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Forage fiber effects on particle size reduction, ruminal stratification, and selective retention in heifers fed highly digestible grass/clover silages[1]

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ABSTRACT: The objective of this study was to investigate the effect of NDF content in highly digestible grass/clover silage on particle size reduction, ruminal stratification, and selective retention in dairy heifers. The reduction in particle size from feed to feces was evaluated and related to feed intake, chewing activity, and apparent digestibility. Four grass/clover harvests (Mixtures of *Lolium perenne, Trifolium pratense*, and *Trifolium repens*) were performed from early May to late August at different maturities, at different regrowth stages, and with different clover proportions, resulting in silages with NDF contents of 312, 360, 371, and 446 g/kg DM, respectively, and decreasing NDF digestibility with greater NDF content. Four rumen-fistulated dairy heifers were fed silage at 90% of ad libitum level as the only feed source in a 4 × 4 Latin square design. Silage, ingested feed boluses, medial and ventral ruminal digesta, and feces samples were washed with neutral detergent in nylon bags of 10-μm pore size, freeze dried, and divided into small (<0.212 mm), medium (0.212 to 1 mm), and large (LP; >1 mm) particles by dry-sieving. Chewing activity, rumen pool size, and apparent digestibility were measured. Intake of NDF increased linearly from 2.3 to 2.8 kg/d with greater NDF content of forages ($P = 0.01$), but silages were exposed to similar eating time ($P = 0.55$) and rumination time per kg NDF ($P = 0.35$). No linear effect of NDF content was found on proportion of LP in ingested feed boluses ($P = 0.31$), medial rumen digesta ($P = 0.95$), ventral rumen digesta ($P = 0.84$), and feces ($P = 0.09$). Greater proportions of DM ($P < 0.001$) and particulate DM ($P = 0.008$) were found in medial ruminal digesta compared with ventral rumen, and differences in DM proportion increased with greater NDF content ($P = 0.02$). Particle size distributions were similar for digesta from the medial and ventral rumen regardless of NDF content of the silages ($P > 0.13$). The LP proportion was >30% of particles in the ventral and medial rumen, whereas in the feces, the LP proportion was <2%. Particle size stratification of the rumen was undetectable regardless of NDF content of the silages, stressing that the retention mechanism of large undigested particles lies elsewhere than with particle entrapment in the rumen mat. In this study, forage particle breakdown, ruminal stratification, and retention of particles in the rumen were not affected by NDF content of highly digestible grass/clover silages.

Key words: cows, NDF, particle size reduction, ruminal stratification, selective particle retention

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INTRODUCTION

Forage NDF degradation, ruminal stratification, and selective retention of undigested forage fiber particles are of great importance for optimal fiber digestion in ruminants. Several authors have reviewed the influential forage and animal factors regarding this topic (Faichney, 1986; Lechner-Doll et al., 1991; Allen, 1996; Zebeli et al., 2012). Briefly, ingested and undigested forage fiber particles are buoyant and of low density because of fermentation gasses entrapped within the

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plant NDF structure. Buoyancy makes particles form a rumen mat towards the dorsal rumen. When forage particles are adequately reduced in size by rumination and fermentation, density increases and the particles move downward to the more liquid ventral rumen and reticulum from where they can escape. Jung and Allen (1995) state that particle buoyancy, breakdown, and passage are related to the physical properties of the NDF of the cell wall, such as tissue origin, shape and size, maturity stage, and forage species. Forage cell wall NDF lignifies with plant maturation, affecting the physical characteristics of particles, and previous research on the formation and function of the rumen mat was mainly based on mature forages of high NDF contents. Increasing attention to including high digestibility forages, of earlier maturity, in diets for dairy cattle raises questions about the physical properties of the ruminal mat and its function in retaining undigested material when forages of low NDF content and high potential NDF digestibility are fed.

The hypothesis was that decreasing NDF content of forages of high digestibility would decrease the ruminal mat formation and, consequently, the retention of large particles (**LP**). The objective was to follow the particle size reduction from feed to feces as an effect of increasing forage NDF when feeding grass/clover silages with high digestibility to dairy heifers. Second, the objective was to evaluate the effect of grass/clover silage NDF on ruminal stratification and particle distribution of digesta between the medial and ventral rumen.

MATERIALS AND METHODS

All experimental procedures complied with Danish Ministry of Justice Law no. 382 (June 10, 1987) Act no. 726 (September 9, 1993), concerning experiments with and the care of animals.

Forages

Grass/clover from a mixed sward of ryegrass (*Lolium perenne*), red clover (*Trifolium pratense*), and white clover (*Trifolium repens*) was harvested in 2009 at Aarhus University, Foulum, Denmark (56°29′ N, 9°35′ E), in 4 different cuts for silage to represent 4 levels of NDF content. Silages were given the abbreviations S1, S2, S3, and S4, according to NDF content, potential NDF digestibility, and NDF degradation rate. They consisted of spring growth at an early vegetative stage, harvested on May 9 (**S1**), first regrowth at beginning heading stage, harvested on June 22 after 42 d regrowth (**S4**), early harvest third regrowth at an early vegetative stage, harvested on August 7 after 7 d regrowth (**S2**), and late harvest third regrowth at a late vegetative stage, harvested on August 23 after 33 d regrowth (**S3**). Crops were pre-wilted in the field to approximately 40% DM, chopped to 19.2 mm theoretical length of cut by a forage harvester (John Deere 6750), mixed and baled in round bales (diameter 125 cm, height 125 cm; Orkel MP 2000 Compactor; Orkel, Fannrem, Norway), and ensiled by wrapping with 11 layers of 25 μm plastic without use of ensiling additives.

Morphological analysis was conducted by manually separating the fresh crop into grass leaves, grass stems including leaf sheaths, red clover leaves, red clover stems including flowers and stipules, white clover leaves, and white clover flower stems including flowers. The leaf and stem fractions were dried for 24 h at 60°C. Proportions are reported on a DM basis.

Animals, Treatments, and Experimental Design

Four rumen-fistulated Jersey heifers with an average initial live weight of 343 ± 32 kg were assigned randomly to 4 dietary treatments in a balanced 4 × 4 Latin square design. The dietary treatments consisted of 1 of the 4 silages fed as the only source of feed, making investigation of the silage effects possible without disturbance from other feed sources.

The experimental periods were 28 d each and included an adaptation period (d 1 to 15) and a data collection period (d 15 to 18). Heifers were fed ad libitum from d 1 to 11 and feed offers and residuals were recorded. On d 12 to 18, a fixed feeding level of 90% of individual ad libitum intake was offered to avoid selection in forage. On d 19 to 28, treatments were continued and feeding levels were further restricted; however, data from d 19 to 28 is not reported in the current paper.

Heifers were kept in individual tie stalls with rubber mats and access to separate, automatic water bowls and cribs for forage. Live weights of the heifers were recorded before each experimental period and at the end of the experiment. Silages were fed twice daily, half the daily amount at 0800 h and half at 1530 h. Mineralized salt (NaCl) blocks (biotin 12 mg/kg, manganese 190 mg/kg, iron 210 mg/kg, copper 80 mg/kg, cobalt 12 mg/kg, zinc 300 mg/kg, iodine 50 mg/kg, selenium 20 mg/kg; Brogaarden, Denmark) were available during the adaptation periods. For apparent total tract digestibility measurement, 5 g of chromic oxide (Cr_2O_3) was administered to the rumen via the fistula as marker before each meal throughout the experiment.

Sample Collection and Chewing Data

Silages were representatively sampled by random grab sampling throughout each bale. Samples were gently mixed, pooled into representative samples of 2 kg per bale, and stored at -20°C for later analysis.

A sample of 150 g feces was collected rectally from each heifer in the morning, at noon, and in the afternoon,

on d 15 to 17. These 9 samples were mixed into 1 representative sample per heifer and period and frozen at -20°C for later analysis.

Samples of ruminal content were taken through the ruminal fistula from the medial and ventral rumen at 1200 h on d 18. This time was chosen so as to wait for the rumen to settle after the morning meal, to distinguish forage effects from effects of particle hydration and swallowed air. All samples were taken by the same person throughout the study. Medial ruminal content was sampled by grabbing 1 large handful of the ruminal mat 5 cm below the mat surface in the midsection of the rumen. Ventral ruminal content was sampled 5 cm above the ventral rumen wall. A 300 mL container was gently introduced by hand along the rumen lateral wall while covering the opening of the container during insertion and withdrawal. Samples were stored at -20°C for later analysis.

The rumen was evacuated manually through the ruminal fistula subsequent to sampling of medial and ventral ruminal content, to be able to relate possible ruminal pool size differences to medial–ventral differences in particle size. The ruminal digesta was placed on a screen with 0.5-cm mesh size and separated into a primarily solid and liquid pool by applying static pressure to the material. Both pools were weighed and a proportional, composite sample was taken for determination of DM. While the rumen was empty, the experimental silage was offered and samples of ingested feed boluses were taken manually through the rumen fistula at the cardiac sphincter. After the collection of boluses, the ruminal content was returned.

Chewing activity was recorded for 72 h continuously from d 15 at 0800h before morning feeding. Jaw movements were recorded by use of a chewing halter (Nørgaard and Hilden, 2004) and modified GigaLog F logger (Controlord, La Farlede, France) logging digitized jaw movement oscillations at 20 Hz. Eating time, rumination time, and total chewing time were analyzed from the recorded jaw movements by methods described in Schleisner et al. (1999). The data analysis distinguishes between eating and ruminating behavior based on the regularity of jaw movement patterns within a defined time period.

Analytical Procedures

Dry matter was determined in fresh silage, ingested feed boluses, ruminal contents, and feces by drying under forced air for 24 h at 60°C. Silage and feces were freeze dried and ground to 1.5 mm before chemical analysis. Ash was determined by combustion at 525°C for 6 h. The contents of NDF, ADF, and ADL were analyzed according to Van Soest et al. (1991). The fractions were analyzed sequentially in triplicate for feeds and duplicate for digesta and fecal samples, using filter bags in an ANKOM apparatus (ANKOM200; ANKOM Technology, Fairport, NY). The analysis was modified by adding thermo-stable α-amylase (Novozymes, Bagsvaerd, Denmark) during neutral detergent boiling, and reported contents of NDF, ADF, and ADL were corrected for residual ash determined after ADL treatment. Total sugar was analyzed by the Luff-Schoorl method (European Community, 1971). Total N contents of silages were analyzed by the Kjeldahl procedure (AOAC, 2012) and CP was calculated as N × 6.25. Determination of crude fat in silage was performed by HCl hydrolysis followed by petroleum ether extraction (AOAC, 2012) using a Soxtec system (FOSS, Hillerød, Denmark). In vitro OM digestibility was performed by anaerobic incubation in rumen fluid for 48 h, followed by incubation of undissolved material with pepsin HCl solution for 48 h (Tilley and Terry, 1963).

Analysis of silage pH and fermentation products was performed using silage extracts. Water (500 mL) was added to thawed samples (50 g) and blended in a Bosch Chopper (Type CNCM13ST1; Ballerup, Denmark). A 40-mL homogenized sample was centrifuged, and the pH was measured in the supernatant before stabilizing with 5% metaphosphoric acid. Concentrations of acetic acid, propionic acid, and butyric acid were analyzed by gas chromatography (Nielsen et al., 2007). Ammonia was analyzed using a Cobas Mira autoanalyzer (Triolab A/S, Brøndby, Denmark) and a kit based on glutamate dehydrogenase (AM 1015; Randox Laboratories Ltd., Crumlin, UK). Glucose and L-lactate were analyzed using a YSI analyzer (YSI 7100; YSI Inc., Yellow Springs, OH).

The content of potentially digestible NDF (**DNDF**) and indigestible NDF (**iNDF**) and the rate of DNDF degradation of the silages were assessed by Dacron bag incubations in situ, as described by Åkerlind et al. (2011). Dry and ground (1.5 mm) silage samples were incubated for 0, 2, 4, 8, 16, 24, 48, 96, 168 (38 μm pore size bags), and 288 h (iNDF, 12 μm pore size bags) in the rumen of 3 cows fed at a maintenance metabolizable energy level. The daily ration consisted of 4 kg/d of grass/clover hay, 2 kg/d barley straw, and 2.8 kg/d pelleted concentrate mixture. Concentrate consisted of 40 kg barley grain, 40 kg oat grain, 10 kg soybean meal, 3 kg rapeseed meal, 3 kg sugar beet molasses, and 4 kg of a commercial mineral mixture (consisting of 6 g/100 g Ca, 10 g/100 g P, 12 g/100 g Mg, 5 g/100 g Na; Type 3; Vitfoss, Gråsten, Denmark) per 100 kg fresh mixture. The chemical composition of the diet was (g/kg DM) crude protein 139, NDF 241, starch 137, crude fat 32. The daily ration was divided into 2 meals of equal size. Bags were presoaked for 20 min in 39°C water immediately before incubation. After incubation, bags were rinsed twice for 12 min in 25°C water in a washing machine. Residues for NDF determination were transferred directly to filter crucibles for analysis of ash-free NDF, using a Fibertec 2010 (FOSS, Hillerød, Denmark), according to Mertens

(2002). Rate of NDF degradation was calculated by nonlinear curve fitting of the degradation profile for up to 168 h incubation time. The rate of degradation was corrected for DNDF, according to Åkerlind et al. (2011). The degradation profiles were fitted and reported without lag time adjustment, as parallel estimations including lag time revealed estimated lag times < 0.6 h.

Chromic oxide content of feces samples was determined colorimetrically after oxidation to chromate, as described by Schürch et al. (1950). Total feces output was calculated from the up-concentration of chromium oxide in feces when compared with chromium oxide amount being fed. Digestibility of OM, NDF, and DNDF was calculated from fecal output of nutrients relative to ingested nutrients.

For determination of particle size distributions of silages, ingested feed boluses, ruminal digesta, and feces, triplicate samples of 10 g thawed, mixed material were transferred into nylon bags of 10-µm pore size with 4.0 mL of liquid soap (Biotex Color, Copenhagen, Denmark). Batches of 18 nylon bags were washed for 2 h at 40°C in a washing machine to remove neutral detergent soluble matter from the mostly fibrous particulate matter. Washed particulate DM (**PDM**) was removed from the bags by gentle flushing with demineralized water. Samples were then freeze-dried. The dry particles were separated into 7 fractions by dry-sieving with a vertical sieve shaker (Retsch AS 200; Retsch GmbH, Haan, Germany) using sieves of 4.75, 2.360, 1.000, 0.500, 0.212, and 0.106 mm aperture and a bottom bowl. To facilitate interpretation of particle size distributions, the weight proportions of the sieving fractions were summarized into groups of small particles (**SP**) < 0.212 mm, medium particles (**MP**) 0.212 to 1.0 mm, and LP > 1.0 mm. The threshold for LP was determined as > 1.0 mm, according to the threshold of >1.18 mm described by Poppi et al. (1981) as the maximum size of particles that can easily escape from the rumen of cows. The geometric mean particle size (**GPS**) was calculated based on a log-normal distribution of particle mass proportions, according to Waldo et al. (1971), using the equation:

$$GPS = \exp\left(\begin{array}{l}\left[A\times\ln(0.05)\right]+\left[B\times\ln\sqrt{(0.106\times0.212)}\right]+\\ \left[C\times\ln\sqrt{(0.212\times0.5)}\right]+\left[D\times\ln\sqrt{(0.5\times1.0)}\right]\\ +\left[E\times\ln\sqrt{(1.0\times2.36)}\right]+\left[F\times\ln\sqrt{(2.36\times4.75)}\right]\\ +\left[G\times\ln\sqrt{(4.75\times9.5)}\right]\end{array}\right),$$

where A = the mass proportion of particles < 0.106 mm, B = the mass proportion retained on the 0.106-mm aperture sieve, C = the mass proportion retained on the 0.212-mm aperture sieve, D = the mass proportion retained on the 0.5-mm aperture sieve, E = the mass proportion retained on the 1.0-mm aperture sieve, F = the mass proportion retained on the 2.36-mm aperture sieve, and G = the mass proportion retained on the 4.75-mm aperture sieve.

Table 1. Morphological composition of forage crops at harvest

Fresh crop composition[2] %	Grass/clover harvest[1], % of DM			
	S1	S2	S3	S4
Ryegrass	71	68	60	63
Leaf	53	66	57	21
Stem	17	2	3	42
White clover	2	4	3	5
Leaf	2	4	2	4
Stem	0	0	1	1
Red clover	27	28	37	32
Leaf	25	25	33	19
Stem	2	3	3	13
Total leaf:stem ratio	80:20	94:6	92:8	44:56
Total grass:clover ratio	71:29	68:32	60:40	63:37

[1]Harvests were S1 (May 9, spring growth), S2 (August 7, third regrowth), S3 (August 23, third regrowth), and S4 (June 22, first regrowth).

[2]Weight proportion of DM of plant parts in harvested fresh crop, determined by morphological separation of plant species and parts into: grass leaves, grass stems including leaf sheaths, red clover leaves, red clover stems including flowers and stipules, white clover leaves, and white clover flower stems including flowers.

Statistical Analysis

All data were analyzed with the MIXED procedure in SAS 9.2 (SAS Inst. Inc., Cary, NC). Differences between ruminal parameters from the medial and ventral rumen were further analyzed by the *t* test procedure. Data on feed intake, digestibility, ruminal evacuation data, particle size distributions, and chewing activity were analyzed by ANOVA; the model included treatment (S1, S2, S3, and S4) and period (1 to 4) as fixed effects and heifer (1 to 4) as random effect. Results are reported as least square means of treatments. Linear and quadratic contrast effects of NDF content of the 4 silages were tested on the basis of orthogonal contrast. The IML procedure and orpol option were used to calculate coefficients from the unequally spaced NDF contents. Treatment effect and linear and quadratic contrast effects are reported and considered significant at $P < 0.05$.

RESULTS

Silages and Feed Intake

The silages were of spring growth, first regrowth in early summer, and third regrowth at 2 different stages of maturity in late summer. Consequently, distribution of grass and clover species, plant maturity stages, and, consequently, leaf:stem ratios were different among the freshly harvested crops (Table 1). As a result, NDF content did not correspond to harvest date. The chemical composition and characteristics of the silages are given in Table 2. The silages were numbered S1 to S4 according to NDF content, S1 and S4 having NDF contents of 312 g/kg DM and 446 g/kg DM, respectively. It

Table 2. Characteristics and chemical composition of experimental grass/clover silage treatments

	Silages[1]			
Item[2]	S1	S2	S3	S4
DM, g/kg	462	445	408	396
Ash	85	104	100	89
NDF	312	360	371	446
ADF	177	200	215	258
ADL	16	30	36	37
iNDF[3]	24	36	45	59
Total sugar[4]	158	8	27	65
Glucose	40	6	16	20
CP	183	269	228	147
Crude fat	33	46	39	29
Characteristics of NDF				
iNDF, % of NDF	7.7	10.0	12.1	13.2
K_dDNDF[5], %/h	10.2	7.2	7.5	5.9
Fermentation products, g/kg DM				
L-lactic acid[6]	12.0	32.4	30.0	22.9
Acetic acid	5.1	12.7	11.2	12.6
Propionic acid	0.02	0	0	0.01
Butyric acid	0.01	0	0	0
Ethanol	6.9	2.0	1.0	3.0
Ammonia N, % of total N	3.9	4.0	4.9	4.2
pH	4.83	4.24	4.37	4.09
Digestible OM[7], g/kg OM	819	785	749	749
Particle proportion of silages, % of PDM[8]				
Very large (>4.75 mm)				
Large (>1.0 mm)	93.0	95.0	91.4	96.1
Medium (0.212 to 1.0 mm)	6.2	4.5	7.6	3.3
Small (<0.212mm)	0.8	0.5	1.1	0.6
GPS[9], mm	3.28	4.40	3.05	3.67

[1]Harvests were S1 (May 9, spring growth), S2 (August 7, third regrowth), S3 (August 23, third regrowth), and S4 (June 22, first regrowth).

[2]Values are means of $n = 4$ samples per silage type. All analyses were performed twice per sample at minimum.

[3]Indigestible NDF (iNDF) estimated by incubation 288 h in situ.

[4]Glucose, fructose, sucrose, and fructan.

[5]K_dDNDF = Rate of degradation of potentially digestible NDF.

[6]Total lactic acid concentration approximates 2 times L-lactic acid.

[7]Digestible organic matter determined in vitro (Tilley and Terry, 1963).

[8]Particulate DM (PDM) is DM residue after washing forage in neutral detergent/DM in forage × 100.

[9]GPS = geometric mean particle size.

can be seen in Table 2 that the potential DNDF fraction and rate of NDF degradation decreased with greater NDF content.

The intake of DM decreased linearly with greater NDF content of the silages ($P = 0.003$; Table 3). However, increasing silage NDF content caused greater NDF intake (NDFI; $P = 0.01$). Categorization of the NDF in a potentially digestible and indigestible fraction from in situ incubation allowed calculation of daily intake of DNDF and iNDF. With greater NDF content of the silages, the iNDF intake increased ($P < 0.001$), whereas DNDF intake tended to increase linearly ($P = 0.06$).

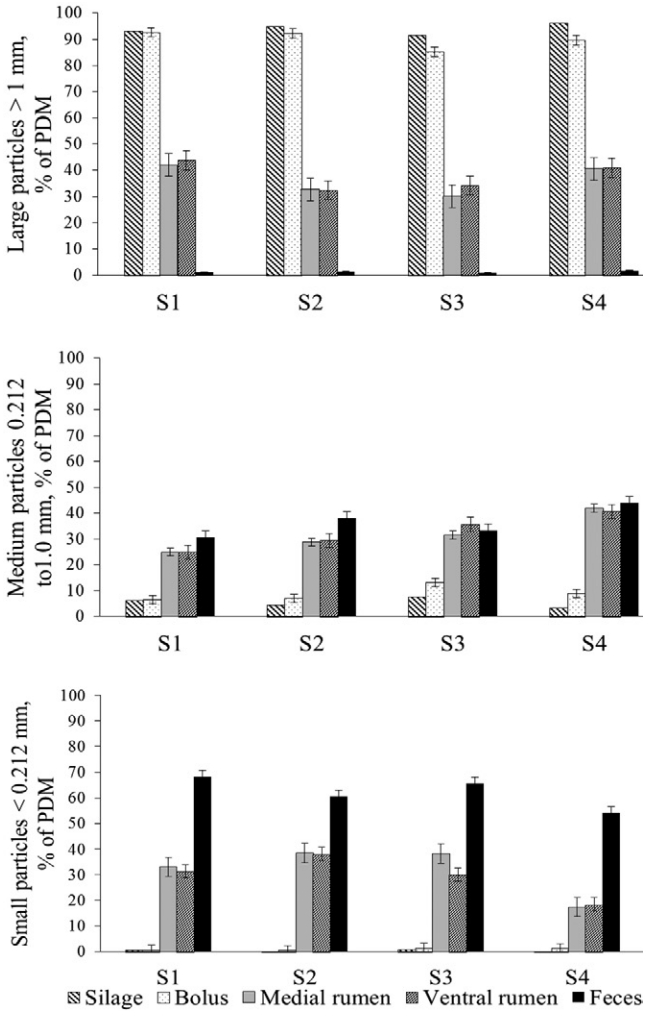

Figure 1. Weight proportions of large (top), medium (middle), and small particles (bottom) in particulate DM (PDM) of silages, ingested feed boluses, medial ruminal digesta, ventral ruminal digesta, and feces from 4 silages of increasing NDF content fed to heifers. Silage harvests were: S1 (May 9, spring growth), S2 (August 7, third regrowth), S3 (August 23, third regrowth), and S4 (June 22, first regrowth). Displayed data are least square means and SEM of treatments ($n = 4$ per type of material).

Particle Size Reduction

Chewing was stimulated by greater NDF content of the silages, with linearly increasing eating time per day ($P = 0.05$; Table 3) and per kg DMI ($P = 0.01$). Rumination time per day also increased linearly ($P = 0.02$), as did rumination time per kg DMI ($P < 0.001$). Consequently, with greater NDF content of the silages, an increase in total chewing time per day and per kg DMI ($P = 0.01$ and $P = 0.002$, respectively) was found. Total chewing time per kg DMI was 86 min for the S1 silage (31% NDF in DM) compared with 124 min for the S4 silage (45% NDF in DM). No effect of increasing NDF content of silages was found on eating time per kg NDFI ($P = 0.52$) or rumination time per kg NDFI ($P = 0.88$), and total chewing activity per kg NDFI was therefore not affected by increasing NDF content ($P = 0.62$).

Table 3. Effect of treatment and NDF content on feed intake, chewing activity, and particle size distribution of ingested feed boluses in heifers

	Treatment[2]					*P*-value[4]		
Item[1]	S1	S2	S3	S4	SEM[3]	Treatment	L	Q
Intake								
DM, kg/d	7.4[a]	7.1[ab]	6.8[b]	6.2[c]	0.3	0.02	0.003	0.92
NDF, kg/d	2.3[a]	2.5[ab]	2.5[ab]	2.8[b]	0.1	0.05	0.01	0.94
DNDF, kg/d	2.2	2.3	2.2	2.4	0.1	0.20	0.06	0.80
iNDF, kg/d	0.18[a]	0.25[b]	0.31[c]	0.36[d]	0.01	<0.001	<0.001	0.03
Eating time								
Min/24h	241	234	239	302	22	0.14	0.05	0.20
Min/kg DMI	33[a]	33[a]	36[a]	49[b]	4	0.05	0.01	0.22
Min/kg NDFI	104	92	96	110	11	0.55	0.52	0.23
Rumination time								
Min/24h	395	397	439	464	24	0.06	0.02	0.87
Min/kg DMI	53[a]	56[ab]	65[bc]	75[c]	3	0.005	<0.001	0.75
Min/kg NDFI	168	156	176	168	9	0.35	0.88	0.87
Total chewing time								
Min/24h	636[a]	631[a]	678[ab]	766[b]	34	0.05	0.01	0.31
Min/kg DMI	86[a]	89[a]	101[a]	124[b]	7	0.01	0.002	0.37
Min/kg NDFI	272	248	272	278	17	0.51	0.62	0.38
Particle proportion of ingested feed, % of PDM[5]								
Large (>1.0 mm)	92.6	92.2	85.2	89.6	1.8	0.11	0.31	0.17
Medium (0.212 to 1.0 mm)	6.6	7.1	13.2	9.2	1.6	0.11	0.32	0.15
Small (<0.212 mm)	0.8	0.7	1.6	1.2	0.3	0.19	0.30	0.44
GPS, mm	3.2[a]	3.01[ab]	1.97[c]	2.45[bc]	0.22	0.01	0.03	0.05

[a–d]Within a row, means without a common superscript differ ($P < 0.05$).

[1]DNDF = potentially digestible NDF; iNDF = indigestible NDF; NDFI = NDF intake; PDM = particulate dry matter; GPS = geometric mean particle size.

[2]Treatment silage harvests were: S1 (May 9, spring growth), S2 (August 7, third regrowth), S3 (August 23, third regrowth), and S4 (June 22, first regrowth).

[3]$n = 4$ per treatment.

[4]Probability of treatment effect and linear (L) and quadratic (Q) contrast effects of the NDF content of the silages.

[5]From ingested feed boluses collected by the cardia during test feeding in rumen evacuated heifers. Particulate DM is DM residue after washing ingested feed boluses/DM in ingested feed boluses × 100.

No effect of increasing NDF content of the silages was found on particle size distributions in ingestive masticate ($P > 0.30$; Table 3) though the GPS of ingested feed boluses decreased with greater NDF content of the silages ($P = 0.03$), reflecting numerically decreasing LP proportions with increasing NDF content ($P = 0.11$). The particle size distributions of ingested feed boluses showed that the silage particles were reduced little in size from the initial mastication during eating (Fig. 1; not analyzed). The LP proportions decreased by 0.4, 2.8, 6.2, and 6.5% points for the S1 to S4 silages. The reduction in LP was reflected in increased proportions of mainly MP of the ingested feed boluses compared with the silages.

The reduction in the proportion of LP from the ingested feed boluses to the medial ruminal content was 50.6, 59.5, 55.1, and 49.0% points for the S1, S2, S3, and S4 silages, respectively (Fig. 1), illustrating the particle size reduction caused by rumination and fermentation.

Ruminal Digesta Characteristics

Table 4 shows the characteristics of, and differences between, digesta from the medial and ventral rumen compartments. The digesta DM contents of the medial and ventral rumen were not affected by greater NDF content of the silages though increasingly more DM was found in the medial rumen compared with the ventral rumen with greater NDF content of the silages ($P = 0.02$, linearly). Particulate DM content increased linearly with greater NDF content of the silages in both the medial rumen ($P = 0.008$) and the ventral rumen ($P < 0.001$). The medial rumen digesta generally contained more (4.6 to 6.1% points) PDM compared with the ventral digesta ($P = 0.008$). The difference between the medial and ventral rumen for PDM was not affected linearly ($P = 0.31$) by greater silage NDF content; however, a tendency for a quadratic effect was shown ($P = 0.09$), peaking for S2 and S3.

The PDM of the medial and ventral rumen (Fig. 1 and Table 4) generally showed similar proportions of LP ($P = 0.34$), MP ($P = 0.58$), and SP ($P = 0.13$), and the GPS was also similar in the 2 rumen compartments ($P = 0.12$).

Table 4. Effect of treatment and NDF concentration on content of DM, particulate DM, and particle size of medial and ventral ruminal digesta from heifers

	Treatment[1]					*P*-value[3]			
Item	S1	S2	S3	S4	SEM[2]	Treatment	L	Q	*t* test
Medial rumen content									
DM, g/kg	121	132	125	132	3	0.15	0.09	0.41	
PDM[4], g/kg DM	518[a]	623[bc]	559[ab]	689[c]	34	0.03	0.008	0.97	
Particle proportion, % of PDM									
Large (>1.0 mm)	42.0	32.7	30.1	40.6	4.3	0.14	0.95	0.03	
Medium (0.212 to 1.0 mm)	25.0[a]	28.8[ab]	31.6[b]	42.0[c]	1.5	0.001	<0.001	0.29	
Small (<0.212 mm)	32.9[a]	38.5[a]	38.2[a]	17.4[b]	3.8	0.005	0.004	0.006	
GPS[5], mm	0.62	0.45	0.40	0.66	0.08	0.06	0.36	0.01	
Ventral rumen content									
DM, g/kg	75	81	78	70	4	0.37	0.30	0.19	
PDM, g/kg DM	479[a]	529[a]	473[a]	684[b]	40	<0.001	<0.001	0.02	
Particle proportion, % of PDM									
Large (>1.0 mm)	43.8	32.4	34.2	40.9	3.6	0.10	0.84	0.02	
Medium (0.212 to 1.0 mm)	24.9[a]	29.5[ab]	35.7[bc]	40.7[c]	2.7	0.009	0.002	0.49	
Small (<0.212 mm)	31.3[a]	38.2[b]	30.0[a]	18.4[c]	2.6	0.004	0.003	0.01	
GPS	0.67[a]	0.45[b]	0.50[bc]	0.66[ac]	0.06	0.03	0.99	0.007	
Medial–ventral content differences									
DM, g/kg	46	51	47	61	3	0.06	0.02	0.42	<0.001
PDM, g/kg DM	39	94	86	5	38	0.24	0.31	0.09	0.008
Particle proportion, % of PDM									
Large (>1.0 mm)	-1.8	0.3	–4.1	-0.3	3.4	0.86	0.83	0.83	0.34
Medium (0.212 to 1.0 mm)	0.2	-0.6	–4.1	1.3	3.1	0.64	0.75	0.34	0.58
Small (<0.212 mm)	1.6	0.3	8.2	-1.0	2.6	0.08	0.40	0.11	0.13
GPS	-0.05	0.01	-0.10	0.01	0.05	0.53	0.38	0.98	0.12

[a–c]Within a row, means without a common superscript differ ($P < 0.05$).

[1]Treatment silage harvests were: S1 (May 9, spring growth), S2 (August 7, third regrowth), S3 (August 23, third regrowth), and S4 (June 22, first regrowth).

[2]$n = 4$ per treatment.

[3]Probability of treatment effect, linear (L) and quadratic (Q) contrast effects of the NDF content of the silages, and *t* test of difference. *t* test was performed to determine if medial–ventral content differences differed from 0.

[4]Particulate DM (PDM) is DM residue after washing ruminal content/DM in fresh ruminal content × 100.

[5]GPS, geometric mean particle size.

A quadratic effect of increasing NDF content on LP proportions was found in both the medial ($P = 0.03$) and the ventral rumen ($P = 0.02$), as digesta PDM from S1 and S4 silages had numerically larger proportions of LP compared with the S2 and S3 silages. The proportion of MP increased with greater NDF content of silages in both the medial ($P < 0.001$) and the ventral rumen ($P = 0.002$). Quadratic effects on SP proportions were significant from medial ($P = 0.006$) and ventral rumen digesta ($P = 0.01$), which is very clear from Fig. 1. Linear effects were also significant in both the medial ($P = 0.004$) and the ventral rumen ($P = 0.003$).

Analysis of the rumen evacuation data showed no difference between the silages on either pool size or content of DM in digesta relative to the rumen pool size, or relative to DMI ($P > 0.20$; Table 5).

Feces Characteristics and Apparent Digestibility

Feces contained linearly decreasing amounts of DM ($P = 0.03$; Table 6) and increasing concentrations of PDM (g/kg DM; $P < 0.001$) and iNDF (g/kg PDM; $P < 0.001$) with greater NDF content of the silages.

An increased proportion of LP in feces ($P = 0.09$), which was generally <1.7% of fecal PDM, was found with greater NDF content of the silages. The fecal proportions of SP decreased linearly ($P = 0.002$), whereas the proportion of MP increased linearly ($P = 0.004$) with greater NDF content of silages. This resulted in a slightly increasing fecal GPS, from 0.15 to 0.19 mm, with greater NDF content ($P < 0.001$). The proportion of LP in feces compared with the proportion of LP in the ventral rumen was 42.7, 31.0, 33.2, and 39.2% points smaller for S1, S2, S3, and S4, respectively (Fig. 1).

Table 6 further shows the apparent total tract digestibility. A lower apparent digestibility of OM ($P = 0.001$), NDF ($P < 0.001$), and DNDF ($P = 0.009$) was found with greater NDF content of the silages, corresponding well to the increasing in situ-determined iNDF fractions with greater NDF content.

Table 5. Effect of treatment and NDF content on ruminal evacuation parameters

	Treatment[1]					*P*-value[3]		
Rumen evacuation data	S1	S2	S3	S4	SEM[2]	Treatment	L	Q
Total pool, kg	47.0	41.9	44.3	46.5	3.2	0.71	0.92	0.33
Solid pool, kg	18.3	17.3	17.2	17.9	1.6	0.95	0.93	0.58
Liquid pool, kg	28.7	24.6	27.1	28.6	1.8	0.48	0.82	0.23
Total digesta DM, % of total pool	9.4	9.7	10.0	9.3	0.3	0.20	0.68	0.06
Total digesta DM, % of DMI	59.4	57.5	65.4	70.2	4.5	0.31	0.13	0.70

[1]Treatment silage harvests were: S1 (May 9, spring growth), S2 (August 7, third regrowth), S3 (August 23, third regrowth), and S4 (June 22, first regrowth).

[2]$n = 4$ per treatment.

[3]Probability of treatment effect and linear (L) and quadratic (Q) contrast effects of the NDF content of the silages.

DISCUSSION

Forage Particle Size Reduction

The present increase in chewing activity, relative to DMI, with increasing NDF content of silages is in agreement with previous research (De Boever et al., 1993; Rinne et al., 2002). The NDF composition in terms of iNDF and DNDF proportion differed between silages in this study; however, this did not affect chewing time per kg NDF because similar chewing time relative to NDFI was found. This agrees with previous studies where effect of maturity stage and, hence, different NDF composition on chewing activity was assessed in ryegrass silage (Rinne et al., 2002), red clover and white clover silages (Kornfelt et al., 2013), and alfalfa and orchard grass hay (Beauchemin and Iwaasa, 1993).

The grass/clover crops were chopped to a 19.2 mm theoretic length of cut, which resulted in silages with 92 to 96% of the particulate matter retainable on a 1-mm screen. Though eating activity per kg NDFI was similar between treatments, the reduction in the LP proportions from silages to ingested feed boluses showed that silages of greater NDF content were subject to a slightly higher degree of comminution from eating activity. This agrees with results found by Ulyatt (1983), who fed roughages of increasing cell wall content to sheep, and Kornfelt et al. (2013), who fed red and white clover silages of different NDF contents. Wilson and Kennedy (1996) suggest that stems are more brittle than leaves of both grass and legumes, causing stem particles to break down more easily during mastication. Ulyatt (1983) suggests that brittleness could be caused by greater lignification of the cell wall, increasing with forage plant maturity. Both suggestions could partly explain the most distinct reduction in particle size during initial mastication in the S4 silage where 56% of the DM in the fresh crop was stem and 13% of the NDF was indigestible.

The reductions in LP proportions from ingested feed boluses to ruminal content were much more distinct compared with reductions in LP proportions from forage to ingested feed boluses. Considering mean LP proportions of the medial and ventral rumen contents, the reduction in the LP fraction from ingested feed boluses to ruminal digesta averaged 53% points. Hence, ruminating activity and fermentation accounted for more particle size reduction compared with chewing during feed intake where the LP proportion was reduced by only 4% points on average.

Silage particles have certain characteristics dependent on forage species, stem vs. leaf origin, and conservation form. These affect reduction in size from mastication and fermentation. The particles rich in NDF are mainly of vascular tissue origin from both leaf and stem in grasses and mainly stem in clover (Wilson and Kennedy, 1996). Fermentation alone can reduce the width of grass leaf particles to the width of the vascular strands, whereas mastication is needed to reduce length of leaf particles and both length and width of grass stem particles (Wilson et al., 1989; Akin, 1989). In clover, leaf particles are highly fermentable, but stem particles are more lignified and break into chunky particles during mastication (Kornfelt et al., 2013). The rumen digesta in this study therefore consisted of slim particles of varying length from grass leaves and chunky particles of clover stem origin. The vertical dry-sieving technique allows passage of particles longer than the aperture size of the sieves and to some degree it sorts particles according to their width, as discussed by Faichney (1986). Because chewing activity per kg NDFI was similar among treatments, the quadratic effect on LP and SP in ruminal contents may have been caused by long and slim grass leaf particles (from especially the S2 and S3 silages) passing the larger sieves and becoming part of the MP and SP fractions.

Ruminal Stratification

Stratification of the ruminal content was present with respect to DM and PDM, with clearly greater DM and PDM contents found in the medial rumen. The DM difference in the rumen is inevitable because of the effect of gravity on liquids. However, the proportion of PDM in wet digesta was twice as high in the medial rumen content compared with the ventral rumen content,

Table 6. Feces composition, particulate DM distribution and apparent digestibility as affected by treatment and NDF concentration

	Treatment[1]					*P*-value[3]		
Item	S1	S2	S3	S4	SEM[2]	Treatment	L	Q
Fecal DM, g/kg	195	190	181	180	4	0.07	0.03	0.35
Fecal PDM[4], g/kg DM	240[a]	293[ab]	342[b]	480[c]	16	<0.001	<0.001	0.38
iNDF, g/kg PDM[5]	316[a]	441[b]	582[c]	742[d]	36	<0.001	<0.001	0.39
Particle proportion, % of PDM								
Large (>1.0 mm)	1.1	1.4	1.0	1.7	0.2	0.20	0.09	0.56
Medium (0.212 to 1.0 mm)	30.7[a]	38.1[b]	33.3[ab]	44.0[c]	2.5	0.02	0.004	0.79
Small (< 0.212 mm)	68.3[a]	60.5[bc]	65.6[ab]	54.2[c]	2.4	0.008	0.002	0.78
GPS[6], mm	0.15[a]	0.18[bc]	0.16[ab]	0.19[c]	0.01	0.002	<0.001	0.40
Apparent digestibility[7], %								
OM	83.3[a]	79.4[b]	75.8[c]	76.6[c]	0.7	0.002	0.001	0.006
NDF	89.0[a]	87.3[a]	82.3[b]	80.3[b]	0.7	<0.001	<0.001	0.24
DNDF	96.4[ab]	96.8[a]	93.7[bc]	92.5[c]	0.7	0.02	0.009	0.74

[a–d]Within a row, means without a common superscript differ ($P < 0.05$).

[1]Treatment silage harvests were: S1 (May 9, spring growth), S2 (August 7, third regrowth), S3 (August 23, third regrowth), and S4 (June 22, first regrowth).

[2]$n = 4$ per treatment.

[3]Probability of treatment effect and linear (L) and quadratic (Q) contrast effects of the NDF content of the silages.

[4]Particulate DM (PDM) is DM residue after washing feces/DM in fresh feces × 100.

[5]Indigestible NDF (iNDF) content of fecal PDM. Derived from excreted fecal DM, estimated from the chromic oxide (Cr_2O_3) marker under the assumption that daily iNDF output in feces = daily iNDF intake.

[6]GPS = geometric mean particle size.

[7]Determined using the Cr_2O_3 digestibility marker.

indicating the presence of a fibrous mat in the medial rumen. The PDM created a more densely packed mat in the medial rumen compared with the ventral rumen holding most of the liquid, as was also found in previous studies on ruminal stratification (Evans et al., 1973; Tafaj et al., 2004; Hummel et al., 2009) where the dorsal or medial rumen contained more DM and NDF compared with the ventral rumen.

Forage particles float when they are buoyant and of low density and sediment when they increase in density and reduce in size (Wilson and Kennedy, 1996). Particle buoyancy is thought to depend on the content of DNDF of a particle, because this fraction of NDF will contribute to the release of fermentation gas during microbial digestion (Jung and Allen, 1995). Further, the degradation rate of DNDF limits the time length when gas is released and therefore influences buoyancy time (Allen, 1996). The general high potential digestibility of NDF found for all silages must have resulted in great amounts of fermentation gas released over a short period of time, likely contributing to particle buoyancy and mat formation. A distinct release of fermentation gas from the mat of the S1, S2, and S3 silages was noticed when a hand was introduced to the mat surface, as if large amounts of fermentation gas were trapped between the long, fine particles as in thick foam. The intake of DNDF was similar among silages, though large variation was found in the potential digestibility of NDF of the silages. At the same time, the rate of DNDF degradation decreased with greater NDF content of the silages; the S1 silage was potentially degraded twice as fast as S4 silage. The fermentation gas produced from the low NDF silages was potentially released faster, thus in theory keeping the associated particles buoyant for a shorter time period. The tendency for more DM and significantly more PDM in the medial rumen with greater NDF content could be a consequence of a slower release of fermentation gas.

Ruminal Distribution and Retention of Particles

Despite a significant PDM stratification of the rumen, the PDM was more or less of equal size distribution in medial and ventral ruminal content within and between treatments, indicating that the position of particles in the rumen was not dependent on particle size. Similar results were found by Tafaj et al. (2004) in cows fed hay (471 and 619 g/kg DM of NDF) and by Ahvenjärvi et al. (2001) in cows fed grass silage (623 g/kg DM of NDF). Both studies showed similar mean particle sizes in the medial and ventral ruminal contents. Other studies found differences in the particle size distributions of the mat and the liquid layer. In a comprehensive study by Sutherland (1988) of sheep fed alfalfa, the dorsal rumen contained a larger proportion of LP > 1.0 mm compared with the ventral rumen until 12 h after feeding. Evans et al. (1973) fed cows hay of 380 g/kg DM of NDF and found a clearly greater proportion of LP > 1.0 mm up to 7.5 h postfeeding, whereas the proportion of small particles < 1.0 mm was more or

less similar over 24 h. Hummel et al. (2009) fed grass and lucerne forages to oxen and found that particle size was generally higher in the dorsal rumen when compared with the ventral rumen, though for grass hay, the ventral rumen contained more LP 3 and 6 h after feeding. In summary, different particle size distributions between the mat and the ventral rumen do not seem to be related to NDF content in these studies, but rather to time postfeeding and forage species specific characteristics.

Besides the amount and rate of fermentation gas release, the buoyancy of particles is dependent on the ability of the particles to retain gas in void spaces. Sutherland (1988) has shown that stem particles predominate in the ruminal mat, most likely because of their cylindrical shape and ability to retain fermentation gas for a prolonged time period. This ability is related to the NDF structure and lignification and has been found to be greater in stems compared with leaves (Sutherland, 1988) and with increasing plant maturity due to increasing lignification of the stem (Akin, 1989). It could therefore be assumed that the quadratic effect on proportion of LP in the medial rumen mat was mainly due to a markedly greater stem proportion of the S1 and S4 silages and a greater NDF content of the S4 silage.

When observing the consistency and appearance of the ruminal content through the ruminal fistula and during ruminal evacuations, great differences were found between the 4 silages. The medial ruminal mat formed by the S1 silage, with the lowest NDF content, was rather soft and spongy, with long, thin particles entangling together. By contrast, the mat from the S4 silage seemed to contain more coarse particles. The ruminal fluid associated with the particles seemed to be of greater viscosity in S1 compared with S4 and hence ruminal fluid from S4 appeared to move more freely through the solid particle mat during ruminal contractions. The observed differences in mat consistency and appearance could be related to the quadratic effect of NDF content observed for proportions of SP and LP in both the medial and the ventral ruminal contents. The LP fraction was almost similar between S1 and S4 silages and a little less in the intermediate silages. Small particles < 0.212 mm in both the ventral and the medial rumen comprised proportions of PDM corresponding to the proportion of leaves in the fresh crop. Wilson et al. (1989) found the width of a vascular strand from the leaves of ryegrass to be 0.04 to 0.1 mm. This could easily pass a 0.212-mm sieve which was the upper limit size for the SP sieving fraction. However, stem or leaf origin of the digesta particles was not assessed in this study, and assuming that the SP fraction was mainly of leaf origin would only be speculative.

The similarity in proportions of SP in the medial and ventral rumen may be due to a combination of the properties of the small particles and particle–particle interactions occurring regardless of NDF content of the silages. Faichney (1986) suggests that the entangled particles of the ruminal mat can to some extent entrap small particles that would otherwise be free to sediment and move to the reticulum for passage to the omasum by a filter-bed effect. Related to this, Hummel et al. (2009) discuss how sedimenting particles were prevented from dropping to the ventral rumen by buoyant particles, which to some extent were also present in the ventral rumen. Poppi et al. (2001) found that small particles generated in the ruminal mat have difficulty escaping, but once they enter the ventral pool they have high probability of passing from the rumen and do not reenter the mat. Considering these findings, the long and slim shape of digesta particles from the grass forage, as discussed earlier, could predispose them to entanglement in the ruminal digesta mat.

The total tract digestibility of NDF and DNDF linearly decreased with greater NDF content of the silages, which corresponded well to the higher proportion of PDM in feces containing increasing amounts of iNDF. However, greater digestibility was not related to less LP in feces. In this study, >30% of particles in the ventral rumen were >1 mm, whereas <2% of feces particles were >1 mm for all silage treatments. Because practically no further particle size reduction occurs after particles leave the rumen (Ahvenjärvi et al., 2001), other mechanisms besides buoyancy of particles and ruminal mat entrapment must have contributed to the retention of LP in the rumen.

Conclusions

Feeding high digestibility silages of increasing NDF content did not systematically affect particle size distribution from forage through the digestive tract to feces. Despite similar particle size distributions of the medial and ventral rumen digesta, very few particles > 1 mm escaped the rumen, indicating that the retention mechanism of LP lies elsewhere than with entrapment of particles in the ruminal mat. In this study of high digestibility grass/clover silages, it was clear that forage particle size reduction and distribution of particles in the medial and ventral rumen were not affected by NDF content of the silages and neither was retention of LP in the rumen.

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