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# Characteristics of boli formed by dairy cows upon ingestion of fresh ryegrass, lucerne or chicory

E. M. K. Minnee[1†], G. C. Waghorn[1], P. Gregorini[2], R. H. Bryant[2] and D. F. Chapman[3]

[1]*DairyNZ, Private Bag 3221, Hamilton 3420, New Zealand;* [2]*Department of Agricultural Sciences, Faculty of Agricultural and Life Sciences, Lincoln University, Lincoln 7647, New Zealand;* [3]*DairyNZ, Canterbury Agriculture and Science Centre, PO Box 85066, Lincoln 7647, New Zealand*

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*This study examined the comminution of fresh herbage, subsequent nutrient release, and the characteristics of swallowed boli from three physically and chemically contrasting forages during ingestive mastication by dairy cows. The extent and pattern of nutrient release will determine their availability to rumen microflora, and potentially influence their efficiency of use. The forages evaluated were perennial ryegrass (ryegrass,* Lolium perenne *L., cv Alto AR37), lucerne (*Medicago sativa *L., cv Torlesse) and chicory (*Cichorium intybus *L., cv Choice). Experimental design was a 3 × 3 cross-over with three forages and three consecutive 1-day measurement periods, conducted twice. Six non-lactating, pregnant, multiparous Holstein-Friesian × Jersey cows (*Bos taurus*) were used, with the first cross-over applied to three mature (10.1 ± 0.61 years old; BW 631 ± 64 kg) cows, and the second to three young (4.8 ± 0.02 years; BW 505 ± 19 kg) cows. Fresh cut forage was offered to the cows following partial rumen evacuation. Swallowed boli were collected directly at the cardia at the commencement, middle and end of the first feeding bout of the first meal of the day. Forage species did not affect the fresh weight of ingested boli (mean 169 g,* $P = 0.605$*) but the proportion of saliva in boli varied between forage. Boli of chicory contained the greatest amount of herbage material and least amount of saliva, whereas ryegrass boli were the opposite. Boli fresh weight tended to increase as time in the meal progressed, but the age of the cow was not shown to affect any boli characteristics or nutrient release. Particle size reduction was affected by forage, with 31%, 38% and 35% of chicory, lucerne and ryegrass herbage reduced to <2 mm. There was little evidence of relationship between comminution and any physical or chemical characteristic of the forage, except in ryegrass where extent of comminution was moderately correlated with herbage strength. Proportional release of herbage soluble carbohydrate exceeded that of N during mastication. Differences in loss of N were moderately correlated with the amount of N in the herbage (*$R^2 = 0.53$*) but herbage comminution was not strongly correlated with release of either N or carbohydrate. These findings illustrate the complex animal × forage interactions that occur during mastication, and that it is not possible to infer nutrient loss from herbage based on herbage characteristics as the driver for this differ between species.*

**Keywords:** cell rupture, comminution, forage, mastication, particle size

## Implications

The release of nutrients and extent of comminution during ingestive mastication by dairy cows varies between forages. This information may aid understanding of how forages are digested, and why differences in nutrient use efficiency between forages exist. Furthermore, it was shown that the cow determines the fresh weight (FW) of the boli ingested, but the forage influences the feed and saliva content of the boli. These findings provide information that may enhance understanding of factors influencing foraging behaviour.

† E-mail: elena.minnee@dairynz.co.nz

## Introduction

Although the chemical composition of herbage determines how much of a given nutrient is consumed, this measure does not describe the pattern of availability of that nutrient during digestion. Ingestive mastication (mastication) is the first step in feed digestion, and while there is a significant body of research into the ruminal digestion of herbage, less is known about the degradation of herbage during mastication. For cows consuming forage, mastication is particularly important for disrupting the outer cuticle layer that forages commonly possess. The cuticle is highly resistant to microbial degradation, so disruption is important for permitting access by microbes to internal degradable plant material (Cheng *et al.*, 1980). A second function of mastication is to reduce

the size of feed particles, to manipulate the feed into a bolus that can be swallowed and to increase surface area available for microbial adhesion and digestion (Ulyatt *et al*., 1986). The contribution of ingestive mastication to herbage particle size reduction is less than that of rumination but can reduce 15% to 60% of ingested dry matter (DM) (by weight) (Pond *et al*., 1984; McLeod and Minson, 1988; Waghorn *et al*., 1989), to particles of a size able to pass from the rumen (Ulyatt *et al*., 1986). The third function of mastication is cell rupture and release of cell nutrients. Waghorn and Clark (2004) reported 50% to 80% of plant cells were ruptured through mastication in ruminants. Most plant nitrogen and all non-fibre carbohydrates (NFC) are stored within the plant cell (Sanderson and Wedin, 1989; Acosta *et al*., 2007), therefore the extent and pattern of cell rupture and subsequent release of nutrients will impact on their delivery to the rumen microflora and host. Studies investigating nutrient release from temperate grass and clover forage masticated by dairy cows, show 20% to 30% of intracellular nitrogen and 30% to 50% of NFC is released during ingestion (Boudon and Peyraud, 2001; Boudon *et al*., 2006; Acosta *et al*., 2007). Differing physical attributes and chemical composition of forages are likely to affect the extent of comminution and nutrient release, however, no clear relationship between these and forage physical or chemical characteristics were reported in previous studies. Further investigation across physically or chemically divergent species to determine which factors influence comminution and nutrient release during mastication is required to improve understanding of forage digestion and nutrient availability.

The rate and extent of nutrient delivery to the rumen is relevant to livestock production systems as this can influence efficiency of forage utilisation (Huntington and Archibeque, 2000; Phuong *et al*., 2013). In forage-based systems, energy is commonly the most limiting nutrient for ruminant production, but nitrogen is often in excess supply (Brookes and Nicol, 2007). Excess dietary N is excreted as urea in urine and is a source of environmental pollution, both as atmospheric ammonia and nitrate in groundwater (Tamminga, 1992). Recently, Minneé *et al*. (2017) showed that cows fed forages that had lesser proportions of soluble N also had lower concentrations of N in their urine. Increasing our knowledge of how different forages are processed during mastication and the extent of nutrient release may improve our understanding of how nutrients are utilised.

The objective of this study was to investigate the effects of mastication by dairy cows on three physically and chemically contrasting forages on boli characteristics, extent of comminution and cell nutrient release from herbage.

## Material and methods

*Experimental treatments and animals*

The experiment compared the characteristics of ingested boli, and cell nutrient release from three temperate forages: perennial ryegrass (ryegrass; *Lolium perenne* L. cv. One50 with AR37 endophyte), chicory (*Cichorium intybus* cv. Choice) and lucerne (*Medicago sativa* cv. Torlesse). Experimental design was a $3 \times 3$ cross-over design with three forages and three consecutive 1-day measurement periods, repeated twice. The three forages were allocated to $n = 18$ experimental units (6 cows $\times$ 3 periods), so that each cow randomly received one of the six possible cross-over permutations. Six non-lactating, pregnant, multiparous Holstein-Friesian $\times$ Jersey crossbred cows were used in the experiment conducted over 2 weeks in May 2015 in Hamilton, New Zealand (37°76'S, 175°36'E, 40 m a.s.l.). In the 1st week, three mature (10.1 ± 0.61 years old; BW 631 ± 64 kg) cows were used, while the three cows used in the 2nd week were young (4.8 ± 0.02 years; BW 505 ± 19 kg) cows, this served to explore whether the reported decline of mastication efficiency with age (Pérez-Barbería and Gordon, 1998) influences comminution and nutrient release from herbage. The cows were fitted with a large ruminal cannula (i.d., 125 mm), and were randomly allocated one forage each day.

*Sward management and animal feeding*

The forages were sown in October 2014, received 200 kg N/ha per year in 10 applications of 20 kg N/ha as SustaiN™ (N46; Ballance Agri-nutrients, Tauranga, New Zealand) and grown without irrigation. The stage of maturity of the swards during the experiment was ~2.75 leaves per tiller for ryegrass, 35 cm sward height for chicory and lucerne was at in the early-bud stage (44 days of regrowth). Fresh herbage was cut and collected each day at ~0800 h using a Jenquip® forage harvester (NZ Agriworks Ltd, Feilding, New Zealand), set to a cut height ~50% of the mean sward height for each forage to simulate the bite depth of the first grazing bout of dairy cows on fresh pasture (Gregorini *et al*., 2017). The cut heights were 10, 20 and 15 cm above ground for ryegrass, chicory and lucerne determined from the mean of 50 measurements of sward height for each forage using a meter ruler.

The afternoon before sampling (~1700 h), cows were removed from grazing perennial ryegrass/white clover swards and fasted overnight (~16 h) with unrestricted access to water. At 0900 h cows were moved to individual tie-stalls with rubber mats, open ventilation and access to water. The cows' rumens were partially emptied by hand via the cannula, enabling access to the cardia. Rumen contents were stored in covered bins to maintain temperature. Following partial rumen emptying, 11 kg of herbage was offered to each cow. As the cows ate, boli were collected and the duration of eating recorded. Any feed not eaten after 30 min was weighed to calculate herbage intake and rate (g DM/min).

*Sampling and measurements on the ingested boli*

Methodology for boli collection was based on that described by Acosta *et al*. (2007) and Boudon *et al*. (2006). Ingestive boli were sampled during the commencement, near the middle and at the end of the first feeding bout of the meal. At each sampling time in the meal, the first bolus collected was discarded, then the next 10 consecutive boli were collected from each cow for measurement. Individual boli were firm enough to be removed intact by hand. Each bolus was weighed, then all 10 boli from each cow were pooled to



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generate a composite sample for each sampling time. After collection from the final sampling time (~30 min after commencement), the stored rumen contents were returned to the rumen and cows were released to graze perennial ryegrass/clover swards.

The composite sample of boli was gently hand mixed. From this, two subsamples of 100 ± 5 g were weighed fresh then dried in a forced-draught oven at 95°C for 48 h before reweighing to determine DM content. Herbage and saliva content of the boli, and the amount of saliva added per 100 g of herbage was calculated using the equations of Reid *et al.* (1962):

$$Herbage\ content(g)\ of\ bolus\ (F) = \left(\frac{b-(y\times B)}{x-y}\right)$$

$$Saliva\ content\ (g)\ of\ bolus\ = B-F$$

where $b$ is the dry weight of the boli (g); $B$ the wet weight of the boli (g); $x$ the DM content of the herbage (g/100 g); $y$ the DM content of the saliva (g/100 g).

The DM of saliva ($y$) was 0.9/100 g, as used by Hart (1983).

A 150 ± 5 g subsample was taken for calculation of cell nutrients released from feed during mastication, using the method described by Boudon and Peyraud (2001). The subsample was placed on cheesecloth and rinsed with 3 l of water with gentle stirring using a spatula to remove released nutrients and saliva. The rinsed residues were freeze-dried and ground in a mill to pass a 1 mm sieve (Christy & Norris Mill, Ipswich, UK), then analysed to determine total N, water soluble carbohydrate concentration (WSC) and NDF in the DM. Methodology for chemical analyses is detailed in the 'Chemical analysis' section. Nutrient release was calculated as the difference between the quantity in the herbage and the quantity remaining in the bolus after rinsing. The calculation of Boudon and Peyraud (2001) assumes no loss of NDF during rinsing, and the loss of N is calculated as follows:

$$Proportion\ of\ N\ released = 1-\left(\frac{Nboli\times NDFfeed}{Nfeed\times NDFboli}\right)\times 100$$

Particle size distribution (PSD) of the boli was measured using a wet sieving apparatus (Turner and Newell Ltd, Manchester, UK) with four counter-rotating sieves, positioned above a stationary (0.075 mm) sieve. Sieve sizes (as the length of the size of the square hole) were 4, 2, 1, 0.5 and 0.075 mm. Subsamples of 25 g (FW) were placed on the top sieve, then 1500 ml of water recirculated through all sieves at 4 l/min for 5 min. Material retained on each sieve was transferred on to weighed filter papers then oven-dried at 60°C for 48 h before weighing to determine the distribution of DM between size fractions. The soluble fraction was defined as the DM passing the 0.075 mm sieve, and was calculated as the difference between the total DM sieved and the sum of the DM retained on the five sieves. The lengths of chicory and perennial ryegrass leaves and lucerne stems that were retained on the top sieve were measured with a ruler to the nearest millimetre. All samples were measured in duplicate.

*Sward characterisation*

On each of the 6 days of boli collection, 2 kg subsamples from the forages offered were collected. Herbage DM content was determined by weighing and oven-drying triplicate samples of 200 ± 5 g wet weight at 95°C for 48 h. A further sub-sample (200 g) was frozen at −20°C, freeze-dried and ground to pass through a 1-mm sieve (Christy & Norris Mill) for chemical analysis. Botanical composition of the herbage was determined by hand-sorting sub-samples (200 g) into green leaf, dead leaf, stem, petiole and unsown species. Sorted samples were dried in a forced-draught oven at 95°C for 48 h to determine composition on a dry weight basis. For morphological measurement, 100 pieces of each feed were randomly sampled and the length of the stem, and length and width of the leaf blade measured using a ruler, or digital callipers where the leaf or stem was less than 20 mm in size (Mitutoyo digimatic CD-8"CS; Mitutoyo Corp., Kawasaki, Japan). Two methods were used to assess the forages' biomechanical properties. The first method determined the resistance of plant material to fracture by measuring the force (Newtons/mm$^2$) required to punch a 2 mm diameter hole in the material using a digital force gauge (DS2 Digital force gauge; Imada Inc., Northbrook, IL, USA). The force to punch of the whole sward was estimated by calculating a weighted average of the force to punch each component (i.e. leaf or stem) and multiplying by the proportion that component contributes to the sward. The second method measured the amount of energy required to mechanically macerate the herbage using a Kreft Compact mincer fitted with a sieve plate with 12 mm holes (Kreft, Solingen, Germany) to provide a PSD similar to rumen digesta (Barrell *et al.*, 2000). To do this, ~700 g of frozen herbage was chopped into 20 mm lengths, then fed into the mincer. A HOBO® analog logger (Onset Computer Corporation, MA, USA) recorded the current (amps) used by the mincer at 1 s intervals.

*Chemical analyses*

Structural carbohydrates, NDF and ADF were measured using the methods of Van Soest *et al.* (1991) and method 973.18 (Association of Official Analytical Chemists (AOAC), 2000), respectively, using Whatman 934-AH glass micro-fibre filters with a 1.5 μm particle retention. Fibre recovered from the ADF measurement was used to determine lignin concentration as per Goering and Van Soest (1970). Water soluble carbohydrate concentration was measured colorimetrically by the method of DuBois *et al.* (1956). Total N concentration was determined by combustion (method 990.03; AOAC, 2000) in a LECO FP-528 Nitrogen analyser (Leco, St Joseph, MI, USA). Nitrogen in the NDF residue (NDIN) was measured in the residue from the NDF procedure also using the LECO FP-528 analyser. Concentration of non-protein N components was assessed by the urease and potentiometric methods (941.04 and 986.31; AOAC, 2000); and ash by heating 1.5 g of sample to 600°C in porcelain crucibles for 4 h (method 942.05; AOAC, 2000).



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*Statistical analyses*

Forage physical and chemical data from each of the 6 measurement days were subjected to one-way ANOVA with week included as a blocking factor and forage as a fixed effect. For intake data, the model included cow as blocking factor and forage as fixed effect. Mixed models approach to repeated measures ANOVA was used for boli data. The model included forage, sampling time and their interaction as fixed effects, week as blocking factor, time of day when first bolus was collected as covariate, and cow as a random factor. Analysis of variance was followed by Tukey's *t*-test for pairwise comparisons. Results are presented as least-squares means and root mean square error, significance is declared if *P*-values $\leqslant 0.05$.

Associations between feed characteristics ($n = 23$) and boli characteristics ($n = 15$) were assessed using Pearson correlations ($r$) including all boli data ($n = 54$). All analyses were performed using Proc Mixed, SAS/STAT 14.3 (SAS Institute Inc., NC, USA).

## Results

*Sward characteristics and chemical composition of forages*

Pre-harvest sward height were 21, 39 and 32 cm above ground level, for chicory, lucerne and ryegrass, respectively. Chicory and ryegrass swards were vegetative, and predominantly green leaf material (Table 1). Lucerne swards were in the early-bud stage, and comprised 55% green leaf and 45% stem (DM basis). The physical and chemical properties of the forages offered are detailed in Table 1. Ryegrass and lucerne had similar mean fragment length which was ~60 mm shorter than chicory ($P < 0.001$). Herbage DM content was least for chicory (76 g/kg) and similar (122–127 g/kg) for lucerne and ryegrass ($P < 0.001$). Measures of the biomechanical properties of the forages suggested that lucerne leaves require ~1/3 of the force to be fractured compared with chicory and ryegrass, but lucerne stem required 10 × the force for leaves to be fractured. Energy required to mechanically macerate the herbage was greatest for ryegrass at 113 J/g FW, and least for chicory, with lucerne intermediate (22 and 55 J/g FW; $P < 0.001$). Total *n* concentration was greatest in lucerne, which also contained the most lignin. Water soluble carbohydrate in chicory was nearly twice that of the other two forages. Ryegrass herbage had the greatest cell wall content (423 compared with 201 and 238 g NDF/g DM from chicory and lucerne) and the most fibre-bound protein.

*Characteristics of ingested boli*

The boli weight, herbage and saliva content of boli, and intake rates of the herbage are presented in Table 2. The average FW of the boli did not differ between forages but their composition did. Boli of chicory contained the most herbage material (g FW), while ryegrass boli had the greatest

**Table 1** *Physical and chemical characteristics of the chicory, lucerne and perennial ryegrass offered to dairy cows (*Bos taurus*)*

| | | Forage | | | |
|---|---|---|---|---|---|
| | Chicory | Lucerne | P. ryegrass | RMSE | *P*-value |
| Mean length of offered forage (mm) | 200[a] | 147[b] | 138[b] | | 18.0 |
| Mean leaf width (mm) | 47[a] | 15[b] | 3[c] | | 1.9 |
| Dry matter (DM, g/kg) | 76[b] | 127a | 122[a] | | 3.1 |
| Botanical composition (%) | | | | | |
| Leaf | 100[a] | 55[b] | 97[a] | 3.6 | < 0.001 |
| Stem | 0[b] | 45[a] | 3[b] | 3.8 | < 0.001 |
| Punch force (Newtons/mm$^2$) | | | | | |
| Leaf | 0.89[a] | 0.32[b] | 0.93[a] | 0.07 | < 0.001 |
| Stem | – | 3.4 | – | 0.50 | |
| Maceration energy (J, joules) | | | | | |
| J/g DM | 294[c] | 430[b] | 915[a] | 57.3 | < 0.001 |
| J/g FW | 22[c] | 54[b] | 112[a] | 6.1 | < 0.001 |
| Chemical composition (g/kg DM) | | | | | |
| Total nitrogen (N) | 31[c] | 49[a] | 38[b] | 0.31 | < 0.001 |
| Urea + ammonia N | 0.32[b] | 0.44[a] | 0.36[ab] | 0.12 | 0.004 |
| Nitrate N | 1.6[b] | 5.0[a] | 1.4[c] | 0.80 | 0.019 |
| Neutral-detergent insoluble nitrogen | 2.5[b] | 1.4[c] | 4.3[a] | 0.45 | < 0.001 |
| Water-soluble carbohydrates | 138[a] | 69[b] | 87[b] | 16.7 | < 0.001 |
| NDF | 201[c] | 238[b] | 423[a] | 23.7 | < 0.001 |
| ADF | 135[c] | 208[b] | 258[a] | 20.2 | < 0.001 |
| Lignin | 28[b] | 42[a] | 24[b] | 6.01 | 0.001 |
| Ash | 163[a] | 100[b] | 111[b] | 4.6 | < 0.001 |

FW = fresh weight.
[a,b,c]Values within a row with different superscripts differ significantly at $P < 0.05$.



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**Table 2** *Weight, herbage and saliva contents of ingested boli, and intake rate of dairy cows (*Bos taurus*) fed chicory, lucerne or perennial ryegrass indoors*

| | Forage | | | | |
|---|---|---|---|---|---|
| | Chicory | Lucerne | P. ryegrass | RMSE | *P*-value |
| Fresh weight of boli (g) | 171 | 162 | 170 | 16.3 | 0.605 |
| Dry weight of boli (g) | 9.0[b] | 14.0[a] | 13.4[a] | 2.18 | < 0.001 |
| Herbage content of boli (g) | 120.3[a] | 107.2[b] | 104.9[b] | 5.5 | 0.030 |
| Saliva content of boli (g) | 47.6[b] | 54.7[b] | 65.4[a] | 4.0 | 0.004 |
| Saliva added per 100 g feed (g)[1] | 42.4[b] | 54.3[ab] | 63.3[a] | 5.5 | 0.013 |
| Intake rate (g FW/min) | 621 | 509 | 579 | 97.7 | 0.187 |
| Intake rate (g DM/min) | 46.5[b] | 64.7[ab] | 70.7[a] | 10.94 | 0.021 |

FW = fresh weight; DW = dry matter.
[a,b]Values within a row with different superscripts differ significantly at $P < 0.05$.
[1]Calculated according to the formula of Reid *et al.* (1962).

**Table 3** *Influence of sampling time during a meal on the weight and herbage content of ingested boli, and the amount of saliva added to herbage from dairy cows (*Bos taurus*) fed chicory, lucerne or perennial ryegrass indoors*

| | Time in meal | | | | |
|---|---|---|---|---|---|
| | Start | Middle | End | RMSE | *P*-value |
| Fresh weight of boli (g) | | | | | |
| Chicory | 145.3[b] | 171.3[a] | 187.1[a] | 16.3 | < 0.001 |
| Lucerne | 149.8[b] | 174.3[a] | 161.6[ab] | 16.3 | 0.047 |
| P. ryegrass | 148.0[b] | 169.9[ab] | 192.3[a] | 16.3 | < 0.001 |
| Dry weight of boli (g) | | | | | |
| Chicory | 8.2 | 9.8 | 8.9 | 2.18 | 0.432 |
| Lucerne | 12.8 | 15.4 | 13.8 | 2.18 | 0.132 |
| P. ryegrass | 11.3[b] | 13.3[ab] | 15.4[a] | 2.18 | 0.013 |
| Herbage content of boli (g) | | | | | |
| Chicory | 100.5[b] | 122.3[ab] | 138.1[a] | 13.8 | < 0.001 |
| Lucerne | 97.6 | 118 | 105.8 | 13.8 | 0.051 |
| P. ryegrass | 89.2[b] | 104.2[ab] | 121.2[a] | 13.8 | 0.002 |
| Saliva added per 100 g herbage (g) | | | | | |
| Chicory | 45.9 | 42.6 | 39.4 | 12.6 | 0.707 |
| Lucerne | 54.0 | 49.0 | 60.0 | 12.6 | 0.333 |
| P. ryegrass | 67.4 | 63.1 | 59.2 | 12.6 | 0.534 |

[a,b]Values within a row with different superscripts differ significantly at $P < 0.05$.

saliva content ($P < 0.01$). Saliva (g) added per 100 g of herbage was greatest when cows consumed ryegrass at 63/100 g of herbage, which was 21 and 9 g more saliva than was added to chicory or lucerne, respectively ($P = 0.013$). Intake rate of fresh herbage (FW; g FW/min) was similar between forages, however, intake rate of DM was greatest for cows consuming ryegrass (71 g DM/min) compared with chicory or lucerne (47 and 65 g DM/min, respectively).

Bolus weight and herbage content in the boli tended to increase as the meal progressed when cows consumed chicory and ryegrass but not when consuming lucerne (Table 3). The amount of saliva in the boli and added to herbage (g/100 g feed) was not affected by time within the meal.

Dry weight of the boli and herbage content in the boli did not differ between cows and no boli characteristics were affected by the age of the cow (results not shown).

*Forage comminution and release of cell nutrients during ingestion*

The PSD in boli (Table 4) show the proportion of large particles (>4 mm) was greatest in chicory, compared with lucerne and ryegrass (0.65, 0.56 and 0.58, respectively, $P < 0.01$), but DM in other fractions was similar. There were no differences in the proportion of large (>4 mm) particles and 'soluble' DM in boli between cows ($P = 0.079$ and $P = 0.345$, respectively).

Further separation of the particles retained on the sieve with 4-mm apertures into groups based on fragment length (Table 4) showed that ryegrass boli had a greater proportion of shorter particles (4 to 40 mm) than chicory or lucerne ($P < 0.001$) which had a similar distribution of particle lengths. Lucerne had the longest median particle length, and ryegrass the shortest. The longest particle length measured in the boli from each forage was a 251 mm lucerne stem, and



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**Table 4** *Proportion of size distribution of masticated particles in ingested boli from cows (*Bos taurus*) offered chicory, lucerne or perennial ryegrass; and the distribution of particle sizes (mm) of those retained on the top sieve (4 mm)*

| | Forage | | | | |
|---|---|---|---|---|---|
| | Chicory | Lucerne | P. ryegrass | RMSE | *P*-value |
| Distribution of particles | | | | | |
| > 4 mm | 0.65[a] | 0.56[b] | 0.58[b] | 0.020 | 0.002 |
| 2– to 4 mm | 0.04[b] | 0.06[a] | 0.07[a] | 0.017 | 0.004 |
| 1 to 2 mm | 0.04[b] | 0.06[a] | 0.06[a] | 0.012 | < 0.001 |
| 0.075 to 1 mm | 0.05[b] | 0.07[a] | 0.05[b] | 0.009 | 0.030 |
| Soluble (<0.075 mm) | 0.24 | 0.26 | 0.24 | 0.053 | 0.290 |
| Distribution of large particles >4 mm | | | | | |
| 4 to 40 mm | 0.68[b] | 0.66[b] | 0.82[a] | 0.10 | < 0.001 |
| 41 to 80 mm | 0.26[a] | 0.24[a] | 0.15[b] | 0.07 | < 0.001 |
| 81 to 120 mm | 0.05[ab] | 0.06[a] | 0.02[b] | 0.03 | 0.003 |
| > 121 mm | 0.03 | 0.04 | 0.01 | 0.04 | 0.097 |
| Median fragment length of large particles >4 mm | 28.9[ab] | 33.0[a] | 22.7[b] | 7.6 | 0.044 |

[a,b]Values within a row with different superscripts differ significantly at $P < 0.05$.

**Table 5** *Proportion of nutrient release from chicory, lucerne and perennial ryegrass during mastication by dairy cows (*Bos taurus*) fed indoors during three sampling times during one meal, and the mean of all times*

| | Forage | | | | | |
|---|---|---|---|---|---|---|
| | Chicory | Lucerne | P. ryegrass | Mean | RMSE | *P*-value |
| Water-soluble carbohydrate | | | | | | |
| Start | 0.30[B] | 0.28 | 0.32 | 0.30 | 0.027 | 0.097 |
| Middle | 0.35[aAB] | 0.26[b] | 0.31[ab] | 0.31 | 0.028 | 0.005 |
| End | 0.39[aA] | 0.28[b] | 0.31[b] | 0.33 | 0.027 | < 0.001 |
| *Mean* | *0.34[a]* | *0.27[b]* | *0.31[a]* | | *0.016* | *0.005* |
| *RMSE* | *0.029* | *0.026* | *0.026* | *0.016* | | |
| *P-value* | *0.034* | *0.817* | *0.971* | *0.269* | | |
| Nitrogen | | | | | | |
| Start | 0.12[b] | 0.26[a] | 0.19[ab] | 0.19 | 0.021 | < 0.001 |
| Middle | 0.16[b] | 0.25[a] | 0.20[ab] | 0.21 | 0.024 | < 0.001 |
| End | 0.15[b] | 0.27[a] | 0.22[ab] | 0.21 | 0.022 | < 0.001 |
| *Mean* | *0.15[c]* | *0.26[a]* | *0.20[b]* | | *0.019* | *0.015* |
| *RMSE* | *0.033* | *0.031* | *0.031* | *0.018* | | |
| *P-value* | *0.371* | *0.801* | *0.616* | *0.378* | | |

[a,b,c,A,B]Values within a row with different lowercase superscripts differ significantly at $P < 0.05$; values within columns with different uppercase superscripts differ significantly at $P < 0.05$.

193 mm chicory and 212 mm ryegrass leaves. Median particle length was unaffected by time in the meal ($P > 0.05$). Particle size distribution in boli was not affected by the age of the cow.

There were no relationships between any of the physical or chemical characteristics measured and the extent of forage comminution, when all forages from all sampling dates ($n = 18$) were included in the analyses. Within ryegrass herbage, however, stem content in the sward and the energy required for maceration explained 23% of the variation in ryegrass PSD. Where stem content and energy required to macerate increased, the proportion of large particles (>4 mm) in macerated material declined ($P < 0.05$). This relationship was not observed in chicory or lucerne.

The proportion of forage WSC released during mastication was greater than that of nitrogen (Table 5; mean 0.31 *v.* 0.20, respectively). The proportion of WSC released from chicory (0.34) was similar to that from ryegrass (0.31); however, the absolute amount lost from chicory was double that of ryegrass and lucerne (48 *v.* 23 and 21 g/ kg DM) because chicory herbage contained more WSC. Nitrogen loss also differed between the forages ($P < 0.001$), with the greatest proportion (0.26) from lucerne. Absolute losses of N were 5, 12 and 8 g N/kg DM from chicory, lucerne and ryegrass, respectively. When considering intake rate (g DM/min) the WSC release was 2.25, 1.38 and 1.66 g/min from chicory, lucerne and ryegrass, respectively, while N loss was 0.22, 0.54 and 0.79 g/min from respective forages. There was a



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moderate correlation between the amount of N released during ingestive mastication and the amount of N in the forage ($R^2 = 0.53$, $n = 52$; $P < 0.001$) but no relationship between WSC concentration and WSC release ($R^2 = 0.17$). In lucerne, nutrient release was strongly correlated with the extent of comminution (PSD), where WSC and N release increased with increasing proportion of small particles in masticated material ($R^2 = 0.68$ and 0.70 for WSC and N loss respectively; $P < 0.001$). This relationship was not observed in the other forages.

Sampling time in the feeding bout had no effect on the release of N during mastication, or of WSC from lucerne and ryegrass. However, the proportion of WSC released from chicory increased as the meal progressed ($P = 0.001$). Results of the associations are presented as heat maps in the Supplementary Figure S1.

## Discussion

Understanding of how herbage is processed during ingestive mastication by dairy cows may aid our understanding of nutrient digestion and utilisation. The study reported here aimed to investigate how different forages are ingested and explore what characteristics of the herbage influence the extent of comminution and nutrient release during ingestive mastication.

### *Effect of forage on boli characteristics and herbage intake rate*

Forage species did not affect the FW of ingested boli of cows fed indoors. Comparison of the physical and chemical characteristics of the three forages used in this study indicate that they were divergent in their physical and chemical characteristics (i.e. fragment size, biomechanical properties and structural fibre concentration). This finding is in agreement with that of Acosta *et al.* (2007) who observed no difference in the FW of boli from cows consuming different forages (ryegrass, tall fescue and white clover). Mean FW of the boli in this experiment was greater than that observed by Acosta *et al.* (2007) (169 *v.* 132 g), but mean BW of cows in this experiment was also greater. The lack of forage effect, but between-cow variation in bolus weight, supports other research that suggest cows will manipulate herbage to an extent which overcomes differences in physical characteristics of forage in order to create a bolus that can be swallowed comfortably and safely, and thus the size of the bolus is determined by the animal (Gill *et al.*, 1966; Prinz and Lucas, 1997).

Although weight of the boli did not differ between forages, the herbage and saliva content of the boli did. The saliva content of ryegrass boli was 11% to 18% greater than the saliva content of chicory and lucerne boli, likely reflecting the functions of saliva which are to create cohesion between particles and provide lubrication for swallowing (Hutchings and Lillford, 1988; Prinz and Lucas, 1997). Chicory herbage had the least amount of saliva added during mastication, despite chicory having longer and wider leaves than the other forages. It is possible the low DM and fibre (NDF) concentration of chicory, which required one-fifth the energy to macerate than ryegrass, eased the manipulation of chicory herbage into a bolus, thus reducing the requirement for added saliva. The results suggest that the requirement of saliva for bolus formation is associated with the biomechanical properties and DM and fibre concentration of herbage. Research to determine whether this relationship applies in subsequent feeding bouts, and the implications on saliva production during the remainder of digestion and rumen buffering would be valuable.

Boli ingested at the start of the meal were smaller (wet weight) than those ingested later in the meal. Our observations agree with those of Gill *et al.* (1966), supporting the idea that the size of bolus swallowed is determined by the animal because the amount of saliva added per unit of herbage remained constant between sampling times. The smaller initial boli are likely a response to hunger levels being greater at the start of the meal, and indicates a possible decline in intake rate as the meal progresses and the animal begins to feel satiated.

Dry matter content of the herbage influenced dry matter intake (DMI) rate (g/min), which is consistent with research using fasted cows fed indoors (Cabrera Estrada *et al.*, 2003). Although cows fed chicory increased their intake rate of fresh forage, they were unable to compensate for the low DM content of the feed and attained a lower DMI compared with cows fed ryegrass and lucerne, both of which had higher DM content. This agrees with other studies that positively correlated DMI of sheep and cows with feed DM content of fresh forage (John and Ulyatt, 1987; Cabrera Estrada *et al.*, 2003; Acosta *et al.*, 2007) and suggests, that in studies that have observed similar DMI between cows fed diets of ryegrass only or ryegrass and chicory (Muir *et al.*, 2015; Minneé *et al.*, 2017) the cow must ingest a larger number of chicory boli to achieve this. Gregorini *et al.* (2013) reported increased number of ingestive mastications by cows grazing chicory relative to those grazing ryegrass in a field study despite similar total grazing time (min/day), suggesting the rate of swallowing individual boli of chicory was more rapid than for ryegrass. Therefore, these studies support the conclusion that the DM content of forage influences DMI rate.

### *Effect of forage on comminution by ingestion and cell nutrient release*

Mastication, as ingestive chewing and rumination, is the main process responsible for reducing feed to fragment sizes that can pass from the rumen. The extent of comminution differed between forage species in this study, where mastication reduced 31%, 38% and 35% of chicory, lucerne and ryegrass herbage to particles less than the 2 mm threshold thought able to pass from the rumen (Ulyatt *et al.*, 1986). This reduction in particle size is less than the 50% reported from cows consuming fresh red clover (Reid *et al.*, 1962), but greater than the 12% to 25% observed for fresh legumes and grasses (McLeod and Minson, 1988; Boudon *et al.*, 2006;



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Acosta *et al*., 2007). The variation in extent of fragment size reduction may be due to the different hunger states of the animals used in the studies. Nevertheless, many of the studies reported that forages with a greater DM and fibre concentration or greater implied 'toughness' tended to be reduced to a smaller particle size compared to lower fibre containing or weaker forages. In agreement with this, ryegrass herbage, in the present study, contained the greatest concentration of fibre and required the greatest energy to macerate, also had the least proportion of large particles in the boli of the three forages investigated. Further investigation of the PSD of the large (>4 mm) fragments in the boli, showed particles in ryegrass boli were shortest in median length and contained a greater proportion of particles between 4 to 40 mm, whereas the weaker and less fibrous chicory forage had the greatest proportion of large particles in the boli. These findings agree with those reported in the literature. Comparing green panic (*Panicum maximum* var. trichoglume) and ryegrass herbage, Wilson *et al*. (1989) showed the more fibrous and tougher green panic was reduced to smaller mean particle size during mastication than the less fibrous ryegrass herbage. Similarly, Lee and Pearce (1984) described the greatest reduction in feed particle size occurred in feeds with the greatest fibre concentration and measure of toughness. These findings indicate that forages with greater fibre concentration or toughness are required to be chewed more extensively to allow bolus formation, resulting in greater fragmentation of forage material. The relationships between herbage fibre concentration and forage comminution were, however, not linear. The fibre concentration of lucerne was about half that of ryegrass yet the proportion of large particles in the boli was similar between forages. Therefore, while forage fibre concentration explains some of the variation in particle size reduction it appears other characteristics of the forage not determined in this study must also contribute, supporting the conclusion of Acosta *et al* (2007), that the process of mastication is complex, and is likely influenced by multiple forage and animal factors.

An important function of mastication is cell rupture and the subsequent release of rapidly fermentable nutrients such as soluble carbohydrates and nitrogen. Mean loss of nutrients (WSC and N) was at the lower end of the range reported for cows fed indoors (30% to 37% and 19% to 26% loss of WSC and N, respectively) (Boudon and Peyraud, 2001; Boudon *et al*., 2006; Acosta *et al*., 2007). The proportional release of WSC was the same for chicory as it was for ryegrass, despite the extent of fragmentation being less in chicory boli than in ryegrass which suggests that the extent of comminution is not the sole determinant of nutrient release during ingestion. A similar conclusion was made by Acosta *et al*. (2007) who compared the nutrient release of white clover (*Trifolium repens* L.) with temperate grass species (*Lolium perenn*e L. and *Festuca arundinacea* Schreb.) during mastication and found no correlation between nutrient release and extent of fragmentation but rather, nutrient release was associated with the toughness of the herbage. Further to this, the greatest absolute amount (g/kg) of WSC released in this study was observed from chicory herbage which contained the greatest concentration of WSC and was also the weakest herbage of the three evaluated, requiring the least energy to macerate. It is possible that during mastication, crushing of the weaker chicory material during manipulation of herbage into a bolus could have caused rupture of cells or disruption of tissue allowing for escape of WSC without a reduction in fragment size. Whereas, the absolute amount of WSC released from the lower WSC containing, and tougher, ryegrass herbage was half that from chicory despite the greater extent of fragmentation of herbage observed in ryegrass boli. Furthermore, the greater loss of N from lucerne herbage might also be explained by its greater N concentration and the very low strength of the lucerne leaf material which is where most plant N is stored (Wilman and Altimimi, 1982). Similarly, Acosta *et al*. (2007) also showed that forages with greater N concentration, particularly a greater intracellular N (Total N − NDIN) concentration as observed in lucerne herbage, tended to release more N. While the toughness of forage and concentration of nutrients explains some of the variation in nutrient release, this study indicates that comminution of herbage is not a main driver for the release of nutrients during mastication.

A greater proportion of water-soluble carbohydrate than nitrogenous compounds was released during mastication, which is consistent with other studies (Boudon and Peyraud, 2001; Boudon *et al*., 2006; Acosta *et al*., 2007). Boudon and Peyraud (2001) suggested that because most plant N is chloroplastic and protein N, which are large molecules and typically associated with organelles, they are far less easily released during ingestive mastication than smaller, soluble carbohydrates which are stored in the cytoplasm or vacuole and can diffuse through pores or across the cell wall of intact cells. However, because proportional release is relative, it is the absolute amount and rate of delivery to the rumen microflora that is important. The amount of WSC released was 10 times greater than N release from chicory, but only two and three times greater in lucerne and ryegrass, respectively. While the amount of N released was greater from lucerne compared with ryegrass, the rate of release (g/min) was 46% faster from ryegrass. Increasing our knowledge of nutrient release, and the factors that affect it, could enable improved explanation of differences in observed livestock production or N excretion in livestock consuming different diets (Hutton *et al*., 2011; Totty *et al*., 2013; Minneé *et al*., 2017). The balance of nutrient availability to the rumen has been suggested to influence the efficiency of nutrient use (Cole and Todd, 2008), particularly in intermittent feeding regimens, such as pastoral grazing where herbage is allocated once or twice a day. Improving our understanding of the consequences of substrate availability, may enable improved management of forages or design of forage mixtures to alter diet composition and effect a greater efficiency of nutrient use and hence reduction in environmental impact.



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## Declaration of interest

The authors declare no potential conflicts of interest associated with this research.

## Ethics statement

Care and use of animals were conducted in accordance with the guidelines issued by the New Zealand Ministry of Primary Industries. The experiment was approved and supervised by the Ruakura Animal Ethics Committee (Hamilton, New Zealand); application No. 13556.

## Software and data repository resources

None of the data were deposited in an official repository.

## Supplementary material

To view supplementary material for this article, please visit https://doi.org/10.1017/S1751731118002938

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