



# Chewing and ruminating with various forage qualities in nonlactating dairy cows

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## ABSTRACT

*This study investigated Italian typical dry forage diets used in the Parmigiano Reggiano PDO (protected designation of origin) area of Italy. The first cutting is typically a mixture of alfalfa and native wild grasses, resulting in a unique forage nutrient content. Alfalfa makes up less than 10% in the first cut because herbicides are not commonly used. Wild grasses are predominant in such conditions, whereas in the subsequent cuts alfalfa content increases as grasses decrease because of agronomical and climatic characteristics. Six multiparous, nonlactating Holstein cows were used in a replicated 3 × 3 Latin square to evaluate 3 different cuttings of alfalfa hay fed as the sole diet source. Eating and ruminating behavior were studied to investigate forage properties related to chewing activity. No differences were found in eating time; however, ruminating time per kilogram of physically effective NDF was greater when cows were fed first-cutting alfalfa than when they were fed the second or fifth cutting, despite similar digestibility and diet particle size of first- and fifth-cutting forage. In vivo digestibility of the diets revealed higher fecal NDF, ADF, and DM digestibility*

*for first- and fifth-cutting hay. A similar trend was observed with the results of the in vitro data, with reduced digestibility for second-cutting forage.*

**Key words:** eating behavior, forage digestibility, forage quality, ruminating

## INTRODUCTION

Dairy cows require a minimum amount of dietary fiber with sufficient particle size to enhance digesta stratification in the reticulorumen. Chewing is strictly related to saliva production, which contains bicarbonate able to buffer rumen pH, preventing digestive disorders such as subacute ruminal acidosis (Owens et al., 1998; NRC, 2001). Mertens introduced the concept of physically effective NDF (peNDF) to better describe the fraction of dietary fiber implicated in the control of the subacute ruminal acidosis (Mertens, 1997).

Studies have shown that increasing peNDF increases chewing activity and ruminal pH (Krause et al., 2003), improves fiber digestion (Yang and Beauchemin, 2007b), and improves total digestibility (Kononoff and Heinrichs, 2003). A relationship between peNDF and milk fat content has also been reported (Yang et al., 2001;

Kononoff and Heinrichs, 2003). Some studies have demonstrated increased chewing activity as a result of increased peNDF intake (Beauchemin, 1991; Krause et al., 2002; Yang and Beauchemin, 2007a), yet increased peNDF intake has shown less correlation to chewing in other studies (Kononoff et al., 2003b).

Several methods could provide an increase of peNDF in the diet and thus a possible increase in chewing activity. Total time spent chewing has been shown to increase as dietary NDF concentration increases (Beauchemin, 1991). This could be a useful mechanism to increase total chewing and thus alter rumen pH and milk fat production in diets with finely chopped forage. In the diets of cows producing milk for Parmigiano Reggiano cheese, only dry hay can be fed as forage; thus, making a TMR requires chopping the hay. The challenge in this situation is achieving a blended diet that also provides enough peNDF to allow adequate chewing and rumination. Therefore the objective of this study was to evaluate effects on eating, rumination, and apparent total-tract digestibility of 3 different chopped alfalfa hays, fed in a fixed amount to dry cows.

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## MATERIALS AND METHODS

This experiment was conducted at the Faculty of Veterinary Medicine of the University of Bologna following approval by the university animal care and use committee. This study was designed to investigate Italian typical dry forage diets used in the Parmigiano Reggiano PDO (protected designation of origin) area of Italy, using the typical forage management system. Forages were all harvested from the same small farm in Medicina (near Bologna, in northern Italy) in 2008: first cut on April 27, second on June 9, and fifth on September 20 (27 d after the fourth). The first cutting is typically a mixture of alfalfa and native wild grasses, resulting in a unique forage nutrient content. Alfalfa makes up less than 10% in the first cut because herbicides are not commonly used in this region of the coun-

try. Wild grasses are predominant in such conditions during the early rainy season, whereas in the subsequent cuttings alfalfa content increases as grasses decrease because of hot and dry climatic characteristics of summer. Forages were harvested and then dried in the field, and the moisture was approximately 10% before baling (New Holland BR 7060, New Holland, PA). Hays were chopped using a Zago TMR mixer (Padova, Italy) and offered twice a day at 10 kg/d (approximately 90% of ad libitum intake based on 1-wk pretrial measurements). Six nonlactating cows were housed in a naturally ventilated tie stall facility using a replicated 3 × 3 Latin square design with 18-d periods with 14 d of adaptation. The cows were nonlactating Holsteins averaging 719 ± 66 kg of BW and 46 ± 8 mo of age. Cows were only fed the forage in question during the study,

covering the maintenance requirement without gaining BW according to NRC requirements (NRC, 2001). Cows were also allowed free-choice water. Eating and ruminating activities were recorded on d 14 through 18 of each period (two 24-h periods/cow) using the Institute of Grassland and Environmental Research Behavior Recorders and Graze Jaw Movement Analysis Software (Ultra Sound Advice, London, UK) and validated for use in this type of situation (Kononoff et al., 2002). Total feces were collected on d 16 and 17 of each period. Feed samples were collected daily and composited for analysis; there were no refusals. Samples were then placed in a forced-air oven at 65°C for 48 h to determine DM. Samples were ground through a 1-mm-screen Wiley Mill (Arthur H. Thomas, Swedesboro, NJ) and analyzed for residue DM, ash (AOAC, 1990), and ADF and

**Table 1. Nutrient composition and particle size analysis of alfalfa hay used in the study**

Item	1st Cutting		2nd Cutting		5th Cutting	
	Mean	SD	Mean	SD	Mean	SD
DM, %	90.95	0.04	90.52	0.48	91.60	0.20
CP, % DM	15.56	0.06	13.90	0.20	22.26	0.09
Ash, % DM	9.45	0.11	7.91	0.12	9.19	0.19
NDF, % DM	47.85	0.67	52.66	3.61	41.62	1.53
ADF, % DM	33.89	0.83	38.82	3.51	32.12	1.63
ADL, % DM	6.29	0.07	8.42	0.65	6.70	0.54
Ca, % DM	1.40	0.06	1.70	0.07	1.43	0.04
P, % DM	0.31	0.03	0.18	0.02	0.37	0.05
24-h NDF digestibility, %	50.44	0.52	39.00	0.43	48.38	0.56
Particles >1.18 mm, <sup>1</sup> % DM	43.75	4.32	58.25	3.19	49.73	2.29
NDF, % DM	58.25	0.25	57.56	0.35	60.16	0.14
ADF, % DM	41.65	0.37	45.36	0.37	48.45	0.48
ADL, % DM	8.32	0.28	10.31	0.15	10.79	0.35
24-h NDF digestibility, %	43.05	0.32	35.00	0.26	35.03	0.16
K <sub>d</sub> <sup>2</sup>	6.63	0.06	5.20	0.12	7.47	0.08
Particle size analysis <sup>3</sup>						
>19 mm, %	14.9	4.7	24.1	7.3	12.5	7.2
19 to 8 mm, %	28.5	3.2	24.0	2.2	26.1	5.3
8 to 1.18 mm, %	30.9	2.4	33.2	2.2	28.3	1.7
<1.18 mm, %	25.7	2.5	18.7	5.3	33.0	12.0
Mean particle size, mm	4.9	3.5	6.3	3.4	4.3	3.5
% >1.18 mm	74	—	81	—	67	—
% >8 mm	43	—	44	—	39	—

<sup>1</sup>Particles >1.18 mm determined by Ro-Tap separator.

<sup>2</sup>Digestibility rate.

<sup>3</sup>Determined by Penn State Particle Separator, DM basis.

NDF (Van Soest et al., 1991) using a Fibertek Extractor Analyzer (Tecator, Skåne län, Sweden) with heat-stable  $\alpha$ -amylase for the NDF procedure. Crude protein was analyzed using the Kjeldahl method (AOAC, 1990). Particle size was determined using the modified Penn State Particle Separator (Kononoff et al., 2003a) and the Ro-Tap separator (American Society of Agricultural Engineers, 1993) to determine peNDF as defined by Mertens (1997). In vitro DM digestibility was determined by modified Tilly and Terry method (Van Soest, 1965[AU1: Add to Refs.]).

Statistical analysis was conducted using PROC MIXED of SAS (SAS Institute Inc., 2006). Repeated measurements for rumen sample particle size were analyzed using the first-order autoregressive covariance structure,

as well as terms for time and interaction of treatment by time. All data are presented as least squares means, and treatment effects are considered significant when  $P < 0.05$  and a trend when  $P < 0.10$ .

## RESULTS AND DISCUSSION

Nutrient composition and particle size data for forages are presented in Table 1. These forages were typical of alfalfa hay grown in northern Italy and represent the result of typical forage management systems used in this region of the country. The first cutting typically has lower lignin because of grass inclusion in the forage and thus greater digestibility. Because of the hot, dry environment without irrigation, grass is a minor component of subsequent cuttings. Second- and

subsequent-cutting forages from this region typically have more NDF and lignin, less protein, and lower digestibility.

All the cows ate the total forage provided because it was restricted to 10 kg to avoid intake variability. The 3 cuttings of hay were prepared to have particles no longer than 4 cm to approximate the typical length included in Parmigiano Reggiano TMR. All forages had similar values for NDF in the fractions  $>1.18$  mm from the Ro-Tap samples. Using the peNDF 1.18 values from the Ro-Tap separator, all forages provided adequate effective fiber as described by Mertens (1997); peNDF was 25.5, 33.6, and 29.9% for first, second, and fifth cuttings, respectively. The Penn State Particle Separator values were in the normal range as would

**Table 2. Meal patterns of dairy cows eating diets containing 3 different cuttings of alfalfa hay**

Item	Cutting			SE	P-value	Contrast <sup>1</sup>		
	1st	2nd	5th			1 vs. 2	1 vs. 5	2 vs. 5
<b>Eating</b>								
Total time, min/d	220.5	261.7	235.5	21.0	0.42	0.63	0.20	0.40
Time/kg of NDF, min/d	46.1	49.7	56.6	4.2	0.25	0.11	0.56	0.28
Time/kg of NDF $>1.18$ , <sup>2</sup> min/d	89.6	81.1	81.9	6.9	0.64	0.45	0.41	0.94
Bouts/d per kg of DMI	1,343.7	1,691.7	1,447.9	136.0	0.24	0.60	0.11	0.24
Bouts/d per kg of NDF	2,808.7	3,212.7	3,479.7	262.4	0.25	0.11	0.31	0.49
Bouts/d per kg of NDF $>1.18$ <sup>2</sup>	5,461.4	5,244.9	5,032.6	432.3	0.79	0.50	0.73	0.74
<b>Ruminating</b>								
Total time, min/d	334.8	358.7	308.0	16.8	0.17	0.29	0.35	0.07
Time/kg of NDF, min/d	70.0	68.1	74.0	3.5	0.50	0.43	0.71	0.26
Time/kg of NDF $>1.18$ , <sup>2</sup> min/d	136.1	111.2	107.1	6.3	0.02	0.01	0.02	0.65
Bouts/d per kg of DMI	1,950.2	2,165.3	1,831.0	117.1	0.19	0.49	0.23	0.08
Bouts/d per kg of NDF	4,076.5	4,112.0	4,400.5	240.6	0.60	0.37	0.92	0.42
Bouts/d per kg of NDF $>1.18$ <sup>2</sup>	7,926.6	6,713.2	6,364.3	417.2	0.07	0.03	0.07	0.57
<b>Boli</b>								
Total boli/d	351.0	381.0	326.8	17.4	0.15	0.35	0.26	0.06
Boli/kg of NDF	73.4	72.4	78.5	3.5	0.45	0.33	0.84	0.25
Boli/kg of NDF $>1.18$ <sup>2</sup>	142.7	118.1	113.6	6.3	0.02	0.01	0.03	0.63
<b>Total chewing time</b>								
Total time, min/d	555.3	620.3	543.5	33.1	0.27	0.81	0.20	0.14
Chewing/kg of NDF, min/d	116.1	117.8	130.6	6.5	0.28	0.15	0.86	0.20
Chewing/kg of NDF $>1.18$ , <sup>2</sup> min/d	225.7	192.3	188.9	11.4	0.10	0.05	0.07	0.84
<b>Total chewing bouts</b>								
Bouts/d per kg of DMI	3,293	3,857	3,278	228.0	0.19	0.96	0.12	0.11
Bouts/d per kg of NDF	6,885	7,324	7,880	441.0	0.33	0.15	0.50	0.40
Bouts/d per kg of NDF $>1.18$ <sup>2</sup>	13,388	11,958	11,397	756.3	0.22	0.10	0.22	0.61

<sup>1</sup>Contrasts comparing first (1), second (2), and fifth (5) cutting.

<sup>2</sup>Particles  $>1.18$  mm determined by Ro-Tap separator.

**Table 3. Least squares means of fecal composition and apparent digestibility of diets containing 3 different cuttings of alfalfa hay**

Item	Cutting			SE	P-value	Contrast <sup>1</sup>		
	1st	2nd	5th			1 vs. 2	1 vs. 5	2 vs. 5
Fecal NDF, kg/d	2.42	2.94	2.19	0.090	<0.01	0.01	0.12	<0.01
Fecal ADF, kg/d	1.83	2.30	1.72	0.054	<0.01	<0.01	0.20	<0.01
DMd, <sup>2</sup> %	63.57	58.73	67.23	1.40	0.01	0.04	0.10	<0.01
NDFd, <sup>2</sup> %	49.37	44.22	47.36	2.02	0.25	0.11	0.50	0.30
ADFd, <sup>2</sup> %	45.83	40.81	46.18	1.56	0.07	0.05	0.88	0.04

<sup>1</sup>Contrasts comparing first (1), second (2), and fifth (5) cutting.

<sup>2</sup>d = apparent digestibility.

be observed for US forages of similar types (Heinrichs et al., 1999), except for more <1.18 mm, which reflected the high degree of leaf shatter in these dry alfalfa hays. The 24-h NDF in vitro digestibility was lowest for the second-cutting forage, likely because of the higher lignin component of the alfalfa. The fifth cutting likely had a greater leaf-to-stem ratio, which was reflected in the amount of finely ground material (<1.18 mm) in this forage and could have improved plant digestibility in the fifth-cutting forage. The first cutting in the Parmigiano Reggiano region of Italy is typically a mixture of alfalfa with native wild grass (typically native *loium* and oat). Alfalfa is almost absent in the first cut because in Italy they typically do not use herbicide, and in the subsequent cut the alfalfa becomes the predominant species.

The chewing (eating and ruminating) data in Table 2 compare the 3 forages when fed as a fixed amount of DM to the nonlactating Holstein cows. There were no differences in any measures of eating patterns for the 3 forages. The first cutting did stimulate greater rumination per unit of peNDF (Mertens, 1997), resulting in 136 min/d versus 111 and 107 min/d for second and fifth cuttings, respectively. Total chewing bouts per kilogram of NDF >1.18 mm also had a tendency to be increased for first-cutting hay. This was also reflected in the daily number of boli per kilogram of NDF >1.18 mm ruminated, which

was significantly influenced by diet; first cutting was highest, with no difference between second and fifth cutting. In vivo digestibility of the diets (Table 3) revealed higher fecal NDF, ADF, and DM digestibility for first- and fifth-cutting hay. A similar trend was observed with the results of the in vitro data, with reduced digestibility for second-cutting forage compared with either of the other forages likely because of the increase in lignin content of that hay.

The differences in plant species and forage analysis of the first cutting likely affected the rumination results. Forage type has been indicated (Zebeli et al., 2008) as a confounding factor in characterizing effects of peNDF on rumen fermentation. In the current study, both highly digestible and poorly digestible alfalfa hay allowed cows to chew and ruminate for similar amounts of time and to ruminate for less time than first-cutting forage with different plant constituency. In summary, this study found no differences in eating time for the 3 hays fed. Ruminating time was not related to overall nutrient composition or digestibility of the hay but varied by cutting, with first-cutting hay promoting more rumination, likely because of differences in forage nutrient content.

## IMPLICATIONS

This study was done to investigate typical dry hay diets used in

the Parmigiano Reggiano area of northern Italy. These forages come from the same fields but because of agronomic practices and climate have some grass in the first crop and only alfalfa in subsequent cuttings. In addition, the hot, dry summer climate tends to result in high lignin levels in alfalfa plants. Eating and ruminating behavior were studied in nonlactating cows to investigate forage properties related to chewing activity. The study found no differences in eating time for 3 different cuttings of hays fed. Ruminating time was not related to overall nutrient composition or digestibility of the hay but varied by cutting, with first-cutting hay promoting more rumination, likely because of differences in forage composition of the hay. Considering these characteristics, it is important for farmers to choose an appropriate allocation of the forages to maintain consistency in ration composition and maximize cow performance.

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