Short communication: Feeding red clover cut in the afternoon or morning to late-lactation dairy cows

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ABSTRACT

Forages cut in the afternoon (p.m.) generally yield a higher concentration of nonstructural carbohydrates (NSC) than those cut in the morning (a.m.). We aimed to compare the effects of p.m.-cut red clover baleage (p.m.-RC) versus a.m.-cut RC baleage (a.m.-RC) on milk yield, concentrations and yields of milk components, and apparent total-tract digestibility of nutrients in late-lactation Holstein cows. Twelve multiparous and 2 primiparous Holstein cows received a total mixed ration containing, on a dry matter (DM) basis, 65% p.m.-RC or a.m.-RC plus 35% concentrate in a cross-over design with 14 d for diet adaptation and 7 d for data and sample collection. One RC field was split in 2 with the first half cut in the afternoon (1600 h) and the second half in the following morning (0600 h). The p.m.-RC and a.m.-RC contained (% of DM): 12.6 versus 9.43% NSC in samples collected before the beginning of the experiment and 7.49 versus 7.79% NSC in samples collected during the sampling periods (i.e., d 14 to 21). The total mixed rations averaged 18.2 and 17.5% NSC for the p.m.-RC and a.m.-RC, respectively. Feeding p.m.-RC or a.m.-RC did not improve feed intake or milk yield and composition in late-lactation dairy cows. However, milk urea N and plasma urea N were both lowest in cows offered p.m.-RC. With the exception of the apparent total-tract digestibility of DM, which was highest in cows fed p.m.-RC, no other changes in nutrient digestibility were observed. Similarly, no treatment effect was observed for the urinary excretion of N and purine derivatives. Further research is needed to better understand NSC losses during storage and the associated effects on baleage quality and animal performance.

Key words: baleage, dairy cow, diurnal cutting management, red clover

Short Communication

In general, forage sources are rich in RDP but poor in NSC [defined herein as total ethanol-soluble carbohydrates (**TESC**) or water-soluble carbohydrates plus starch] leading to unbalanced supplies of NH₃ and fermentable energy in the rumen. Plant C fixation occurs at a greater rate than C exportation during daylight resulting in accumulation of NSC in tissues of grass and legume species as the day progresses (Bowden et al., 1968; Lechtenberg et al., 1971; Gordon, 1996). Pelletier et al. (2010a) compared the NSC concentration of 8 cool-season forage crops cut at a.m. versus p.m. and reported that red clover (**RC**; *Trifolium pratense* L.) and tall fescue [*Schedonorus phoenix* (Scop.) Holub] showed the greatest concentrations of NSC across cutting times and growth periods. We reported previously that p.m.-cut alfalfa (*Medicago sativa* L.) baleage fed as the sole feed source to late-lactation Holstein cows significantly increased milk yield, milk N efficiency, and omasal flow of microbial NAN compared with a.m.-cut alfalfa baleage (Brito et al., 2008, 2009). Although research-based information about the use of alfalfa with enhanced concentration of NSC offered as baleage (Brito et al., 2008, 2009, 2014) or hay (Yari et al., 2014) to lactating dairy cows is growing, we are not aware of studies in which the effects of p.m.-cut RC baleage on milk yield and nutrient utilization have been investigated. We hypothesized that, compared with a TMR containing a.m.-cut RC baleage (**a.m.-RC**), a TMR with p.m.-cut RC baleage (**p.m.-RC**) would improve milk yield and N utilization in late-lactation dairy cows due to the enhanced supply of ruminal fermentable energy. The objectives of this study were to compare the effects of daytime cutting management (p.m.-RC versus a.m.-RC baleage) on milk yield, concentrations and yields of milk components, and apparent total-tract digestibilities of nutrients in late-lactation Holstein cows.

Care and handling of the animals used in the current study were conducted as outlined in the guidelines of the University of New Hampshire Institutional Animal Care and Use Committee (Institutional Animal Care

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and Use Committee protocol no. 100702). The 42-d-long experiment was conducted at the University of New Hampshire Fairchild Dairy Teaching and Research Center (Durham, NH; 43°14'N, 70°95'W) from March 9 to April 18, 2011. Cows were housed in a naturally ventilated tie stall barn and had access to water throughout the experiment.

The red clover (second cutting at 50% flowering stage) used in the current study was grown in 1 field [4 ha; 75% red clover stand and 25% others (i.e., grass and weeds)] at the Agriculture and Agri-Food Canada, Dairy and Swine Research and Development Centre located in Sherbrooke, QC, Canada (45°24'N, 71°54'W). The first summer regrowth of half of the field (74% RC herbage) was cut in the afternoon on July 26, 2010 (1600 h; p.m.-RC), which was a sunny day (maximum, minimum, and mean temperature of 24.4, 15.1, and 19.8°C, respectively) with a global radiation of 24 MJ/m². Forage was raked and baled at 1300 and 1715 h, respectively, on July 27, 2010. The second half of the field (75.2% RC herbage) was cut on July 27, 2010, in the morning (0600 h; a.m.-RC), and raked and baled at 1700 and 1745 h, respectively, on the same day. The concentrations of NSC in the RC herbage averaged 9.40% (p.m.-RC) and 7.22% (a.m.-RC) at cutting, and 9.09% (p.m.-RC) and 7.94% (a.m.-RC) at baling. A total of 53 bales (27 p.m. and 26 a.m. = 12,900 kg of DM) were made and later transported to the University of New Hampshire. The time elapsed between red clover baling and feeding (d 1 of the study) was 267 d. The ranking and paring procedures, as well as the sampling methodology used for RC bales, were similar to those reported for alfalfa bales (Brito et al., 2008, 2014). Bales fed during the sampling period (i.e., d 14 to 21) period were chopped before feeding with representative samples collected and processed according to Pelletier et al. (2010b). In brief, samples were pretreated (i.e., heated in a microwave oven for 1 min at maximum intensity to reach approximately 70°C) and then oven-dried at 55°C for 48 h before TESC and starch analyses.

Twelve multiparous and 2 primiparous Holstein cows averaging (mean ± SD) 238 ± 23.3 DIM and 693 ± 67.1 kg of BW at the beginning of the experiment were blocked by DIM, parity, and milk yield. Within each block, cows were randomly assigned to 1 out of 2 TMR (65:35 forage-to-concentrate ratio) containing either p.m.-RC or a.m.-RC baleage in a crossover design. Each experimental period lasted 21 d with 14 d for diet adaptation and 7 d for data and sample collection. The TMR was offered to cows individually in tie stalls twice a day (0500 and 1400 h) with the amount of feed adjusted daily to yield refusals equal to approximately 5 to 10% of intake. Cows were milked twice daily (0430

and 1600 h) with milk yield recorded throughout the duration of the study. Body weight was recorded after the afternoon milking for 3 consecutive days at the beginning of the experiment and during the last 3 d of each period to compute ADG.

During each sampling period, samples of TMR and refusals were collected daily, pooled by period, and stored at -20°C until analyses. Samples were then thawed at room temperature, dried (55°C, 48 h; forced-air oven), and ground to pass through a 1-mm screen (Wiley mill, Arthur H. Thomas, Philadelphia, PA). Samples of TMR and baleage were analyzed for absolute DM (105°C), ash, total N, NDF, ADF, and lignin following the methods reported by Brito et al. (2015), starch and TESC (Brito et al., 2014), and indigestible ADF (Brito and Broderick, 2006). In addition, TMR was analyzed for crude fat, NDIN, and ADIN (Brito et al., 2015).

Milk samples were collected for 4 consecutive milkings (d 18, 19, and 20) beginning in the afternoon milking of d 18 and were preserved, pooled, and analyzed for fat, protein, lactose, and MUN according to Brito et al. (2015). Blood samples were collected for 2 consecutive days (d 20 and 21) approximately 5 h after the morning feeding and processed and analyzed for plasma urea N (**PUN**) as described elsewhere (Brito et al., 2015). Fecal grab samples and spot urinary samples were collected for 4 consecutive days (d 15 to 18) at approximately 6-h intervals, yielding a total of 12 sampling points. Feces and urine were processed following the methodology reported previously (Brito et al., 2015). Pooled fecal samples were analyzed for DM, ash, total N, NDF, ADF, and indigestible ADF according to the methods reported earlier (Brito and Broderick, 2006; Brito et al., 2015). Pooled urine samples were analyzed for creatinine and urea N using colorimetric methods (Brito et al., 2015), and for uric acid and allantoin by HPLC (Beckman Instruments, San Ramon, CA; Balcells et al., 1992). Fecal output of DM (indigestible ADF as the internal marker) was determined according to Cochran et al. (1986). Urinary volume was estimated assuming a constant creatinine excretion rate of 29 mg/kg of BW (Valadares et al., 1999).

Data were analyzed using the MIXED procedure of SAS (version 9.4, SAS Institute, Inc., Cary, NC) according to a crossover design. The following model was fitted for all variables:

$$Y_{ijkl} = \mu + S_i + C_j(S)_i + P_k + T_l + E_{ijkl},$$

where Y_{ijkl} = dependent variable, μ = overall mean, S_i = mean effect of the i th crossover sequence group, $C_j(S)_i$ = mean effect of j th cow nested within i th sequence, P_k = mean effect of k th period, T_l = mean effect

Table 1. Nutrient composition (% of DM, unless otherwise noted) of p.m.- and a.m.-cut red clover baleage collected 267 d before d 1 of the study (n = 7/treatment) and on d 14 to 21 of the sampling periods (n = 7/treatment)

Item	Red clover baleage (before study)		Red clover baleage (during study)	
	p.m.-cut	a.m.-cut	p.m.-cut	a.m.-cut
DM, % of fresh matter	47.5	45.4	42.9	42.2
OM	NA ¹	NA	89.2	90.0
CP	NA	NA	18.6	19.0
NDF	NA	NA	39.7	41.3
ADF	NA	NA	30.3	31.4
TESC ²	11.8	8.49	7.09	7.20
Starch	0.88	0.94	0.39	0.59
NSC (TESC plus starch)	12.6	9.43	7.49	7.79
Lignin	NA	NA	5.17	5.13

¹NA = not analyzed.²TESC = total ethanol-soluble carbohydrates.

lth treatment, and E_{ijkl} = random residual variation. All terms were considered fixed except $C_j(S)_i$ and E_{ijkl} , which were considered random. All reported values are least squares means. Differences between least squares means were reported only if the *F*-test for treatment was significant at $P \leq 0.05$. Trends were declared at $0.05 < P \leq 0.10$.

The RC baleage and TMR nutrient compositions are presented in Tables 1 and 2, respectively. The concentrations of TESC (+28.1%) and NSC (+25.2%) were substantially higher, whereas starch values did not change in samples of p.m.-RC versus a.m.-RC baleage collected before the beginning of the experiment (Table 1). The concentrations of TESC (mean = 7.15%) and NSC (mean = 7.64%) were similar between the 2 baleage sources, but the starch concentration was much lower (−51.3%) in the p.m.-RC compared with the a.m.-RC baleage during the sampling periods (i.e., d 14 to 21; Table 1). Owens et al. (2002) observed that the concentration of NSC in fresh RC and unwilted RC silage increased when cutting time was delayed from a.m. to p.m., but they also reported substantial losses in the concentration of NSC during ensiling. Whereas in the study of Owens et al. (2002) the concentration of NSC was highest in the p.m.-cut silages, Tremblay et al. (2014) observed that the NSC concentration in alfalfa silage did not differ across cutting times in most growth cycles. Compared with Owens et al. (2002), it is conceivable that the longer storage (current study) or ensiling time (Tremblay et al., 2014) resulted in more pronounced losses of NSC narrowing and eliminating differences between cutting times. In addition to storage time, RC baleage used herein was chopped before being incorporated into the TMR. According to McDonald et al. (1991), chopping increases the availability of water-soluble carbohydrates, thus enhancing fermentation.

Intake of DM did not differ significantly in cows fed p.m.-RC or a.m.-RC and averaged 20.8 kg/d (Table 3). As a result, milk yield, concentrations and yields of milk components (except MUN), and feed efficiency were not affected by treatments (Table 3). Brito et al. (2014) fed diets containing high- or low-NSC alfalfa baleage supplemented with a corn-based concentrate (approximately 10 kg/cow daily) and observed no effects on yields of milk and milk components in early-lactation dairy cows. On the other hand, Brito et al. (2008) observed significant increases in DMI and yields of milk and milk fat and protein in late-lactation dairy cows offered p.m.- rather than a.m.-cut alfalfa baleage

Table 2. Nutrient (% of DM, unless otherwise noted) composition of TMR¹ containing p.m.- or a.m.-cut red clover baleage offered to late-lactation Holstein cows fed high-forage diets

Item	TMR	
	p.m.-cut red clover	a.m.-cut red clover
DM, % of fresh matter	51.34	52.52
OM	88.81	89.50
CP	18.63	19.23
NDF	36.65	36.95
ADF	27.35	28.50
NDIN	0.84	0.80
ADIN	0.26	0.24
TESC ²	7.01	6.22
Starch	11.2	11.3
NSC (TESC plus starch)	18.2	17.5
Lignin	4.65	5.55
Crude fat	2.98	3.35

¹The TMR contained (% of diet DM): p.m.- or a.m.-cut red clover baleage (65%), steam-flaked corn (4.27%), dried and ground corn (11.1%), dried sugarcane molasses (1.18%), dried citrus pulp (8.1%), soyhulls (3.41%), corn dried distillers grains with solubles (0.18%), solvent soybean meal (1.73%), expelled canola meal (0.55%), ProvAA_L (2.23%; Lys and Met supplement; Perdue AgSolutions LLC, Salisbury, MD), urea (0.05%), minerals and vitamin premix (2.20%).

²TESC = total ethanol-soluble carbohydrates.

as the sole feed source. The discrepancies in animal production between our 2 latest studies (current and Brito et al., 2014) and the earliest (Brito et al., 2008) may be explained as follows: (1) the NSC concentration of the high-NSC/p.m. baleage decreased substantially in the time elapsed between baling and the beginning of the study; (2) DMI and NSC intakes did not differ significantly between treatments; and (3) concentrate fed provided enough ruminally fermentable energy to meet microbial and animal requirements, thus diluting the contribution of forage NSC to total dietary NSC.

The concentrations of MUN ($P < 0.001$) and PUN ($P < 0.001$) were both lowest with feeding p.m.-RC (Table 3). Decreased concentration of MUN is entirely explained by the lower concentration of CP in p.m.-RC versus a.m.-RC (Table 2). It is well established the relationship between dietary CP concentration and MUN, as well as the high correlation between MUN and PUN (Broderick and Clayton, 1997; Nousiainen et al., 2004). Apparent total-tract digestibilities of DM ($P = 0.05$) and OM ($P = 0.09$) were highest with p.m.-RC, whereas NDF, ADF, and N did not differ significantly between treatments. Similarly, Brito et al. (2008) ob-

served increased apparent total-tract OM digestibility in late-lactation cows fed p.m.- versus a.m.-cut alfalfa baleage, but no effect on NDF digestibility. Because DM and OM digestibilities decreased without an effect on fiber digestibility, it is conceivable that TESC and NSC were more digestible in the rumen with p.m.-RC and p.m.-alfalfa baleages. In fact, ruminal digestibility of TESC was significantly higher in Holstein cows fed diets containing high- rather than low-NSC diet (Brito et al., 2014). Urinary excretions of total N, urea N, and purine derivatives did not differ significantly between treatments despite the lowest ($P < 0.01$) N intake in cows fed p.m.-RC (Table 3).

In summary, the significant difference in NSC concentration between the p.m.-RC and a.m.-RC baleage in samples collected before the beginning of the study was not maintained in samples collected during the sampling periods. Decreased MUN and PUN in cows fed p.m.-RC versus a.m.-RC is explained by lower dietary CP and N intake. Future research is needed to better understand NSC losses during storage in RC baleage and the associated effects on baleage fermentation profile and animal performance.

Table 3. Effects of feeding TMR containing red clover cut in the afternoon (p.m.-cut) or morning (a.m.-cut) and harvested as baleage on milk yield and composition, intake and apparent total-tract digestibility of nutrients, plasma concentration of urea-N (PUN), and urinary N and purine derivatives (PD) excretion in late-lactation Holstein cows fed high-forage diets

Item	TMR		SEM	$P > F^1$
	p.m.-cut red clover	a.m.-cut red clover		
DMI, kg/d	20.7	20.9	0.49	0.26
N intake, g/d	615	644	14.8	<0.01
Milk yield, kg/d	27.7	28.3	1.18	0.28
Milk yield/DMI, kg/kg	1.34	1.36	0.06	0.58
Milk N/N intake, %	23.1	22.4	0.73	0.39
Milk fat, %	4.04	4.02	0.10	0.82
Milk fat, kg/d	1.10	1.10	0.04	0.90
Milk protein, %	3.26	3.27	0.08	0.67
Milk protein, kg/d	0.89	0.90	0.03	0.69
Milk lactose, %	4.73	4.74	0.03	0.90
Milk lactose, kg/d	1.30	1.31	0.06	0.78
MUN, mg/dL	12.1	13.7	0.31	<0.001
PUN, mg/dL	14.8	17.1	0.50	<0.001
ADG, kg/d	0.55	0.73	0.15	0.06
DM digestibility, %	71.9	69.8	0.65	0.05
OM digestibility, %	73.6	72.0	0.64	0.09
NDF digestibility, %	66.5	64.6	1.02	0.23
ADF digestibility, %	64.7	64.5	1.05	0.89
N digestibility, %	65.9	64.8	0.90	0.41
Urinary N excretion, g/d	308	315	12.3	0.29
Urinary N excretion, % N intake	49.7	48.9	1.45	0.51
Urinary urea N, g/d	250	255	11.9	0.45
Urinary allantoin excretion, ² mmol/d	561	555	24.8	0.79
Urinary uric acid excretion, ² mmol/d	104	95.2	8.41	0.06
Urinary PD excretion, ² mmol/d	666	650	30.8	0.47

¹Probability of treatment effect (p.m.- vs. a.m.-cut-red clover baleage TMR); significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

²Calculated assuming a constant creatinine excretion rate of 29 mg/kg of BW (Valadares et al., 1999).

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