\text{MRT measures} = \mu + \text{Water Intake} + \text{Treatment}_i + \text{Animal}_j + \text{Round}_k + e;$$

The relationship between MRT measures and chewing measures was investigated by LMM as:

$$\text{MRT measures} = \mu + \text{Chewing measures} + \text{Treatment}_i + \text{Animal}_j + \text{Round}_k + e;$$

where $\mu$ is the overall mean, treatment is the fixed factor ($i = 1, 2, 3, 4$), round is a random factor, as is animal to account for repeated measures ($j, k = 1, 2, 3, 4$), e is the random residual error. In order to compare the inter-individual variability of our study with that of Dado and Allen (1994), we calculated the coefficient of variation between our four cows.

## 3 | RESULTS

The animals maintained a relatively constant BW during the experiment irrespective of pilocarpine dosage. As planned, no significant differences between treatments were observed for feed intake (data not shown). However, water intake (kg/day) was higher at the highest pilocarpine dose (data not shown).

Total ingestion time, rumination times and the ratio of rumination to ingestion were not affected by treatment; the same was true for total and rumination chewing frequency as well as chewing intensity (data not shown). However, the individual animals differed distinctively in these characteristics (Table 2). The chewing intensity (chews per DMI) was significantly, positively related to time spent chewing per DMI, for ingestion, rumination and total chewing behaviour ($p < 0.001$). However, there were clear differences between animals, which are explained by the differences in chewing frequency (Figure 1). The coefficient of variation between the four animals was low to moderate, ranging between 7.4% and 13.7%, for most MRT and chewing frequency measures. By contrast, it was higher, ranging between 12.8% and 25.1%, for measures of chewing time and chewing intensity. The only MRT measure with a similarly high coefficient of variation was that for the distal GIT at 20.0%. For each chewing measure, the coefficient of variation was higher for rumination than for ingestive chewing, indicating that the four cows were particularly variable in their rumination behaviour (Table 2).

Marker elimination showed the typical cattle pattern, with a clear distinction of solute, small and large particle excretion (Figure 2). MRT measures were different among the individuals (Table 2). BM did not have a significant effect in the models on chewing or MRT measures (Table 3), although total chewing intensity tended to decrease when BM increased ($p = 0.084$). Drinking water intake had no effect on MRT measures (Table 4).

Significant negative relationships were evident between some chewing and MRT measures, for example, between rumination chewing intensity and MRT RR of fluid (Figure 3a) and between total chewing intensity and MRT RR of large particles (Figure 3b). Rumination chewing frequency was negatively related to the MRT GIT of large particles, but there was only a trend for a negative relation with the MRT GIT of fluid and small particles (Table 5). The same negative relation was also seen for total chewing frequency with large particle MRT GIT. For the RR, total as well as rumination chewing intensity had a negative relation with the fluid, small and large particle MRT RR, while chewing frequency showed no relation on MRT RR of fluid, small and large particles (Table 5). The MRT in the distal GIT was not related to the chewing measures (Table 5).

When using the time spent chewing rather than the number of chews, MRT RR was negatively related with rumination time but not with total chewing time per DMI; no relationships were detected between the rest of MRT measures and measures of chewing time per DMI (Table 5). Chewing intensity, as based on the number of chews, had a distinctively higher correlation to MRT measures than chewing time per DMI (Figure 3b,c).

## 4 | DISCUSSION

The results of our pilot study suggest that individual differences in chewing intensity as well as chewing frequency exist when fed the same hay-only diet, and that chewing activity may affect fluid and particle MRT in the RR. In contrast to MRT measurements, chewing measurements take much less time and effort. Future studies need to corroborate their suitability as a proxy for MRT measurements, at least in the sense of a ranking of phenotypes. In doing so, it should be further assessed whether it is necessary to count the number of chews, or whether a measure of 'time spent chewing' is already sufficient. Our preliminary data suggest that, due to individual differences in chewing frequency, 'time spent chewing' is a less informative measure than the actual number of chews made in that time. This is relevant because many recent studies that include a measure of chewing behaviour usually only report (and possibly,

**TABLE 2** Individual means (±SD) and the inter-individual coefficient of variation for body weight, intake, chewing and MRT measures.

| | Animal | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | $p^a$ | CV (%) |
| Body weight (kg) | $683^a \pm 5$ | $688^a \pm 15$ | $843^b \pm 10$ | $788^c \pm 13$ | <0.001 | 10.4 |
| Hay intake (kg/day) | $11.1^a \pm 0.5$ | $11.1^a \pm 0.2$ | $13.1^b \pm 0.4$ | $12.0^c \pm 0.2$ | <0.001 | 8.0 |
| DMI (kg/day) | $11.8^a \pm 0.3$ | $11.7^a \pm 0.5$ | $13.7^b \pm 0.2$ | $12.6^c \pm 0.3$ | <0.001 | 7.4 |
| Water intake (kg/day) | $56.5^a \pm 8.7$ | $60.0^a \pm 5.2$ | $69.2^b \pm 2.7$ | $54.9^a \pm 5.0$ | <0.001 | 10.6 |
| Ingestion time (min/kg DMI) | $32.3^a \pm 3.8$ | $26.3^{ab} \pm 4.5$ | $22.9^b \pm 1.6$ | $25.7^b \pm 2.3$ | 0.009 | 14.8 |
| Rumination time (min/kg DMI) | $37.3^{ab} \pm 1.8$ | $48.8^c \pm 3.8$ | $32.3^a \pm 1.1$ | $40.2^b \pm 2.1$ | <0.001 | 17.4 |
| Total chewing time (min/kg DMI) | $69.5^{ab} \pm 4.3$ | $75.2^a \pm 5.5$ | $55.1^c \pm 1.2$ | $65.8^b \pm 3.3$ | <0.001 | 12.8 |
| Ratio rumination:ingestion time | $1.17^a \pm 0.2$ | $1.89^{bc} \pm 0.3$ | $1.42^a \pm 0.1$ | $1.57^{ac} \pm 0.2$ | 0.007 | 19.9 |
| Ingestion chewing intensity (/kg DMI) | $2131^a \pm 228$ | $1839^{ab} \pm 294$ | $1627^b \pm 84$ | $1543^b \pm 130$ | 0.004[b] | 14.7 |
| Rumination chewing intensity (/kg DMI) | $2426^a \pm 155$ | $3574^b \pm 395$ | $2299^a \pm 106$ | $2133^a \pm 113$ | <0.001 | 25.1 |
| Total chewing intensity (/kg DMI) | $4557^a \pm 289$ | $5412^b \pm 502$ | $3926^c \pm 92$ | $3676^c \pm 184$ | <0.001 | 17.6 |
| Ingestion chewing frequency (/min) | $66.1^a \pm 1.4$ | $69.9^b \pm 0.9$ | $71.2^b \pm 1.7$ | $60.1^c \pm 2.5$ | <0.001 | 7.4 |
| Rumination chewing frequency (/min) | $65.1^a \pm 1.3$ | $73.1^b \pm 2.9$ | $71.2^b \pm 1.5$ | $53.1^c \pm 1.2$ | <0.001 | 7.4 |
| Total chewing frequency (/min) | $65.5^a \pm 0.4$ | $71.9^b \pm 1.8$ | $71.2^b \pm 1.5$ | $55.8^c \pm 1.6$ | <0.001 | 13.7 |
| MRT GIT Co (h) | $26.3^{ac} \pm 1.4$ | $21.1^b \pm 1.5$ | $25.3^c \pm 2.1$ | $29.5^d \pm 0.5$ | <0.001[b] | 13.6 |
| MRT GIT Cr (h) | $46.8^{ac} \pm 1.9$ | $39.2^b \pm 2.8$ | $46.7^c \pm 2.5$ | $53.3^d \pm 2.2$ | <0.001 | 12.4 |
| MRT GIT La (h) | $55.1^{ac} \pm 2.1$ | $47.7^b \pm 2.8$ | $55.8^c \pm 1.0$ | $64.2^d \pm 1.5$ | <0.001 | 12.1 |
| MRT RR Co (h) | $13.8^a \pm 0.1$ | $11.8^b \pm 0.8$ | $13.4^a \pm 0.6$ | $14.1^a \pm 0.4$ | <0.001 | 7.7 |
| MRT RR Cr (h) | $34.4^a \pm 0.9$ | $29.8^b \pm 2.1$ | $34.7^a \pm 2.1$ | $37.9^a \pm 2.4$ | 0.005 | 9.7 |
| MRT RR La (h) | $42.7^{ac} \pm 1.3$ | $38.3^b \pm 2.5$ | $43.9^c \pm 1.4$ | $48.8^d \pm 1.7$ | 0.001 | 9.9 |
| MRT distal GIT (h) | $12.4^a \pm 1.5$ | $9.4^b \pm 1.3$ | $12.0^a \pm 1.8$ | $15.4^c \pm 0.2$ | <0.001 | 20.0 |

*Note*: Different superscripts indicate significant differences ($p < 0.05$) between individuals.

Abbreviations: DMI, dry matter intake; GIT, gastrointestinal tract; MRT, mean retention time; RR, reticulorumen.

[a]*p*-value for the fixed factor 'animal' in linear mixed models.

[b]ln-transformed data.

also only record) chewing times (Byskov et al., 2017; Watt et al., 2015; Zetouni et al., 2018.

## 4.1 | Factors influencing MRT and chewing activity

Intake level is considered the primary factor that affects MRT; a higher relative intake level is generally accompanied by a decrease of fluid and particle MRT (Lechner-Doll et al., 1991; Mudgal et al., 1982; Shaver et al., 1986; Thornton & Minson, 1972). A higher intake level is also related to an increased gut fill, which partly, but not completely, mitigates the MRT-decreasing effect of intake (Findeisen et al., 2021). The diet type and physical form also exert an influence on MRT (Shaver et al., 1988; Udén, 1988; Zebeli et al., 2007); however, in order to ensure a diet characteristic is really a factor of influence, an effect of diet on food intake level must be excluded (Levey & Martínez del Rio, 1999). In the present study, diet composition as well as the relative food intake level was kept constant throughout, excluding these potential factors. Although BM as such, which varied among the experimental animals of the present study, was repeatedly discussed to be positively related to MRT (Gordon & Illius, 1994; Illius & Gordon, 1992), the effect might have been overestimated (Abraham et al., 2021; Clauss et al., 2007; Müller et al., 2013; Steuer et al., 2011). Our pilot results show that even for two individuals with similar BM and food intake, the MRT can still differ considerably.

Intake level was also suggested to be related to chewing behaviour; a higher relative intake level often leads to a decreased chewing intensity (Bae et al., 1981; Dias et al., 2011; Welch & Smith, 1969). Physical diet characteristics also affect chewing activity. A reduced diet particle size was related to either a decreased rumination time (Beauchemin et al., 2003; Krause et al., 2002) or a decreased total chewing intensity (Kononoff et al., 2003). Body weight also might affect chewing measures, as studies showed that larger individuals or species spent less time chewing per ingested cell wall constituents (Bae et al., 1983; Druzinsky, 1993; Shipley et al., 1994; Welch, 1982). Our preliminary results were not consistent with these findings, even though total chewing intensity tended to

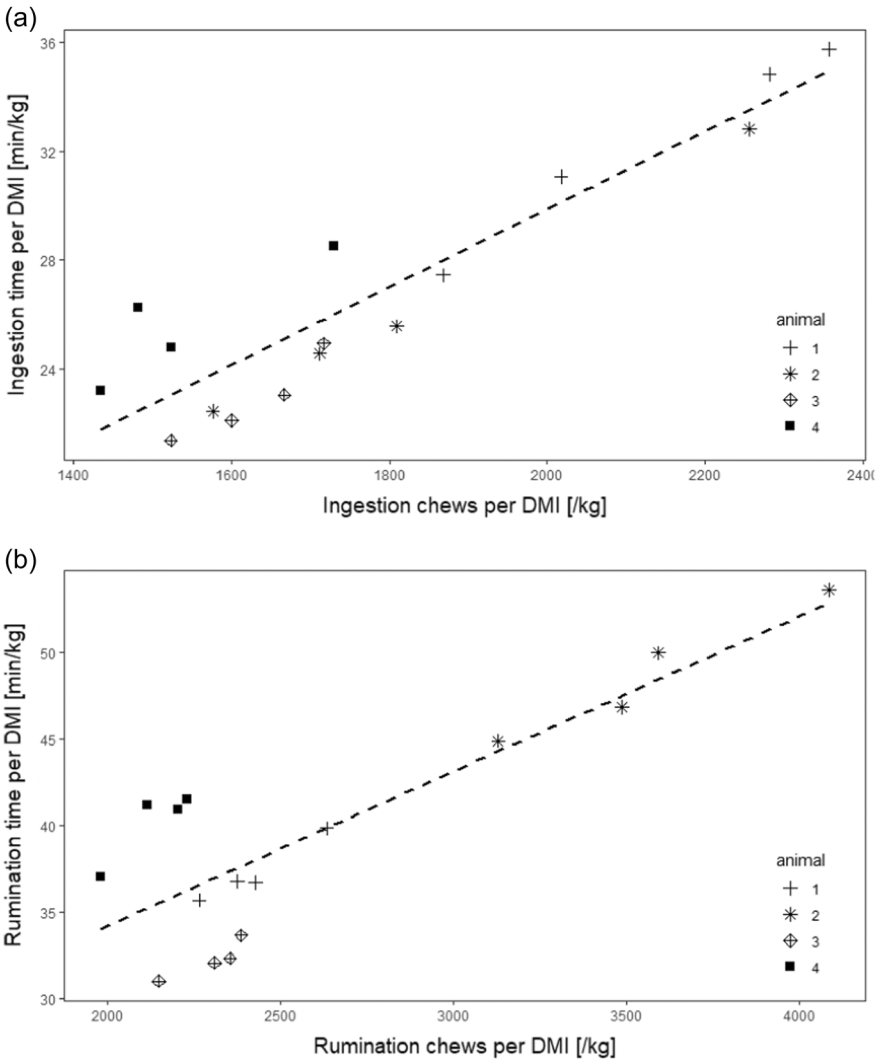


**FIGURE 1** Relationship between (a) the time spent chewing during ingestion (per kg dry matter intake [DMI]) and the ingestive chewing intensity (number of ingestive chews per kg DMI) and (b) the time spent chewing during rumination (per kg DMI) and the rumination chewing intensity (number of rumination chews per kg DMI). Note the difference between animals 3 and 4, which is due to the distinct difference in chewing frequency between these animals (Table 2).

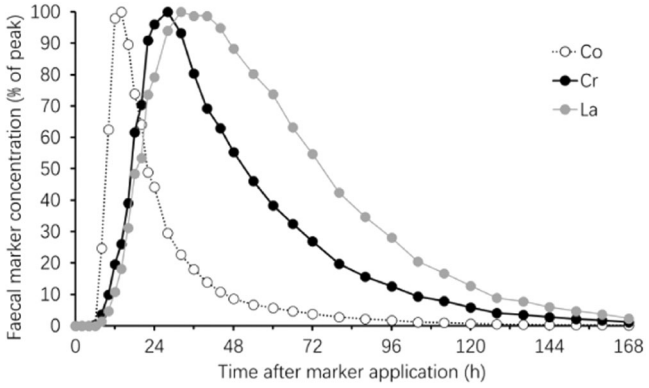


**FIGURE 2** Faecal marker elimination pattern based on the average of the four individual cattle (Co for solute marker, Cr for 2 mm particle marker, La for 1 cm particle marker).

negatively relate to BM. As indicated by Janis et al. (2010) when using a small sample of horses and cattle, chewing frequency may much more likely lead to misrepresentation in low sample size datasets than chewing intensity.

Apart from BM, the dental status (e.g., whether all teeth have already erupted or not) could also be relevant to chewing behaviour (Grandl et al., 2018), although this has not been studied systematically in domestic ruminants. This is a general phenomenon in the cattle literature. Although dental abnormalities or pathologies had a high occurrence in the few studies that addressed this issue (Borsanelli et al., 2016, 2021; Fadden et al., 2016; Fiedler, 1967; Ingham, 2001; Probst et al., 2016; Scheler, 1953; Simmerstetter, 1994), and although it is known from humans (e.g., Helkimo et al., 1978; Ikebe et al., 2011; Van der Bilt et al., 1993) as well as from ruminants (Grandl et al., 2018; Pérez-Barbería & Gordon, 1998) that a lack of chewing surface is compensated by increased chewing intensity, dental status is typically not assessed in studies that measure chewing behaviour. In theory, this makes chewing behaviour an ambiguous signal: a high chewing intensity can indicate both—a specimen with healthy teeth and an above-average propensity for chewing, or a specimen with a compromised dental status and a compensatory chewing investment. The present pilot study is no exception: While no chewing problems were observed in the animals, their dental status was not recorded, and an influence on the chewing behaviour cannot be excluded.

**TABLE 3** Effect of body weight on chewing and MRT measures using LMMs, with treatment as fixed factor, animal and round as random factor.

| Dependent variable | | Body weight (kg) Estimate (standard error) | $p^a$ | Treatment effect $p^b$ |
|---|---|---|---|---|
| Chewing frequency (/min) | Total chewing | −0.025 (0.029) | 0.403 | 0.145 |
| | Rumination | −0.056 (0.040) | 0.186 | 0.352 |
| Chewing intensity (/kg DMI) | Total chewing | −10.07 (3.884) | 0.084 | 0.807 |
| | Rumination | −6.18 (3.803) | 0.177 | 0.965 |
| MRT (h) | GIT Co | −0.001 (0.019) | 0.952 | **0.002**[c] |
| | GIT Cr | 0.024 (0.036) | 0.539 | 0.132 |
| | GIT La | 0.008 (0.039) | 0.837 | 0.292 |
| | RR Co | 0.004 (0.007) | 0.656 | 0.122 |
| | RR Cr | 0.025 (0.022) | 0.339 | 0.323 |
| | RR La | 0.027 (0.026) | 0.368 | 0.700 |
| | Distal GIT | −0.016 (0.019) | 0.425 | **0.034** |

*Note*: Significant *p*-values indicated in bold.

Abbreviations: DMI, dry matter intake; GIT, gastrointestinal tract; LMM, linear mixed model; MRT, mean retention time; RR, reticulorumen.

[a]*p*-value for the independent variable 'body weight' in LMMs.

[b]*p*-value for the effect of pilocarpine treatment.

[c]ln-transformed data.

**TABLE 4** Effect of water intake on MRT measures using LMMs, with treatment as fixed factor, animal and round as random factor.

| Dependent variable | | Water intake (kg) Estimate (standard error) | $p^a$ | Treatment effect $p^b$ |
|---|---|---|---|---|
| MRT (h) | GIT Co | −0.064 (0.074) | 0.413 | **0.003**[c] |
| | GIT Cr | 0.063 (0.129) | 0.660 | 0.154 |
| | GIT La | −0.036 (0.128) | 0.798 | 0.315 |
| | RR Co | 0.037 (0.027) | 0.249 | 0.076 |
| | RR Cr | 0.032 (0.124) | 0.808 | 0.422 |
| | RR La | −0.040 (0.111) | 0.727 | 0.823 |
| | Distal GIT | −0.013 (0.070) | 0.862 | **0.034** |

*Note*: Significant *p*-values indicated in bold.

Abbreviations: GIT, gastrointestinal tract; LMM, linear mixed model; MRT, mean retention time; RR, reticulorumen.

[a]*p*-value for the independent variable 'water intake' in LMMs.

[b]*p*-value for the effect of pilocarpine treatment.

[c]ln-transformed data.

Therefore, when investigating the effect of chewing on MRT, it is necessary to control both intake level and diet composition and account for BM effects (and ensure dental integrity). To our knowledge, the effect of differences in chewing behaviour between individuals on a consistent diet on MRT measures has not been reported so far. Our results show that for two animals with similar body weight, the chewing behaviour can vary considerably, and the chewing measures can significantly relate to the MRT measures. The low number of animals makes this a pilot finding. Dado and Allen (1994) reported inter-individual variability in measures of chewing activity in six primiparous and six multiparous cows. The coefficients of variation of the multiparous cows for ingestion, rumination and total chewing time per DMI are, at 14.1%, 18.9% and 13.6%, very similar to those of our four multiparous cows (14.8%, 17.4% and 12.8% respectively; Table 2). These findings support the concept that individual cattle may vary distinctively in chewing characteristics.

The chewing behaviour of ruminants differs considerably between ingestive and rumination mastication; the latter is more uniform and consistent (Deswysen & Ehrlein, 1981; Dittmann et al., 2017), usually represents the larger proportion of chewing activity (Beauchemin, 2018), and is responsible for the majority of particle size reduction (McLeod & Minson, 1988). Therefore, it is justified to not only assess the effect of total chewing activity on retention measures, but also the effect of rumination activity only. This appears especially justified as individual cows apparently differed most in rumination chewing behaviour, as indicated by the coefficients of variation in Table 2.

## 4.2 | Relationship between chewing activity and MRT

The relationship between chewing activity and MRT found in the present study may have several reasons (López-Paredes et al., 2020; Watt et al., 2015), and these are different for MRT of particles and MRT of fluid.

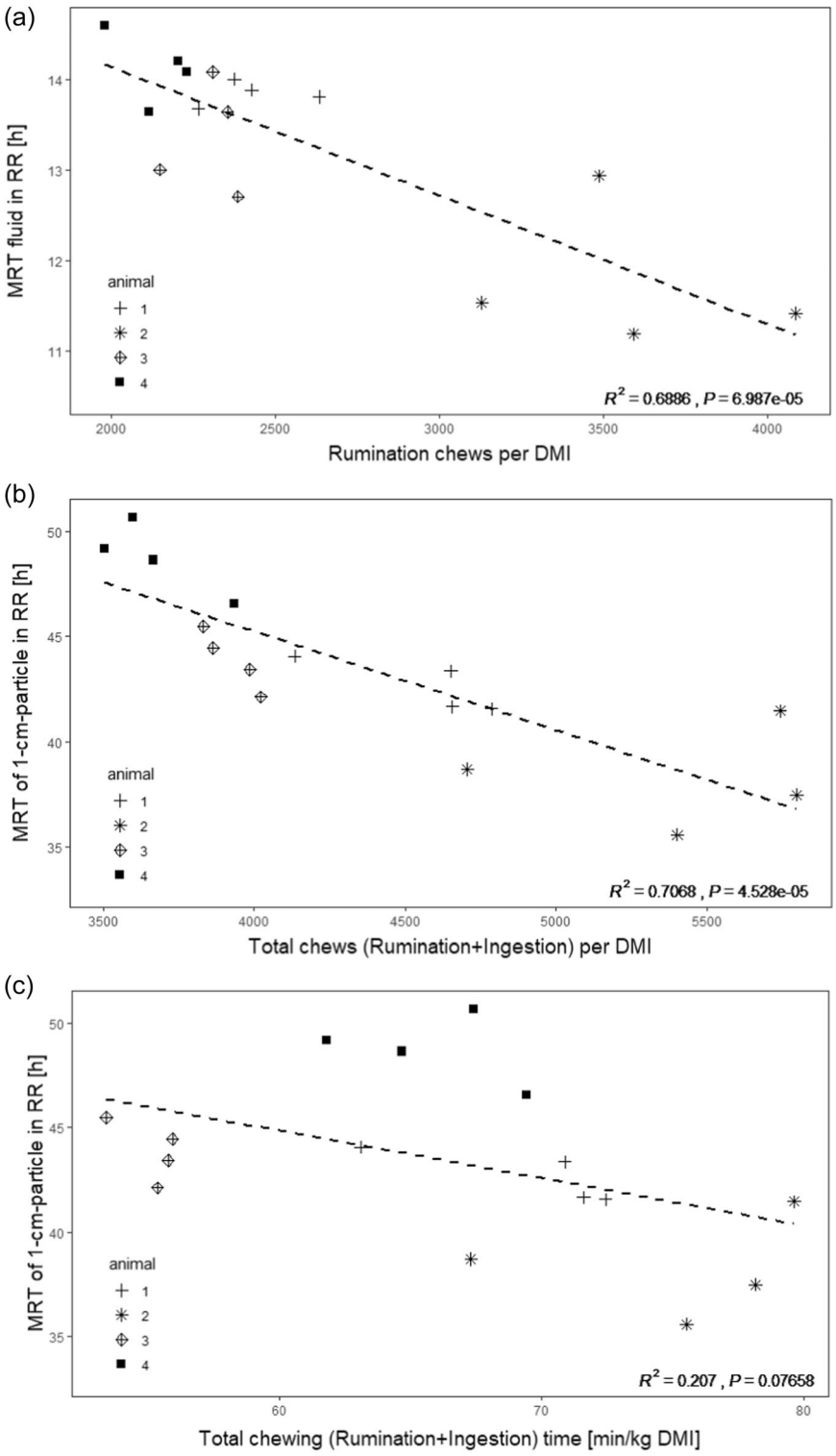


**FIGURE 3** Relationship between (a) MRT fluid in RR (MRT: mean retention time; RR: reticulorumen) and rumination chews per dry matter intake (DMI), (b) MRT of 1-cm-particle in RR and total chews per DMI, and (c) MRT of 1-cm-particle in RR and total chewing time per DMI; simple linear regression coefficient of determination ($R^2$) as well as *p*-value are indicated; for a full statistical evaluation, see Table 5.

Evidently, in mammalian herbivores, chewing plays the predominant role in the reduction of particle size. Kennedy (1985) collected the regurgitated material during rumination and found that about 70% of the large particles in the mouth were comminuted to small particles during one cycle of rumination, and rumination contributed about 85% of the comminution of the large particles which disappear from the RR. A higher chewing intensity should simply facilitate a faster passage of particulate matter from the RR.

Compared to the escape of particles, the passage of fluid is based on different mechanisms. Liquid is dependent to a large extent on absorption and secretion at various sites of the GIT. Absorption is mainly influenced by osmolality; water absorption occurs secondarily to osmotic gradients generated by the active transport of ions and solutes, and passively by hydrostatic or oncotic forces (Masyuk et al., 2002). In studies that investigated both drinking water intake and the MRT of fluid (in the RR or the GIT) in ruminants, no correlation was found (Bernabucci et al., 2009;

**TABLE 5** Effect of chewing measures on MRT measures using LMMs, with treatment as fixed factor, animal and round as random factor.

| Dependent variable | Chewing measure | | Estimate (standard error) | $p$[a] | Treatment effect $p$[b] |
|---|---|---|---|---|---|
| MRT GIT Co (h) | Chewing frequency (/min) | Total chewing | −0.209 (0.144) | 0.283 | **0.006**[c] |
| | | Rumination | −0.230 (0.102) | *0.079* | **0.002** |
| | Chewing intensity (/kg DMI) | Total chewing | 0.000 (0.001) | 0.958 | **0.004**[c] |
| | | Rumination | 0.000 (0.001) | 0.998 | **0.006**[c] |
| | Chewing time (min/kg DMI) | Total chewing | −0.008 (0.060) | 0.741 | **0.004**[c] |
| | | Rumination | 0.016 (0.078) | 0.702 | **0.004**[c] |
| MRT GIT Cr (h) | Chewing frequency (/min) | Total chewing | −0.660 (0.241) | *0.066* | **0.041** |
| | | Rumination | −0.544 (0.182) | *0.052* | **0.035** |
| | Chewing intensity (/kg DMI) | Total chewing | −0.004 (0.002) | *0.056* | 0.108 |
| | | Rumination | −0.004 (0.002) | 0.121 | 0.129 |
| | Chewing time (min/kg DMI) | Total chewing | −0.115 (0.162) | 0.493 | 0.172 |
| | | Rumination | −0.061 (0.229) | 0.794 | 0.181 |
| MRT GIT La (h) | Chewing frequency (/min) | Total chewing | −0.874 (0.240) | **0.017** | **0.022** |
| | | Rumination | −0.636 (0.187) | **0.026** | **0.044** |
| | Chewing intensity (/kg DMI) | Total chewing | −0.003 (0.002) | 0.143 | 0.235 |
| | | Rumination | −0.004 (0.002) | *0.066* | 0.159 |
| | Chewing time (min/kg DMI) | Total chewing | −0.088 (0.148) | 0.569 | 0.334 |
| | | Rumination | −0.244 (0.202) | 0.256 | 0.266 |
| MRT RR Co (h) | Chewing frequency (/min) | Total chewing | −0.106 (0.058) | 0.151 | *0.053* |
| | | Rumination | −0.081 (0.046) | 0.160 | *0.060* |
| | Chewing intensity (/kg DMI) | Total chewing | −0.001 (0.000) | *0.055* | *0.055*[c] |
| | | Rumination | −0.001 (0.000) | **0.005** | **0.026** |
| | Chewing time (min/kg DMI) | Total chewing | −0.039 (0.034) | 0.282 | *0.099* |
| | | Rumination | −0.113 (0.033) | **0.008** | **0.036** |
| MRT RR Cr (h) | Chewing frequency (/min) | Total chewing | −0.415 (0.170) | 0.107 | 0.171 |
| | | Rumination | −0.326 (0.129) | *0.094* | 0.170 |
| | Chewing intensity (/kg DMI) | Total chewing | −0.004 (0.001) | **0.019** | 0.177 |
| | | Rumination | −0.004 (0.001) | **0.025** | 0.250 |
| | Chewing time (min/kg DMI) | Total chewing | −0.173 (0.127) | 0.204 | 0.321 |
| | | Rumination | −0.211 (0.170) | 0.249 | 0.415 |
| MRT RR La (h) | Chewing frequency (/min) | Total chewing | −0.625 (0.201) | *0.061* | 0.257 |
| | | Rumination | −0.420 (0.143) | *0.056* | 0.426 |
| | Chewing intensity (/kg DMI) | Total chewing | −0.004 (0.001) | **0.022** | 0.513 |
| | | Rumination | −0.005 (0.002) | **0.021** | 0.606 |
| | Chewing time (min/kg DMI) | Total chewing | −0.159 (0.134) | 0.263 | 0.670 |
| | | Rumination | −0.311 (0.184) | 0.121 | 0.778 |

**TABLE 5** (Continued)

| Dependent variable | Chewing measure | | Estimate (standard error) | $p^a$ | Treatment effect $p^b$ |
|---|---|---|---|---|---|
| MRT distal GIT (h) | Chewing frequency (/min) | Total chewing | −0.260 (0.093) | 0.103 | **0.030** |
| | | Rumination | −0.226 (0.068) | *0.071* | **0.027** |
| | Chewing intensity (/kg DMI) | Total chewing | 0.000 (0.000) | 0.968 | **0.044** |
| | | Rumination | 0.000 (0.001) | 0.905 | *0.072* |
| | Chewing time (min/kg DMI) | Total chewing | 0.047 (0.072) | 0.526 | **0.037** |
| | | Rumination | 0.069 (0.100) | 0.504 | **0.043** |

*Note*: Significant *p*-values indicated in bold.

Abbreviations: DMI, dry matter intake; GIT, gastrointestinal tract; LMM, linear mixed model; MRT, mean retention time; RR, reticulorumen.

[a]*p*-value for the independent variable 'chewing measure' in LMMs.

[b]*p*-value for the effect of pilocarpine treatment.

[c]ln-transformed data.

Hebel et al., 2011), indicating that drinking water intake is no relevant factor influencing RR fluid passage. Adding mineral salts or hypertonic solutions to the RR, but not (hypotonic) water, typically decreases RR fluid retention (Harrison et al., 1975; Rogers & Davis, 1982; Rogers et al., 1979), suggesting that the absence of an effect of drinking water intake, as observed in the present study, is probably due to its rapid absorption. By contrast, saliva production is linked to fluid MRT. This was demonstrated repeatedly by the pharmacological stimulation of salivation (Bird et al., 1993; Froetschel et al., 1987; Wiedmeier, Arambel, Lamb, et al., 1987; Wiedmeier, Arambel, & Walters, 1987), including the present study. A physiological factor that regulates saliva inflow into the RR is chewing behaviour. Chewing activity, whether for ingestion or rumination, is linked to a high salivary flow (Méot et al., 1997). Compared to the 'resting flow', salivation rate increaases by 2–4 times in cattle during ingestion and rumination (Bailey, 1961). Therefore, diets that require more chewing trigger more salivary flow (Đuric et al., 1994; Kaufmann & Orth, 1966). Individual differences in saliva production in cattle have been reported and linked to the occurrence of frothy bloat (Gurnsey et al., 1980). Chewing behaviour stimulates salivation through the masticatory-salivary reflex, which is based on intra-oral mechanoreceptors (Hector & Linden, 1999). Cattle can produce more than 180 L saliva per day (Van Soest, 1994), which is around three times the amount of drinking water consumed (in our study 50–70 L/d). Individual differences in chewing behaviour could hence contribute greatly to saliva inflow and, subsequently, the fluid MRT.

## 5 | CONCLUSION

Our results underline the relevance of using the number of chews rather than chewing time as the quantity when relating chewing behaviour to other data. In future studies, the dental status of animals should ideally be accounted for to exclude it as a main causative factor for differences in chewing intensity. Chewing behaviour is a potential trait that is definitively easier to acquire than measurements of MRT, and may only require some standardised test meal offered for a limited period of time. While it would be premature to suggest predictive equations for MRT from chewing measures based on the present study, our pilot results may justify more detailed studies into the link between chewing behaviour and digestive physiology to use the former as a predictor for the latter.

### AUTHOR CONTRIBUTIONS

Melissa Terranova, Michael Kreuzer, Jürgen Hummel and Marcus Clauss designed the study, Xiaoyu Zhang, Yang Li and Marcus Clauss performed the study, Sylvia Ortmann, Michael Kreuzer and Jürgen Hummel supervised the laboratory analyses, Xiaoyu Zhang and Marcus Clauss analysed the data, Xiaoyu Zhang and Marcus Clauss wrote the manuscript with input from all co-authors.

### ACKNOWLEDGEMENTS

The authors are grateful to C. Kunz, N. Perez (ETH Zurich), A. Sünder, C. Kramer, K. Sauerland, A. von Gaza and I. Zumbrägel (University of Göttingen) for the assistance in the lab, H. Renfer, M. Klöppel (Strickhof), S. Amelchanka, W. Pendl, R. Jendly, S. Meese, H.-R. Wettstein (AgroVet-Strickhof), A. Birkinshaw (ETH Zurich) and R. Berthel (Agroscope) for the assistance in the animal experiment. Open access funding provided by Universitat Zurich.

### CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

### DATA AVAILABILITY STATEMENT

The original data measured in this study are available as an electronic supplement linked to this article.

### ORCID

*Xiaoyu Zhang* http://orcid.org/0000-0003-3577-1107
*Melissa Terranova* https://orcid.org/0000-0003-4152-8429
*Sylvia Ortmann* https://orcid.org/0000-0003-2520-6251
*Michael Kreuzer* https://orcid.org/0000-0003-2500-7543

*Jürgen Hummel* http://orcid.org/0000-0002-8876-7745
*Marcus Clauss* http://orcid.org/0000-0003-3841-6207

## REFERENCES

Abraham, A.J., Prys-Jones, T.O., De Cuyper, A., Ridenour, C., Hempson, G. P., Hocking, T., Clauss, M., & Doughty, C. E. (2021). Improved estimation of gut passage time considerably affects trait-based dispersal models. *Functional Ecology*, *35*, 860–869.

Bae, D.H., Welch, J.G., & Gilman, B.E. (1983). Mastication and rumination in relation to body size of cattle. *Journal of Dairy Science*, *66*, 2137–2141.

Bae, D. H., Welch, J. G., & Smith, A. M. (1981). Efficiency of mastication in relation to hay intake by cattle. *Journal of Animal Science*, *52*, 1371–1375.

Bailey, C. B. (1961). Saliva secretion and its relation to feeding in cattle. 3. The rate of secretion of mixed saliva in the cow during eating, with an estimate of the magnitude of the total daily secretion of mixed saliva. *British Journal of Nutrition*, *15*, 443–451.

Bartocci, S., Amici, A., Verna, M., Terramoccia, S., & Martillotti, F. (1997). Solid and fluid passage rate in buffalo, cattle and sheep fed diets with different forage to concentrate ratios. *Livestock Production Science*, *52*, 201–208.

Beauchemin, K. A. (2018). Invited review: Current perspectives on eating and rumination activity in dairy cows. *Journal of Dairy Science*, *101*, 4762–4784.

Beauchemin, K. A., Yang, W. Z., & Rode, L. M. (2003). Effects of particle size of alfalfa-based dairy cow diets on chewing activity, ruminal fermentation, and milk production. *Journal of Dairy Science*, *86*, 630–643.

Bernabucci, U., Lacetera, N., Danieli, P. P., Bani, P., Nardone, A., & Ronchi, B. (2009). Influence of different periods of exposure to hot environment on rumen function and diet digestibility in sheep. *International Journal of Biometeorology*, *53*, 387–395.

Bird, A. R., Croom, W. J., Bailey, J. V., O'Sullivan, B. M., Hagler, W. M., Gordon, G. L., & Martin, P. R. (1993). Tropical pasture hay utilization with slaframine and cottonseed meal: Ruminal characteristics and digesta passage in wethers. *Journal of Animal Science*, *71*, 1634–1640.

Borsanelli, A. C., Viora, L., Lappin, D. F., Bennett, D., King, G., Dutra, I. S., & Riggio, M. P. (2016). Periodontal lesions in slaughtered cattle in the west of Scotland. *Veterinary Record*, *179*, 652.

Borsanelli, A. C., Viora, L., Parkin, T., Lappin, D. F., Bennett, D., King, G., Dutra, I. S., & Riggio, M. P. (2021). Risk factors for bovine periodontal disease—A preliminary study. *Animal*, *15*, 100121.

Braun, U., Trösch, L., Nydegger, F., & Hässig, M. (2013). Evaluation of eating and rumination behaviour in cows using a noseband pressure sensor. *BMC Veterinary Research*, *9*, 1–7.

Byskov, M. V., Fogh, A., & Løvendahl, P. (2017). Genetic parameters of rumination time and feed efficiency traits in primiparous Holstein cows under research and commercial conditions. *Journal of Dairy Science*, *100*, 9635–9642.

Clauss, M., Schwarm, A., Ortmann, S., Streich, W. J., & Hummel, J. (2007). A case of non-scaling in mammalian physiology? Body size, digestive capacity, food intake, and ingesta passage in mammalian herbivores. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *148*, 249–265.

Colucci, P. E., Chase, L. E., & Van Soest, P. J. (1982). Feed intake, apparent diet digestibility, and rate of particulate passage in dairy cattle. *Journal of Dairy Science*, *65*, 1445–1456.

Dado, R. G., & Allen, M. S. (1994). Variation in and relationships among feeding, chewing, and drinking variables for lactating dairy cows. *Journal of Dairy Science*, *77*, 132–144.

Deswysen, A. G., & Ehrlein, H. J. (1981). Silage intake, rumination and pseudo-rumination activity in sheep studied by radiography and jaw movement recordings. *British Journal of Nutrition*, *46*, 321–335.

Dias, R. S., Patino, H. O., López, S., Prates, E., Swanson, K. C., & France, J. (2011). Relationships between chewing behavior, digestibility, and digesta passage kinetics in steers fed oat hay at restricted and ad libitum intakes. *Journal of Animal Science*, *89*, 1873–1880.

Dittmann, M. T., Kreuzer, M., Runge, U., & Clauss, M. (2017). Ingestive mastication in horses resembles rumination but not ingestive mastication in cattle and camels. *Journal of Experimental Zoology A*, *327*, 98–109.

Druzinsky, R. E. (1993). The time allometry of mammalian chewing movements: Chewing frequency scales with body mass in mammals. *Journal of Theoretical Biology*, *160*, 427–440.

Đuric, M., Zhao, G. Y., Ørskov, E. R., & Chen, X. B. (1994). Indirect measurement of saliva secretion in sheep fed diets of different structures and the effect of such diets on ruminal fluid kinetics and fermentation pattern. *Experimental Physiology*, *79*, 823–830.

Fadden, A. N., Poulsen, K. P., Vanegas, J., Mecham, J., Bildfell, R., & Stieger-Vanegas, S. M. (2016). Dental pathology in conventionally fed and pasture managed dairy cattle. *Veterinary Record*, *178*, 19.

Fiedler, J. (1967). *Untersuchungen über Zahn-und Gebissanomalien sowie über den Zangenwechsel bei Rindern* (Dissertation) HU Berlin.

Findeisen, E., Südekum, K.-H., Hummel, J., & Clauss, M. (2021). Increasing feed intake in domestic goats (*Capra hircus*): Measured effects on chewing intensity are probably driven by escape of few, large particles from the forestomach. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *257*, 110972.

Frei, S., Ortmann, S., Reutlinger, C., Kreuzer, M., Hatt, J.-M., & Clauss, M. (2015). Comparative digesta retention patterns in ratites. *The Auk: Ornithological Advances*, *132*, 119–131.

Froetschel, M. A., Croom, W. J., Hagler, W. M., Argenzio, R. A., Liacos, J. A., & Broquist, H. P. (1987). Effects of slaframine on ruminant digestive function: Liquid turnover rate and fermentation patterns in sheep and cattle. *Journal of Animal Science*, *64*, 1241–1248.

Gindri, M., Moraes, L. E., & Teixeira, I. A. M. A. (2021). Disentangling digesta passage rate in the gastrointestinal tract of male and female growing goats fed varying feeding levels using multivariate and metanalytical approaches. *Livestock Science*, *251*, 104645.

Goopy, J. P., Donaldson, A., Hegarty, R., Vercoe, P. E., Haynes, F., Barnett, M., & Oddy, V. H. (2014). Low-methane yield sheep have smaller rumens and shorter rumen retention time. *British Journal of Nutrition*, *111*, 578–585.

Gordon, I. J., & Illius, A. W. (1994). The functional significance of the browser-grazer dichotomy in African ruminants. *Oecologia*, *98*, 167–175.

Grandl, F., Schwarm, A., Ortmann, S., Furger, M., Kreuzer, M., & Clauss, M. (2018). Kinetics of solutes and particles of different size in the digestive tract of cattle of 0.5 to 10 years of age, and relationships with methane production. *Journal of Animal Physiology and Animal Nutrition*, *102*, 639–651.

Grovum, W. L., & Williams, V. J. (1973). Rate of passage of digesta in sheep. 4. Passage of marker through the alimentary tract and the biological relevance of rate-constants derived from the changes in concentration of marker in faeces. *British Journal of Nutrition*, *30*, 313–329.

Gurnsey, M. P., Jones, W. T., & Reid, C. S. W. (1980). A method for investigating salivation in cattle using pilocarpine as a sialagogue. *New Zealand Journal of Agricultural Research*, *23*, 33–41.

Harrison, D. G., Beever, D. E., Thomson, D. J., & Osbourn, D. F. (1975). Manipulation of rumen fermentation in sheep by increasing the rate of flow of water from the rumen. *Journal of Agricultural Science, Cambridge*, *85*, 93–101.

Hebel, C., Ortmann, S., Hammer, S., Hammer, C., Fritz, J., Hummel, J., & Clauss, M. (2011). Solute and particle retention in the digestive tract of the Phillip's dikdik (Madoqua saltiana phillipsi), a very small browsing ruminant: Biological and methodological implications.

*Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *159*, 284–290.

Hector, M. P., & Linden, R. W. A. (1999). Reflexes of salivary secretion. In J. R. Garrett, J. Ekström, & L. C. Anderson (Eds.), *Neural mechanisms of salivary gland secretion* (pp. 196–217). Karger.

Hegarty, R. S. (2004). Genotype differences and their impact on digestive tract function of ruminants: A review. *Australian Journal of Experimental Agriculture*, *44*, 459–467.

Helkimo, E., Carlsson, G. E., & Helkimo, M. (1978). Chewing efficiency and state of dentition: A methodologic study. *Acta Odontologica Scandinavica*, *36*, 33–41.

Huhtanen, P., & Kukkonen, U. (1995). Comparison of methods, markers, sampling sites and models for estimating digesta passage kinetics in cattle fed at two levels of intake. *Animal Feed Science and Technology*, *52*, 141–158.

Ikebe, K., Matsuda, K. I., Kagawa, R., Enoki, K., Yoshida, M., Maeda, Y., & Nokubi, T. (2011). Association of masticatory performance with age, gender, number of teeth, occlusal force and salivary flow in Japanese older adults: Is ageing a risk factor for masticatory dysfunction? *Archives of Oral Biology*, *56*, 991–996.

Illius, A. W., & Gordon, I. J. (1992). Modelling the nutritional ecology of ungulate herbivores: Evolution of body size and competitive interactions. *Oecologia*, *89*, 428–434.

Ingham, B. (2001). Abattoir survey of dental defects in cull cows. *Veterinary Record*, *148*, 739–742.

Janis, C. M., Constable, E. C., Houpt, K. A., Streich, W. J., & Clauss, M. (2010). Comparative ingestive mastication in domestic horses and cattle: A pilot investigation. *Journal of Animal Physiology and Animal Nutrition*, *94*, 402–409.

Janssen, P. H. (2010). Influence of hydrogen on rumen methane formation and fermentation balances through microbial growth kinetics and fermentation thermodynamics. *Animal Feed Science and Technology*, *160*, 1–22.

Kaufmann, V. W., & Orth, A. (1966). Untersuchungen über Einflüsse des Futters und der Pansen fermentation auf die Speichelsekretion. *Journal of Animal Physiology and Animal Nutrition*, *21*, 110–120.

Kennedy, P. M. (1985). Effect of rumination on reduction of particle size of rumen digesta by cattle. *Australian Journal of Agricultural Research*, *36*, 819–828.

Kennedy, P. M., & Milligan, L. P. (1978). Effects of cold exposure on digestion, microbial synthesis and nitrogen transformations in sheep. *British Journal of Nutrition*, *39*, 105–117.

Kononoff, P. J., Heinrichs, A. J., & Lehman, H. A. (2003). The effect of corn silage particle size on eating behavior, chewing activities, and rumen fermentation in lactating dairy cows. *Journal of Dairy Science*, *86*, 3343–3353.

Krause, K. M., Combs, D. K., & Beauchemin, K. A. (2002). Effects of forage particle size and grain fermentability in midlactation cows. II. Ruminal pH and chewing activity. *Journal of Dairy Science*, *85*, 1947–1957.

Lechner-Doll, M., Kaske, M., & Engelhardt, W. V. (1991). Factors affecting the mean retention time of particles in the forestomach of ruminants and camelids. In T. Tsuda, Y. Sasaki & R. Kawashima, *Physiological aspects of digestion and metabolism in ruminants* (Vol. 176, pp. 455–482). Elsevier.

Lechner-Doll, M., Rutagwenda, T., Schwartz, H. J., Schultka, W., & von Engelhardt, W. (1990). Seasonal changes of ingesta mean retention time and forestomach fluid volume in indigenous camels, cattle, sheep and goats grazing in a thornbush savanna pasture in Kenya. *Journal of Agricultural Science (Cambridge)*, *115*, 409–420.

Levey, D. J., & Martínez del Rio, C. (1999). Test, rejection, and reformulation of a chemical reactor-based model of gut function in a fruit-eating bird. *Physiological and Biochemical Zoology*, *72*, 369–383.

Linden, D. R., Titgemeyer, E. C., Olson, K. C., & Anderson, D. E. (2014). Effects of gestation and lactation on forage intake, digestion, and passage rates of primiparous beef heifers and multiparous beef cows. *Journal of Animal Science*, *92*, 2141–2151.

López-Paredes, J., Goiri, I., Atxaerandio, R., García-Rodríguez, A., Ugarte, E., Jiménez-Montero, J. A., Alenda, R., & González-Recio, O. (2020). Mitigation of greenhouse gases in dairy cattle via genetic selection: 1. Genetic parameters of direct methane using noninvasive methods and proxies of methane. *Journal of Dairy Science*, *103*, 7199–7209.

Mambrini, M., & Peyraud, J. L. (1997). Retention time of feed particles and liquids in the stomachs and intestines of dairy cows. Direct measurement and calculation based on fecal collection. *Reproduction, Nutrition, Development*, *37*, 427–442.

Masyuk, A. I., Marinelli, R. A., & LaRusso, N. F. (2002). Water transport by epithelia of the digestive tract. *Gastroenterology*, *122*, 545–562.

McLeod, M. N., & Minson, D. J. (1988). Large particle breakdown by cattle eating ryegrass and alfalfa. *Journal of Animal Science*, *66*, 992–999.

Méot, F., Cirio, A., & Biovin, R. (1997). Parotid secretion daily patterns and measurement with ultrasonic flow probes in conscious sheep. *Experimental Physiology*, *82*, 905–923.

Mudgal, V. D., Dixon, R. M., Kennedy, P. M., & Milligan, L. P. (1982). Effect of two intake levels on retention times of liquid, particle and microbial markers in the rumen of sheep. *Journal of Animal Science*, *54*, 1051–1055.

Müller, D. W., Codron, D., Meloro, C., Munn, A., Schwarm, A., Hummel, J., & Clauss, M. (2013). Assessing the Jarman–Bell principle: Scaling of intake, digestibility, retention time and gut fill with body mass in mammalian herbivores. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *164*, 129–140.

Pérez-Barbería, F. J., & Gordon, I. J. (1998). The influence of molar occlusal surface area on the voluntary intake, digestion, chewing behaviour and diet selection of red deer. *Journal of Zoology*, *245*, 307–316.

Pinares-Patiño, C. S., Ebrahimi, S. H., McEwan, J. C., Dodds, K. G., Clark, H., & Luo, D. ( 2011). *Is rumen retention time implicated in sheep differences in methane emission*. Proceedings of the New Zealand Society of Animal Production. New Zealand Society of Animal Production, pp. 219–222.

Probst, J. K., Kauf, P., Schellenberger, F., & Spengler Neff, A. (2016). Postmortale Untersuchung zur Zahngesundheit Schweizer Milchkühe. *Tierärztliche Umschau*, *71*, 409–415.

Przybyło, M., Hummel, J., Ortmann, S., Codron, D., Kohlschein, G.-M., Kilga, D., Smithyman, J., Przybyło, U., Świerk, S., Hammer, S., Hatte, J.-M., Górkaa, P., & Clauss, M. (2019). Digesta passage in nondomestic ruminants: Separation mechanisms in 'moose-type' and 'cattle-type' species, and seemingly atypical browsers. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *235*, 180–192.

Rogers, J. A., & Davis, C. L. (1982). Effects of intraruminal infusions of mineral salts on volatile fatty acid production in steers fed high-grain and high-roughage diets. *Journal of Dairy Science*, *65*, 955–962.

Rogers, J. A., Marks, B. C., Davis, C. L., & Clark, J. H. (1979). Alteration of rumen fermentation in steers by increasing rumen fluid dilution rate with mineral salts. *Journal of Dairy Science*, *62*, 1599–1605.

Scheler, W. (1953). *Anomalien am Rindergebiss* (Dissertation). FU Berlin.

Shaver, R. D., Nytes, A. J., Satter, L. D., & Jorgensen, N. A. (1986). Influence of amount of feed intake and forage physical form on digestion and passage of prebloom alfalfa hay in dairy cows. *Journal of Dairy Science*, *69*, 1545–1559.

Shaver, R. D., Nytes, A. J., Satter, L. D., & Jorgensen, N. A. (1988). Influence of feed intake, forage physical form, and forage fiber content on particle size of masticated forage, ruminal digesta, and feces of dairy cows. *Journal of Dairy Science*, *71*, 1566–1572.

Shipley, L. A., Gross, J. E., Spalinger, D. E., Hobbs, N. T., & Wunder, B. A. (1994). The scaling of intake rate in mammalian herbivores. *The American Naturalist*, *143*, 1055–1082.

Simmerstetter, C. (1994). *Untersuchung über Art und Vorkommen von Zahn- und Gebissanomalien beim Rind* (Dissertation). Veterinary University Vienna.

Smuts, M., Meissner, H. H., & Cronjé, P. B. (1995). Retention time of digesta in the rumen: Its repeatability and relationship with wool production of Merino rams. *Journal of Animal Science*, *73*, 206–210.

Steuer, P., Südekum, K.-H., Müller, D. W. H., Franz, R., Kaandorp, J., Clauss, M., & Hummel, J. (2011). Is there an influence of body mass on digesta mean retention time in herbivores? A comparative study on ungulates. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *160*, 355–364.

Thielemans, M. F., François, E., Bodart, C., Thewis, A., Dupont, F., Lallemand, L., & Malburny, C. (1978). Mesure du transit gastrointestinal chez le porc à l'aide des radiolanthanides. Comparaison avec le mouton. *Annales de Biologie Animale, Biochimie, Biophysique*, *18*, 237–247.

Thompson, B. C., Dellow, D. W., & Barry, T. N. (1989). The effect of selection for fleece weight upon urea metabolism and digestive function in Romney sheep. *Australian Journal of Agricultural Research*, *40*, 1065–1074.

Thornton, R. F., & Minson, D. J. (1972). The relationship between voluntary intake and mean apparent retention time in the rumen. *Australian Journal of Agricultural Research*, *23*, 871–877.

Udén, P. (1988). The effect of grinding and pelleting hay on digestibility, fermentation rate, digesta passage and rumen and faecal particle size in cows. *Animal Feed Science and Technology*, *19*, 145–157.

Udén, P., Colucci, P. E., & Van Soest, P. J. (1980). Investigation of chromium, cerium and cobalt as markers in digesta. Rate of passage studies. *Journal of the Science of Food and Agriculture*, *31*, 625–632.

Van der Bilt, A., Olthoff, L. W., Bosman, F., & Oosterhaven, S. P. (1993). The effect of missing postcanine teeth on chewing performance in man. *Archives of Oral Biology*, *38*, 423–429.

Van Soest, P. J. (1994). Nutritional ecology of the ruminant (2nd ed.). Cornell University Press.

VDLUFA. (2006). *Bd. III: Die Chemische Untersuchung von Futtermitteln, Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten. Handbuch der landwirtschaftlichen Versuchs- und Untersuchungsmethodik (VDLUFA-Methodenbuch).* VDLUFA-Verlag.

Watt, L. J., Clark, C. E. F., Krebs, G. L., Petzel, C. E., Nielsen, S., & Utsumi, S. A. (2015). Differential rumination, intake, and enteric methane production of dairy cows in a pasture-based automatic milking system. *Journal of Dairy Science*, *98*, 7248–7263.

Welch, J. G. (1982). Rumination, particle size and passage from the rumen. *Journal of Animal Science*, *54*, 885–894.

Welch, J. G., & Smith, A. M. (1969). Effect of varying amounts of forage intake on rumination. *Journal of Animal Science*, *28*, 827–830.

Wiedmeier, R. D., Arambel, M. J., Lamb, R. C., & Marcinkowski, D. P. (1987). Effect of mineral salts, carbachol, and pilocarpine on nutrient digestibility and ruminal characteristics in cattle. *Journal of Dairy Science*, *70*, 592–600.

Wiedmeier, R. D., Arambel, M. J., & Walters, J. L. (1987). Effect of orally administered pilocarpine on ruminal characteristics and nutrient digestibility in cattle. *Journal of Dairy Science*, *70*, 284–289.

Wylie, M. J., Ellis, W. C., Matis, J. H., Bailey, E. M., James, W. D., & Beever, D. E. (2000). The flow of forage particles and solutes through segments of the digestive tracts of cattle. *British Journal of Nutrition*, *83*, 295–306.

Zebeli, Q., Tafaj, M., Weber, I., Dijkstra, J., Steingass, H., & Drochner, W. (2007). Effects of varying dietary forage particle size in two concentrate levels on chewing activity, ruminal mat characteristics, and passage in dairy cows. *Journal of Dairy Science*, *90*, 1929–1942.

Zetouni, L., Difford, G. F., Lassen, J., Byskov, M. V., Norberg, E., & Løvendahl, P. (2018). Is rumination time an indicator of methane production in dairy cows? *Journal of Dairy Science*, *101*, 11074–11085.

## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**How to cite this article:** Zhang, X., Li, Y., Terranova, M., Ortmann, S., Kreuzer, M., Hummel, J., & Clauss, M. (2023). Individual differences in digesta retention and their relation to chewing in cattle—A pilot investigation. *Journal of Animal Physiology and Animal Nutrition*, 107, 394–406. https://doi.org/10.1111/jpn.13733