Alfalfa Cut at Sundown and Harvested as Baleage Improves Milk Yield of Late-Lactation Dairy Cows¹

A. F. Brito,* G. F. Tremblay,† A. Bertrand,† Y. Castonguay,† G. Bélanger,† R. Michaud,† H. Lapierre,* C. Benchaar,* H. V. Petit,* D. R. Ouellet,* and R. Berthiaume*²

*Dairy and Swine Research and Development Centre, Agriculture and Agri-Food Canada, Sherbrooke, Québec, Canada J1M 1Z3

†Soils and Crops Research and Development Centre, Agriculture and Agri-Food Canada, Québec, Québec, Canada G1V 2J3

ABSTRACT

Alfalfa (*Medicago sativa* L.) cut at sundown has been shown to contain greater concentration of total nonstructural carbohydrates (TNC) than that cut at sunup. Fourteen multiparous (8 ruminally cannulated) and 2 primiparous lactating dairy cows were randomly assigned to 2 treatments in a crossover design (2 periods of 24 d) to investigate the effects of alfalfa daytime cutting management on ruminal metabolism, nutrient digestibility, N balance, and milk yield. Half of each alfalfa field (total of 3 fields) was cut at sundown (PM) after a sunny day, whereas the second half was cut at sunup (AM) on the following day. Both PM and AM cuts were field-wilted and harvested as baleage (531 ± 15.0 g of dry matter/kg of fresh matter). Bales (PM and AM) were ranked according to their concentrations of TNC, paired, and each pair of PM and AM baleages was then assigned to each experimental day (total of 48 d). The difference in TNC concentration between PM and AM baleages fed during the 10 d of data and sample collection varied from -10 to 50 g/kg of dry matter. Each pair of baleage was fed ad libitum to cows once daily with no concentrate. Ruminal molar proportion of acetate and total volatile fatty acid concentration were greater in animals fed the AM baleage, whereas the proportion of valerate was greater with PM baleage; no other significant changes in ruminal molar proportions of volatile fatty acids were observed between forage treatments. Digestible organic matter intake, organic matter digestibility, and plasma Lys concentration were significantly greater in cows fed PM alfalfa, suggesting that more nutrients were available for milk synthesis. Significantly lower body weight gain and retained N as a proportion of N intake were observed in cows fed PM alfalfa, thus suggesting that nutrients were channeled to milk synthesis rather than to body

reserves. Intake of dry matter (+1.0 kg/d), and yields of milk (+1.0 kg/d), milk fat (+70 g/d), and milk protein (+40 g/d) were significantly greater in cows fed PM vs. AM alfalfa. Concentration of milk urea N and excretion of urea N as a proportion of total urinary N were significantly reduced, and milk N efficiency was increased when feeding PM vs. AM alfalfa, indicating an improvement in N utilization. Increasing the TNC concentration of alfalfa by shifting forage cutting from sunup to sundown improved N utilization and milk production in late-lactation dairy cows.

Key words: alfalfa baleage, dairy cow, diurnal cutting, total nonstructural carbohydrate

INTRODUCTION

It is well documented that the concentration of total nonstructural carbohydrates (TNC) varies diurnally in forage because of the plants' potential to accumulate carbohydrates during the photoperiod (Bowden et al., 1968; Gordon, 1996; Burns et al., 2007). In eastern Canada, alfalfa is the most widely cultivated forage legume because of its high yield and CP concentration. However, between 44 and 87% of the CP in alfalfa silage is degraded to NPN in the silo (Papadopoulos and McKersie, 1983; Muck, 1987), which may lead to excessive ammonia formation in the rumen. Thus, an increase in alfalfa TNC to balance the supplies of fermentable energy and RDP in the rumen may enhance ammonia capture by ruminal microbes and improve N utilization in dairy cows. In Europe, reducing the ratio of CP to soluble sugars by feeding ryegrass (*Lolium perenne* L.) cultivars bred for high water-soluble carbohydrates (WSC) significantly improved N utilization and milk yield in late-lactation dairy cows (Miller et al., 2001). Daytime cutting management (p.m. vs. a.m. cut) also offers an alternative to increase TNC concentration of grass (tall fescue, *Lolium arundinaceum*) and legume (alfalfa) as reported by Fisher et al. (1999, 2002). Investigating the variation in ruminant preference for alfalfa hays, Fisher et al. (2002) and Burns et al. (2005) observed that shifting hay mowing from sunup to

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²Corresponding author: berthiaume@agr.gc.ca

sundown was effective in increasing DMI in trials conducted with sheep, goats, and steers. Notwithstanding these few examples, the potential for improving the nutritional value and efficiency of N utilization by feeding alfalfa with improved TNC concentration to lactating dairy cows remains largely unexploited.

Therefore, we hypothesized that feeding p.m.-cut (high TNC) vs. a.m.-cut alfalfa (low TNC) would improve N utilization, DMI, and milk yield in late-lactation dairy cows fed only forage. The objectives of this study were to compare the effects of daytime cutting management (p.m. vs. a.m. cut) on ruminal metabolism, apparent total tract digestibility of nutrients, N balance, and yield and composition of milk.

MATERIALS AND METHODS

Animals

Fourteen [8 ruminally cannulated (RC)] multiparous Holstein cows averaging (mean $\pm$ SD) 171 ± 71 DIM and 643 ± 47 kg of BW and 2 primiparous Holstein cows averaging 196 ± 52 DIM and 594 ± 51 kg of BW at the beginning of the trial were blocked by DIM and parity and, within each block, randomly assigned to 2 treatments in a crossover design with 2 experimental periods. Each period lasted 24 d (total of 48 d) and consisted of 14 d for diet adaptation and 10 d for data and sample collection. Cows were housed in tie stalls and had free access to water throughout the experiment. Care and handling of the animals were conducted as outlined in the guidelines of the Canadian Council on Animal Care (1993), and the study was approved by the Institutional Animal Care Committee of the Dairy and Swine Research and Development Centre.

Alfalfa Harvest

Alfalfa (*Medicago sativa* L. 'AC Caribou') was grown in 3 well-established fields (total of 14 ha) located at the Normandin Research Farm of Agriculture and Agri-Food Canada ($48^{\circ}51'N$, $72^{\circ}32'W$) in the province of Québec, Canada. The spring growth of half of each field was cut after a sunny day on June 12, 2006, at sundown (between 1830 and 2145 h; **PM** alfalfa), whereas the second half was cut on June 13, 2006, at sunup (between 0630 and 1030 h; **AM** alfalfa) at the late bud stage of development. The DM yield of the 3 alfalfa fields averaged approximately 2.5 Mg/ha. Forage was cut using a conventional mower conditioner, tedded, and field-wilted to about 531 g of DM/kg of fresh matter, which was attained after approximately 48 and 30 h of field drying for PM and AM alfalfa, respectively. Between 1300 and 1600 h in the afternoon

of June 14, 2006, PM and then AM alfalfa cuts were harvested as baleages using a large rectangular baler (New Idea model 7333; Agco Corporation, Duluth, GA) and wrapped with stretch plastic using a bale wrapper (Équipement Anderson model 680-S; Chesterville, Québec, Canada). A total of 148 bales (72 PM and 76 AM) were made and later transported to the Dairy and Swine Research and Development Centre of Agriculture and Agri-Food Canada located in Sherbrooke ($45^{\circ}24'N$, $71^{\circ}54'W$), Québec, for the animal study. The daily global solar radiation measured during the 3 harvest days was 35.7 (June 12), 33.2 (June 13), and 35.6 MJ/m² (June 14). These values were greater than the historical climate solar radiation data of approximately 20.5 MJ/m² registered for the month of June (Environment Canada, 1982) at the harvest site. The time elapsed between alfalfa baling and feeding to the cows (d 1 of the study) was 100 d.

Animal Feeding

Three weeks before the beginning of the trial, approximately 200 g of baleage from all harvested bales was sampled using an electrical drill fitted with a metal core bale sampler, dried at 55°C for 48 h (forced-air oven), ground to pass through a 1-mm screen (Wiley mill, Arthur H. Thomas, Philadelphia, PA), and individually analyzed for total soluble carbohydrates (reducing sugars), starch, and pinitol. Pinitol is a cyclitol nonreducing sugar (Streeter et al., 2001) found in several legume species (Smith and Phillips, 1980) and possibly involved in helping plants to survive against adverse environmental conditions (McManus et al., 2000; Streeter et al., 2001) including freezing stress in alfalfa (Bertrand et al., 2007). Water-soluble carbohydrates were obtained by combining reducing sugars and pinitol, whereas TNC were calculated as the sum of WSC and starch. The PM and AM bales were then ranked according to their TNC concentration and paired; each pair of PM and AM baleages was randomly assigned to each experimental day (total of 48 d). The difference in TNC concentration between PM and AM alfalfa within each pair of bales fed during the 10 d of data and sample collection varied from -10 to 50 g/kg of dry matter with an average value of 23 g/kg of DM. Each pair of baleages was fed for ad libitum intake to the cows once daily at approximately 0800 h with no concentrate. Orts were collected daily at about 0700 h, and the amount of feed offered to the cows was adjusted daily to yield refusals equal to approximately 5 to 10% of intake. A mineral and vitamin premix was supplemented daily as a top-dress of approximately 250 g mixed with the forage at feeding and provided (per kilogram of DM) 0.24 g of Ca, 9.07 g of P, 7.86 g of

Mg, 18.1 g of Na, 17.5 g of Cl, 3.02 g of K, 3.63 mg of S, 121 mg of Fe, 3,932 mg of Mn, 3,871 mg of Zn, 968 mg of Cu, 121 mg of I, 72.6 mg of Co, 28 mg of Se, 750,000 IU of vitamin A, 148,200 IU of vitamin D, and 3,800 IU of vitamin E. Water intake was measured continuously throughout the trial.

Alfalfa Sampling and Analyses

Soluble carbohydrates were extracted with methanol:chloroform:water as described by Bertrand et al. (2006). Briefly, approximately 0.25 g (dry weight) of ground baleage was extracted in 6 mL of methanol:chloroform:water (12:5:3, vol/vol/vol) and heated for 20 min at 65°C to stop enzymatic activity. Tubes were left cooling at 4°C overnight followed by 10 min of centrifugation at $1,500 \times g$ to collect the supernatant. For phase separation, 0.25 mL of water was added to a 1-mL subsample of supernatant; tubes were shaken and centrifuged for 3 min at $21,000 \times g$ to collect the upper phase. The aqueous fraction was evaporated to dryness using a Savant Speed Vac vacuum concentrator/centrifugal evaporator system (model SC210A, Fisher Scientific, Ottawa, ON, Canada), and then solubilized in 1 mL of a 13.3 μM EDTA disodium-calcium salt solution, and kept frozen at -80°C until analysis of reducing soluble sugars by colorimetry using the *p*-hydroxybenzoic acid hydrazide method of Blakeney and Mutton (1980). The nonsoluble residues left after extraction were washed twice with methanol and used for starch quantification as glucose equivalent with the *p*-hydroxybenzoic acid hydrazide method after gelatinization at 100°C and digestion for 90 min with amyloglucosidase (Sigma A7255, Sigma Chemical Co., St. Louis, MO). The nonreducing sugar pinitol was quantified by HPLC (Waters, Milford, MA) following a 3-min centrifugation at $21,000 \times g$ using a Bio-Rad Aminex column (HPX-87P, 7.8×300 mm, Bio-Rad, Hercules, CA) and detected on a refractive index detector as described by Bigras and Bertrand (2006).

Ground samples of PM and AM baleages used for the chemical analyses described below were pooled per treatment from d 15 to 24 by mixing equal amounts of DM. Baleage samples were then analyzed for analytical DM (105°C for 16 h; AOAC, 1990) and ash with a thermogravimetric analyzer (model TGA-601; Leco Corporation, St. Joseph, MI); total N, neutral detergent insoluble N, and ADIN using micro-Kjeldahl analysis (Kjeltec 2400 instrument; Foss Analytical, Hillerød, Denmark; AOAC, 1990); NPN by extraction with TCA following the procedure of Licitra et al. (1996); NDF and ADF with a Ankom²⁰⁰ fiber analyzer (Ankom Technology, Fairport, NY) using heat-stable α -amylase and sodium sulfite according to Van Soest et al. (1991);

and ether extract with a Soxtec system HT6 apparatus (Tecator, Fisher Scientific, Montreal, Canada). Concentrations of AA in the baleages were determined by the isotope dilution method of Calder et al. (1999) after a 24-h acid hydrolysis with 6 *N* HCl-phenol at 110°C (AOAC, 2000) as described by Borucki Castro et al. (2007). A second acid hydrolysis using performic acid oxidation was performed for the analysis of Met (AOAC, 2000). A mixture of labeled AA (¹³C and ¹⁵N AA isotope standards; CDN isotopes, Pointe-Claire, QC, Canada; Cambridge Isotope Laboratories Inc., Andover, MA) was used as an internal standard. Amino acid enrichment was quantified using a GC-MS (Hewlett-Packard model GC6890-MS5973, Agilent Technologies, Wilmington, DE). The PM and AM baleage samples used for extraction were collected shortly after opening the bale, pooled from d 15 to 24, and stored at -20°C. Baleage extracts were prepared by mixing 20 g of thawed PM or AM alfalfa samples in 100 mL of distilled water followed by agitation and then 2 h of maceration at room temperature with occasional agitation. The pH of the extracts was measured with a pH meter (Accumet pH meter model 815 MP, Fisher Scientific, Fairlawn, NJ) followed by the addition of 100 mL of a 0.1 *M* solution of HCl and an additional 24 h of maceration at 4°C with occasional agitation. The extracts were filtered with Whatman no. 54 filter paper (catalog no. 1454-150, Whatman International Ltd., Maidstone, UK) and the filtrates were frozen at -20°C for later analysis of VFA with a GLC instrument (Hewlett-Packard 6890N, Hewlett-Packard Inc., Montreal, QC, Canada) equipped with a flame-ionization detector and a 7683B model autosampler as described by Delbecchi et al. (2001). Orts collected from the 8 RC cows were pooled by cow from d 20 to 24 (total collection period), stored at -20°C, freeze-dried, and ground to pass through a 1-mm Wiley mill screen. Samples were then analyzed for analytical DM, ash, total N, NDF, and ADF using the procedures described for the baleages.

Digestive Tract Sampling and Analyses

Samples of whole ruminal contents (about 200 mL) were taken from the ventral sac of the rumen of the 8 RC cows at 0 (prefeeding), 1, 2, 3, 4, 6, 8, 12, and 24 h postfeeding from d 23 to 24 of each period and strained through 2 layers of cheesecloth; pH measurement (pH/temp meter 199 Model No 3D, Fisher Scientific, Pittsburgh, PA) followed immediately. Two 10-mL samples were then preserved by addition of 0.2 mL of 50% H₂SO₄ and stored at -20°C until analysis. Samples were thawed at room temperature, centrifuged (25,200 $\times g$, 15 min, 4°C), and supernatants analyzed for ammonia using the indophenol-blue method of Novozam-

sky et al. (1974) and for VFA using GLC as described previously.

Total collection of urine and feces was done in the last 5 d of each period (d 20 to 24) using the 8 RC cows. Urine was collected in stainless steel containers via a Gooch tube (BF Goodrich Co., Kitchener, ON, Canada) and acidified with concentrated H_2SO_4 to maintain pH <3.0, whereas feces were collected in preweighed plastic-lined plywood boxes. Urine and feces were weighed daily and representative samples (approximately 2% of the total weight) were taken and frozen at $-20^\circ C$. After freeze-drying, fecal samples were ground to pass through a 1-mm Wiley mill screen, pooled per day by mixing equal amounts of DM, and analyzed for analytical DM, ash, NDF, and ADF using the procedures described previously. Representative fresh samples of urine and feces were also taken during total collection and analyzed individually ($n = 5$ samples/period) for total N using micro-Kjeldahl analysis as described earlier. Concentration of urea N in urine was determined colorimetrically using diacetyl-monoxime with a Technicon autoanalyzer (industrial method no. 339-01; Technicon Instruments, Tarrytown, NY).

Blood Sampling and Analyses

Blood samples were collected into Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) approximately 4 h after feeding from the coccygeal artery or vein of cows on d 24 of each period. Vacutainer tubes containing sodium heparin were used for AA and urea N analyses, whereas tubes without anticoagulant were used for NEFA determination. After sampling, tubes were kept on ice and centrifuged ($2,000 \times g$, 12 min, $4^\circ C$). For analysis of AA, 0.2 g of an internal standard solution of stable isotope-labeled AA (Martineau et al., 2007) was gravimetrically added to 1.0 g of plasma before storage at $-80^\circ C$. The remaining plasma was stored at $-20^\circ C$ for later analysis. Urea N was analyzed with the colorimetric method used for urinary urea N and NEFA were analyzed with a commercial kit (Wako Pure Chemical Industries Ltd., Osaka, Japan) according to the procedure of McCutcheon and Bauman (1986). Plasma concentrations of individual AA were determined by the isotope dilution method as described previously.

Animal Performance and Milk Analyses

Cows were milked twice daily at approximately 0600 and 2000 h, and milk yield was recorded at each milking. Milk samples from a.m. and p.m. milkings were collected from d 19 (p.m.) to 24 (a.m.) of each period, preserved in tubes containing 2-bromo-2-nitropropan

1,3 diol, and kept at $4^\circ C$ until shipped for determination of fat, lactose, and MUN by infrared analysis (Valacta, Ste-Anne-de-Bellevue, QC, Canada). Fresh (unfrozen) samples of milk were analyzed for total N by combustion (Leco model FP-428 Nitrogen Determinator, Leco, St. Joseph, MI). Milk samples without preservative were collected from the 8 RC cows, pooled on a yield basis, and kept frozen at $-20^\circ C$ until analyzed for milk fatty acids (FA) profile. Milk FA were prepared, extracted, and methylated according to Petit (2002). Fatty acid methyl ester profiles were measured by a GLC instrument according to the methods described by Delbecchi et al. (2001). Concentrations and yields of milk components and MUN were computed as the weighted means from a.m. and p.m. milk yields on each test day. Feed efficiency was computed by dividing mean milk yield by mean DMI over the last 10 d of each period. Apparent efficiency of utilization of feed N (assuming no retention or mobilization of body N) was calculated by dividing mean milk N secretion (milk N concentration $\times$ milk yield) by mean N intake. Body weights were recorded on 3 consecutive days at the beginning of the experiment and at the end of each period to compute BW change.

Statistical Analyses

Data were analyzed using the MIXED procedure of SAS (SAS Institute, 1999–2000) according to a cross-over design. The following model was fitted for all variables with no repeated measures over time:

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

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 l th 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. Significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

The following model was fitted for variables with repeated measures over time (ruminal pH, ammonia, and VFA):

$$Y_{ijklm} = \mu + S_i + C_j(S)_i + P_k + T_l + E1_{ijkl} + H_m + HT_{ml} + E2_{ijklm},$$

where Y_{ijklm} = 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 of l th treatment, $E1_{ijkl}$ = whole-plot random residual variation, H_m = mean effect of m th hour postfeeding analyzed as repeated measurements, HT_{ml} = interaction between m th and l th treatment, and $E2_{ijklm}$ = subplot random residual variation. The spatial covariance structure SP(POW) had the lowest Akaike information criterion and was retained in the final model. The subject of the repeated measurements was defined as cow (period). All terms were considered fixed, except $C_j(S)_i$, $E1_{ijkl}$, and $E2_{ijklm}$, which were considered random. All reported values are least squares means. Significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Forage Composition

Compared with AM baleage, PM baleage concentrations of starch, WSC, and TNC were increased by 50 ($P < 0.01$), 19 ($P < 0.01$), and 22% ($P < 0.01$), respectively (Table 1). Others (Fisher et al., 2002; Burns et al., 2005) comparing PM- vs. AM-cut alfalfa hay reported average increases of 21 and 20% in the concentrations of starch and TNC, respectively. Although the percentage increase in TNC concentration was similar among studies, that of starch was about 2.4-fold greater in the present trial compared with Fisher et al. (2002) and Burns et al. (2005). We also observed 2- to 3-fold greater concentrations of starch and TNC within PM and AM alfalfa baleage compared with values published by this same group of researchers (Fisher et al., 2002; Burns et al., 2005) using PM and AM alfalfa hays. Many factors such as time of cut (Owens et al., 1999; Burns et

al., 2007), day length, temperature, and plant maturity (Van Soest, 1994), and mode of forage conservation (e.g., baleage vs. hay) can influence accumulation of sugars in plants. Therefore, differences among studies in forage composition of TNC are expected. The concentrations of CP, NDF, and ADF (Table 1) were all numerically lower in PM than in AM alfalfa in part because of the dilution effect associated with increased concentrations of starch, WSC, and TNC (Table 2) in the former forage.

The numerically lower pH and fermentation acids of PM vs. AM alfalfa (Table 2) indicate that the former forage was more extensively fermented possibly because of its increased TNC concentration (Table 1). Baleage pH averaged 5.88 across treatments and was much higher than pH values reported for alfalfa silage, which averaged 4.80 across the studies of Brito and Broderick (2006, 2007) and Broderick et al. (2007). This was expected considering the greater DM concentration and particle length of baleages compared with silages. According to Van Soest (1994), wilting of grass or legume forage reduces organic acid formation during conservation with consequent stabilization at higher pH, whereas chopping (McDonald et al., 1991) increases the availability of WSC and, therefore, enhances organic acid formation. Approximately half of total CP was converted to NPN in both alfalfa baleages (Table 2), which agrees with previous studies using alfalfa silage (Brito and Broderick, 2006, 2007; Broderick et al., 2007). Concentrations of individual AA, branched-chain AA, essential AA, nonessential AA, and total AA were all numerically lower in PM compared with AM alfalfa (Table 3). As previously discussed, increased TNC in PM alfalfa diluted its total N concentration including N fractions such as AA.

Table 1. Nonstructural carbohydrate composition of baleages harvested from alfalfa cut at sundown (PM) or sunup (AM)

Item ¹	Time of cutting		SED ²	P-value ³
	PM	AM		
DM, g/kg of fresh matter	537	524	15.4	0.44
	g/kg of DM			
TNC ⁴	128	105	3.00	<0.01
Total reducing sugars	89.0	67.5	2.30	<0.01
Pinitol	22.0	26.0	0.80	<0.01
Starch	17.1	11.4	0.67	<0.01
WSC ⁵	111	93.5	2.50	<0.01

¹Samples of PM and AM alfalfa baleages were analyzed individually for DM and sugar fractions from d 15 to 24 in each period (n = 10 bales/treatment).

²SED = standard error of the least squares means difference.

³Two-sample *t*-test for probability of treatment effect (PM vs. AM).

⁴Total nonstructural carbohydrates = total reducing sugars + pinitol (nonreducing sugar) + starch.

⁵Water-soluble carbohydrates = total reducing sugars + pinitol.

Table 2. Chemical composition of baleages harvested from alfalfa cut at sundown (PM) or sunup (AM)

Item ¹	Time of cutting	
	PM	AM
pH	5.75	6.01
	g/kg of DM	
Ash	113	117
CP	179	189
NPN ²	90.0	89.0
NDIN ³	3.40	3.72
ADIN	0.97	1.07
Ether extract	31.2	31.2
NDF	391	408
ADF	287	302
Hemicellulose ⁴	104	106
NFC ⁵	307	277
Acetic acid	1.65	2.58
Propionic acid	0.032	0.047
Butyric acid	0.035	0.055
Isobutyric acid	0.013	0.016
Valeric acid	0.006	0.007
Isovaleric acid	0.021	0.030

¹Samples of PM and AM alfalfa baleages used for the chemical analyses of variables presented in this table were pooled from d 15 to 24 in each period.

²NPN = soluble N in TCA solution $\times$ 6.25 (Licitra et al., 1996).

³NDIN = neutral detergent insoluble N.

⁴Hemicellulose = NDF – ADF.

⁵NFC = 1,000 – [(NDF – NDIN $\times$ 6.25) + CP + ether extract + ash]; NRC, 2001.

Ruminal Metabolism and Nutrient Digestibility

A significant ($P = 0.02$) time of sampling by treatment interaction was observed for ruminal pH (Figure 1). Therefore, ruminal pH was significantly higher comparing PM vs. AM alfalfa at 2, 3, 4, 6, and 8 h post-feeding (Figure 1), possibly because the ruminal molar proportion of acetate and total VFA concentration were lower ($P = 0.02$) when cows received the former rather than the latter forage treatment (Table 4). Mean ruminal pH over the 24-h feeding cycle tended ($P = 0.09$) to be higher when cows were fed PM rather than AM baleage (Table 4), which was somewhat unexpected. Peyraud et al. (1997) and Taweel et al. (2005) did not find differences in ruminal pH feeding high- vs. low-WSC perennial ryegrass to lactating dairy cows whereas Lee et al. (2002) reported a significant increase for this same variable when steers were fed ryegrass with improved sugar concentration. We were expecting a rapid increase in the ruminal concentration of readily fermentable sugars when feeding alfalfa with enhanced TNC concentration, which could potentially reduce ruminal pH. However, ruminal pH is influenced not only by VFA production but also by the rate of digestion, rate of release of soluble contents from the plant cells, and rate of salivation (Taweel et al., 2005). According

Table 3. Amino acid composition (g/kg of DM) of baleages harvested from alfalfa cut at sundown (PM) or sunup (AM)

Item ¹	Time of cutting	
	PM	AM
Essential AA		
His	1.98	2.50
Ile	5.77	5.99
Leu	10.4	10.8
Lys	7.50	8.02
Met	2.77	2.87
Phe	6.59	6.94
Thr	6.27	6.82
Val	6.86	7.14
BCAA ²	23.0	23.9
Total	48.1	51.1
Nonessential AA		
Ala	8.21	8.55
Glx ³	10.7	12.4
Gly	6.66	7.04
Pro	2.30	2.63
Ser	6.71	7.25
Tyr	4.81	5.13
Total	39.4	43.0
Total AA	87.5	94.0

¹Samples of PM and AM alfalfa baleages used for the chemical analyses of AA presented in this table were pooled from d 15 to 24 in each period.

²BCAA = branched-chain AA (Ile + Leu + Val).

³Glx = Glu + Gln.

to Mertens (1997), chewing activity stimulates saliva production, which can increase the buffering capacity of the rumen and prevent lower ruminal pH. In fact, Huntington and Burns (2007) showed that steers fed PM-cut gamagrass (*Tripsacum dactyloides* L.) and switchgrass (*Panicum virgatum* L.) baleages spent more time ruminating and eating and less time resting than those fed the AM-cut counterparts. Gregorini et al. (2006, 2008) demonstrated that steers allocated to PM vs. AM strip-grazing pasture had longer and more intense grazing bouts, suggesting increased saliva production. Alternatively, the ruminal disappearance of VFA may have been greater in cows fed PM baleage, resulting in a faster clearance of VFA from the rumen, which is supported by the increased DMI (Table 7).

Except for the ruminal molar proportion of acetate and concentration of total VFA, which were greater ($P = 0.02$) in AM baleage, and that of valerate, which was greater ($P < 0.01$) in PM baleage, no other significant changes in ruminal VFA were observed by feeding dairy cows alfalfa with contrasting TNC concentrations (Table 4); ruminal isobutyrate tended to increase ($P = 0.07$) in cows fed PM-cut alfalfa. The molar proportion of ruminal acetate was also significantly lower in lactating dairy cows (Peyraud et al., 1997) and steers (Lee et al., 2002) fed high- vs. low-WSC ryegrass diets. When sugar concentration was increased in alfalfa

Table 4. Ruminal metabolism in dairy cows fed baleages harvested from alfalfa cut at sundown (PM) or sunup (AM)

Item	Time of cutting		SED ¹	<i>P</i> > <i>F</i> ²
	PM	AM		
pH	6.41	6.34	0.04	0.09
Ammonia N, mg/dL	23.9	23.7	1.05	0.86
Total VFA, mM	87.9	91.8	1.27	0.02
VFA, mol/100 mol				
Acetate (A)	72.2	72.8	0.16	0.02
Propionate (P)	17.4	17.2	0.15	0.15
Butyrate (B)	6.94	6.89	0.10	0.62
Isobutyrate	1.11	1.04	0.03	0.07
Valerate	1.08	0.98	0.02	<0.01
Isovalerate	1.23	1.17	0.06	0.36
A:P	4.17	4.27	0.04	0.05
P/(A + B)	0.22	0.21	0.002	0.08

¹SED = standard error of the least squares means difference.²Probability of treatment effect (PM vs. AM).

(current study) and ryegrass (Peyraud et al., 1997; Lee et al., 2002), the NDF concentration was concomitantly reduced, which might explain the observed decrease in ruminal acetate because it is the main end-product of cellulose fermentation in the rumen.

Greater concentration of sugars in forage plants is expected to elicit a shift in ruminal fermentation toward propionate but that was not the case in the present study (Table 4) or in the studies of Peyraud et al. (1997) and Taweel et al. (2005). However, Lee et al. (2002) reported a significant increase in the concentration of ruminal propionate when steers were fed a diet containing high- vs. low-WSC grass, which agrees with results of Berthiaume et al. (2007) who fed high- vs. low-TNC alfalfa to dual-flow continuous culture fermenters. It is important to emphasize that differences in sugar concentrations between forage treatments in the studies of Lee et al. (2002) and Berthiaume et al. (2007) were much more pronounced compared with those in the current study and other reports (Peyraud et al., 1997; Taweel et al., 2005). Although we observed a trend ($P = 0.08$; Table 4) for greater glucogenic:lipogenic VFA ratio in cows fed PM alfalfa, the difference between treatments was numerically small and probably biologically unimportant. Because the ruminal concentration of acetate decreased and that of propionate did not change between treatments, the acetate:propionate ratio declined ($P = 0.05$) in cows offered PM-cut alfalfa (Table 4).

Concentration of ruminal ammonia did not differ between forage treatments (Table 4), which agrees with Trevaskis et al. (2001) who fed PM vs. AM biennial ryegrass (*Lolium multiflorum*) or PM vs. AM kikuyu grass (*Pennisetum clandestinum*) to sheep. Increased TNC is expected to improve the ability of ruminal mi-

crobes to capture ammonia and use it as an N source to synthesize microbial protein (Rook et al., 1987). In addition, less dietary AA are expected to be deaminated and used as energy sources, which may also reduce ammonia formation in the rumen (Nocek and Russell, 1988). Although Taweel et al. (2005) reported only a trend ($P = 0.17$) for a reduction of ruminal ammonia when feeding high- vs. low-WSC ryegrass to lactating cows, Lee et al. (2002) observed a significant reduction for this variable in steers fed grass with improved sugar concentration. Berthiaume et al. (2007) also showed a significant decline of 23% in the concentration of ammonia feeding dual-flow continuous culture fermenters high- vs. low-TNC alfalfa. However, as mentioned

Figure 1. Diurnal variation of ruminal pH (means $\pm$ SED) in dairy cows fed baleages harvested from alfalfa cut at sundown (PM) or sunup (AM). A significant time of sampling $\times$ treatment interaction ($P = 0.02$) was observed and differences between treatments at each sampling time (*) were found at: 2 h ($P = 0.02$), 3 h ($P = 0.05$), 4 h ($P = 0.05$), 6 h ($P = 0.05$), and 8 h ($P < 0.01$) postfeeding.

Table 5. Intake and apparent total-tract digestibility of nutrients in dairy cows fed baleages harvested from alfalfa cut at sundown (PM) or sunup (AM)

Item ¹	Time of cutting		SED ²	<i>P</i> > <i>F</i> ³
	PM	AM		
DM				
Intake, kg/d	19.2	18.5	0.41	0.11
Digestible intake, kg/d	12.3	11.7	0.30	0.09
Digestibility, %	64.3	63.3	0.59	0.16
OM				
Intake, kg/d	17.1	16.3	0.36	0.07
Digestible intake, kg/d	11.1	10.4	0.25	0.03
Digestibility, %	65.4	63.7	0.46	0.01
NDF				
Intake, kg/d	7.45	7.39	0.18	0.76
Digestible intake, kg/d	3.52	3.56	0.11	0.74
Digestibility, %	47.3	48.0	0.97	0.50
N				
Intake, g/d	553	569	11.5	0.29
Digestible intake, g/d	380	398	8.22	0.07
Digestibility, %	68.7	69.9	0.51	0.05

¹Data from the 8 ruminally cannulated cows.²SED = standard error of the least squares means difference.³Probability of treatment effect (PM vs. AM).

earlier, the difference in WSC concentration comparing high- vs. low-sugar forage was much more pronounced in the studies of Lee et al. (2002) and Berthiaume et al. (2007) than in the studies of Trevaskis et al. (2001), Taweel et al. (2005), and the current trial. Yet the CP to WSC ratios in forages with increased WSC concentrations were 1.92 (Trevaskis et al., 2001), 0.43 (Lee et al., 2002), 0.86 (Taweel et al., 2005), and 1.61 (present trial). A high CP:WSC ratio or an excess of RDP supply may overcome the capacity of ruminal microbes to efficiently capture ammonia leading to its accumulation in the rumen. Although the CP:WSC ratio of the high-TNC alfalfa used in vitro (Berthiaume et al., 2007) was 2-fold greater than that of our in vivo trial, ammonia was significantly reduced compared with the control in the former experiment showing that microbes efficiently captured it despite the apparent excess supply of RDP. It must be emphasized that in the controlled conditions of in vitro systems there is no absorption of ammonia or recycling of urea, which are 2 important factors influencing the concentration of ammonia in the rumen.

Total-tract digestibility of DM did not differ and averaged 64% across treatments (Table 5). However, digestible DMI tended ($P = 0.09$) to be greater in cows fed PM- vs. AM-cut alfalfa. Burns et al. (2005) also observed no difference in apparent digestibility of DM with steers fed PM or AM alfalfa hay. Conversely, Miller et al. (2001) and Moorby et al. (2006) reported significant increases in DM digestion and digestible DMI by feeding high- vs. low-WSC ryegrass to dairy cows and associated these responses to improved con-

centration of sugars and concomitant reduction of NDF in the grass. Total-tract OM digestibility ($P = 0.01$) and digestible OM intake ($P = 0.03$) were both greater in cows fed PM- vs. AM-cut alfalfa (Table 5), which agrees with previous reports (Miller et al., 2001; Moorby et al., 2006).

Apparent total-tract digestibilities of NDF (Table 5) and ADF (data not shown) did not differ across forage treatments. Other researchers (Burns et al., 2005; Huntington and Burns, 2007) also found no difference in fiber digestibility with steers fed alfalfa and tropical grasses cut at sundown or sunup. Total-tract digestibility of NDF was significantly increased when feeding late-lactation dairy cows high- vs. low-WSC ryegrass (Miller et al., 2001) but no difference was observed when a similar high-sugar grass diet was fed to early-lactation cows in a subsequent trial (Moorby et al., 2006). Dilution of NDF concentration due to increased WSC in the high-WSC grass may explain the improved NDF digestibility reported by Miller et al. (2001) although an even more pronounced dilution did not change it in the study of Moorby et al. (2006). It is possible that the significant increase in DMI observed by Moorby et al. (2006) with early-lactation cows fed high-WSC ryegrass reduced ruminal retention time of particles counteracting the positive effect of fiber dilution on NDF digestibility. On the other hand, DMI did not differ between high- and low-WSC grass in late-lactation cows (Miller et al., 2001). Apparent total-tract digestibility of N was significantly lower ($P = 0.05$) in cows fed PM-cut alfalfa than in those fed AM-cut alfalfa (Table 5). However, the numerical difference in

Table 6. Nitrogen metabolism in dairy cows fed baleages harvested from alfalfa cut at sundown (PM) or sunup (AM)

Item ¹	Time of cutting		SED ²	<i>P</i> > <i>F</i> ³
	PM	AM		
N intake, g/d	553	569	11.5	0.29
N output and retention, g/d				
Milk	100	95.2	2.02	0.07
Urinary	262	262	8.33	0.99
Fecal	173	171	4.31	0.64
Apparently retained	17.5	40.3	9.98	0.06
N output and retention, % N intake				
Urinary	47.7	46.2	1.37	0.32
Fecal	31.3	30.1	0.51	0.05
Apparently retained	2.84	6.92	1.72	0.05
Manure N ⁴				
Excretion, g/d	436	433	10.8	0.84
Excretion, % N intake	79.0	76.3	1.55	0.13
Urinary urea N				
Excretion, g/d	221	228	7.21	0.34
Excretion, % urinary N	84.0	86.3	0.78	0.03
Excretion, % manure N	50.6	52.4	0.89	0.08
Excretion, % N intake	40.1	40.1	1.36	0.98
N efficiency				
Milk N, % of N intake	18.2	16.8	0.34	<0.01
Milk produced:manure N excreted, kg:kg	46.8	45.4	0.97	0.18

¹Data from the 8 ruminally cannulated cows.²SED = standard error of the least squares means difference.³Probability of treatment effect (PM vs. AM).⁴Manure N = fecal N + urinary N.

N digestibility between treatments was too small (1.2 percentage units) and possibly biologically meaningless. Conversely, Burns et al. (2005) observed no effect of cutting time on N digestibility when sheep, goats, and steers were fed alfalfa hay with contrasting TNC concentrations.

Nitrogen Balance and Metabolism

Intake and amount of N excreted in urine and feces did not differ between baleages and averaged 561, 262, and 172 g/d, respectively (Table 6). Amount of N secreted in milk tended ($P = 0.07$) to be greater when cows were fed PM vs. AM baleage, whereas the opposite was observed for N apparently retained (Table 6). We expected a significant reduction in urinary N excretion when feeding PM baleage, although it is important to note that cows were fed only alfalfa with about half of its total CP contributed by NPN (Table 2). In fact, urinary N excretion was reduced by 25% when lactating dairy cows were fed silages harvested from low-NPN red clover vs. high-NPN alfalfa (Broderick et al., 2007). Moorby et al. (2006) reported no difference in urinary N output probably because N intake was greater in cows fed high- vs. low-WSC grass. However, a significant reduction was observed when urinary N output was expressed as a proportion of N intake (Moorby et al.,

2006). Huntington and Burns (2007) observed no effect of cutting time on fecal excretion of N when feeding steers diets containing grass baleages with contrasting TNC concentrations, which agrees with data from the current trial.

Excretion of N in urine expressed as a proportion of N intake did not differ between forage treatments (Table 6). However, excretion of fecal N was higher ($P = 0.05$), whereas the opposite ($P = 0.05$) was observed for retained N, both expressed as proportions of N intake, when cows were fed PM vs. AM alfalfa (Table 6). Considering that 18 and 40 g of N, respectively, were retained daily in cows fed PM vs. AM baleage in the current study, an accretion of 0.64 and 1.5 kg/d of BW would be expected assuming that body tissues contain 17% protein (NRC, 2001). However, BW gain (Table 7) averaged 0.23 kg/d when cows were fed PM alfalfa and 0.55 kg/d when they received AM alfalfa indicating that the amount of N apparently retained in the body tissues appears to be overestimated by a factor of 3. Spanghero and Kowalski (1997), analyzing data from 35 published studies, consistently observed high values of retained N (mean = 39 g/d) in lactating dairy cows and concluded that improved methodological accuracy in experimental procedures could reduce overestimation of N balance. Therefore, to minimize errors associated with N volatilization during the drying and freezing processes,

Table 7. Intake, milk yield and composition, plasma metabolites, and BW gain in dairy cows fed baleages harvested from alfalfa cut at sundown (PM) or sunup (AM)

Item	Time of cutting		SED ¹	<i>P</i> > <i>F</i> ²
	PM	AM		
DMI, kg/d	19.9	19.0	0.23	<0.01
Water intake, kg/d	137	132	1.09	<0.01
Milk yield, kg/d	20.1	19.2	0.18	<0.01
Milk yield:DMI	1.01	1.02	0.01	0.46
4% FCM, kg/d	20.3	18.8	0.30	<0.01
4% FCM:DMI	1.03	1.01	0.02	0.24
ECM, ³ kg/d	21.8	20.2	0.31	<0.01
ECM:DMI	1.10	1.08	0.02	0.21
Milk fat, %	4.04	3.96	0.08	0.31
Milk fat, kg/d	0.82	0.75	0.02	<0.01
Milk protein, %	3.18	3.16	0.02	0.26
Milk protein, kg/d	0.64	0.60	0.01	<0.01
Milk lactose, %	4.15	4.10	0.03	0.12
Milk lactose, kg/d	0.85	0.78	0.01	<0.01
MUN, mg/dL	17.2	18.9	0.27	<0.01
PUN, ⁴ mg/dL	23.4	24.3	0.72	0.23
NEFA, mEq/L	147	138	13.9	0.51
BW gain, kg/d	0.23	0.55	0.08	<0.01

¹SED = standard error of the least squares means difference.²Probability of treatment effect (PM vs. AM).³ECM = [0.327 × milk yield (kg/d)] + [12.95 × fat yield (kg/d)] + [7.2 × protein yield (kg/d)] (Orth, 1992).⁴PUN = plasma urea N.

we analyzed N concentration in fresh samples of milk, urine, and feces. Using this methodological approach, a mean value of 29 g of N was retained daily indicating that N balance still overestimated and that other unknown sources of N losses were not accounted for. Brito and colleagues (Bruto et al., 2008), investigating whether N losses from breath and sweat could improve N balance measurements, observed average losses of 2.29 (breath) and 0.48 g of N/d (sweat) in cows fed red clover silage, suggesting that these sources of N output were too small to significantly improve the accuracy of N balance studies.

Despite the lack of a significant effect in the amount of urea N excreted in urine between forage treatments, cows fed PM-cut alfalfa either decreased ($P = 0.03$) or tended to decrease ($P = 0.08$) the excretion of urea N as proportions of urinary and manure N outputs, respectively (Table 6). According to Monteny and Erisman (1998), urinary urea N is the major source of ammonia emissions from dairy systems. Therefore, strategies to reduce not only the amount but also the contribution of urea N to total urinary N are crucial to minimizing the negative environmental impact of dairy farms. Milk N efficiency (milk N output/N intake) was greater ($P < 0.01$) in cows fed PM vs. AM baleage indicating that the cutting strategy to enhance TNC concentration in alfalfa improved N utilization (Table 6). Miller et al. (2001) and Moorby et al. (2006) observed significant increases in the secretion of N in milk when lactating

dairy cows were fed diets containing perennial ryegrass bred for greater WSC concentration. However, only Miller et al. (2001) reported a concomitant improvement in milk N efficiency. Milk N efficiency was much lower in the current study than in some literature reports (Miller et al., 2001; Moorby et al., 2006; Tas et al., 2006b), reflecting the much greater dietary CP in our trial as only alfalfa was fed. In fact, Olmos Colmenero and Broderick (2006) observed a significant linear decrease in milk N efficiency when the dietary CP varied from 13.5 to 19.4%. Nitrogen efficiency expressed as kilograms of milk produced/kilograms of manure N did not differ between treatments (Table 6).

Animal Production and Milk Fatty Acid Profiles

Cows fed PM baleage ingested 0.9 kg/d more DM ($P < 0.01$) than those fed AM baleage (Table 7), which may be related to the significant increase in TNC when alfalfa was cut at sundown (Table 1). In fact, Fisher et al. (1999, 2002) identified TNC, or at least one of its constituents, as an important contributor to ruminant's preference when tall fescue or alfalfa hay mowing was shifted from sunup to sundown. These previous findings were corroborated by Burns et al. (2005) and Huntington and Burns (2007) who observed significant increases of DMI in steers fed PM alfalfa hay or PM tropical grasses rather than their AM counterparts. Dry matter intake was also significantly improved by

feeding early-lactation dairy cows perennial ryegrass bred for high WSC concentration (Moorby et al., 2006). Conversely, Miller et al. (2001) found no difference in DMI when late-lactation dairy cows were fed high- vs. low-WSC ryegrass. Although cows from the present study and those from Miller et al. (2001) were in late lactation, cows from the current trial produced more milk (19.7 vs. 14.0 kg/d) and probably had greater nutritional requirements. Water intake paralleled DMI and was 5 L/d greater ($P < 0.01$) in animals fed PM-cut alfalfa compared with those fed AM-cut alfalfa (Table 7).

The observed increase ($P < 0.01$) in milk yield when cows were fed PM rather than AM baleage was expected because DMI was also enhanced as discussed earlier (Table 7). Therefore, it appears that more nutrients were available for milk synthesis considering that both total-tract OM digestion ($P = 0.01$) and intake of digestible OM ($P = 0.03$) were increased when cows received PM- vs. AM-cut alfalfa (Table 5). Miller et al. (2001) also observed a significant increase in milk yield (+2.7 kg/d) when late-lactation dairy cows were fed high- vs. low-WSC ryegrass but that was not accompanied by a similar response in DMI. However, digestible DMI and total-tract DM digestion were both greater when cows were fed the high-WSC grass diet. Fat-corrected milk also increased ($P < 0.01$; Table 7) when feeding PM vs. AM baleage, further indicating that the management strategy to shift alfalfa cutting from sunup to sundown was effective to improve milk production in the current trial. Milk components (%) were unaffected by treatments (Table 7), which agrees with previous reports (Miller et al., 2001; Taweel et al., 2005; Moorby et al., 2006). Yields of milk fat and milk lactose were increased by 70 g/d and that of milk protein by 40 g/d comparing PM vs. AM baleage ($P < 0.01$; Table 7). This increase in yield of milk components was accompanied by increases ($P < 0.01$; Table 7) in milk yield (+1.0 kg/d), 4% FCM (+1.5 kg/d), and ECM (+1.6 kg/d) suggesting that more nutrients were supplied to the udders of cows fed PM-cut alfalfa, as previously discussed.

The concentration of urea N in milk was lower ($P < 0.01$; Table 7) when cows were fed PM- compared with AM-cut alfalfa, suggesting an improvement in N utilization. Concentration of MUN in response to increased WSC in perennial ryegrass has been variable as reported by Tas et al. (2006a,b). However, Taweel et al. (2005) observed that MUN was reduced when cows were fed the high-WSC cultivar, which was related to lower CP and greater WSC concentrations. Cows fed AM baleage gained 0.32 kg/d more BW than those fed PM baleage ($P < 0.01$; Table 7), which is in agreement with the greater retention of N when animals were fed the AM-cut alfalfa (Table 6). Feed efficiency expressed

as milk yield/DMI, 4% FCM/DMI, and ECM/DMI did not differ between forage treatments, indicating no benefit of increasing alfalfa TNC concentration on feed conversion (Table 7).

Except for the concentrations of C14:1 ($P = 0.01$), C16:1 ($P < 0.01$), and C20:4n-6 ($P = 0.03$) that were lower when cows were fed PM baleage, no other significant changes in the milk FA profile were observed between forage treatments (Table 8). Concentrations of C18:0 and total saturated FA tended ($P = 0.10$) to be greater when cows were fed PM-cut instead of AM-cut alfalfa. It is expected that increased availability of rapidly fermentable carbohydrates would reduce ruminal pH, leading to incomplete biohydrogenation of dietary FA. In fact, we observed a significant reduction of pH in dual-flow continuous culture fermenters fed high- vs. low-TNC alfalfa (Berthiaume et al., 2007). However, in the current in vivo study, ruminal pH was lower in cows fed AM-cut alfalfa at several sampling times after feeding (Figure 1), which may explain the increased concentrations of C14:1, C16:1, and C20:4n-6.

Plasma Metabolites

Plasma concentrations of NEFA (Table 7) did not differ between treatments, suggesting minimal or no mobilization of fat from the adipose tissue, which is expected for late-lactation cows producing 20 kg of milk per day. The concentration of plasma urea N (PUN) paralleled that of MUN but treatment means did not differ (Table 7), which was somewhat surprising. However, PUN was determined in plasma samples taken at a single time (approximately 4 h after feeding), whereas MUN values were derived from both daily milk samples on test days (from d 19 to 24). Moreover, the SED of PUN was about 2.6-fold greater than that of MUN, showing the greater variability of a single PUN measurement compared with an integrated milk sample. According to Gustafsson and Palmquist (1993), plasma and milk urea equilibrate rapidly but PUN rises and falls throughout the day, reflecting the dynamic nature of N metabolism.

With the exception of plasma concentrations of Lys and Thr that were both enhanced ($P = 0.02$) when cows were fed PM- rather than AM-cut alfalfa, no other significant changes in plasma concentration of individual essential AA were observed (Table 9); plasma Met tended ($P = 0.07$) to be greater when cows were fed PM baleage. Studies have shown that Lys and Met are the first 2 limiting AA for yields of milk and milk protein in typical North American diets (King et al., 1990; Schwab et al., 1992a,b), whereas His has been suggested as the first-limiting AA in grass silage-based diets (Kim et al., 1999, 2000; Korhonen et al., 2000). However, stud-

Table 8. Fatty acid profile of milk fat (% of total fatty acids) in dairy cows fed baleages harvested from alfalfa cut at sundown (PM) or sunup (AM)

Item ¹	Time of cutting		SED ²	P > F ³
	PM	AM		
C10:0	2.86	2.86	0.07	0.97
C12:0	3.11	3.14	0.07	0.61
C14:0	13.4	13.3	0.17	0.71
C14:1	0.61	0.72	0.03	0.01
C16:0	41.9	41.3	1.21	0.63
C16:1	1.31	1.46	0.03	<0.01
C18:0	12.3	11.3	0.51	0.10
C18:1- <i>trans</i>	2.89	3.34	0.56	0.46
C18:1n-9 <i>cis</i>	16.8	17.6	0.57	0.20
C18:1n-7 <i>cis</i>	0.67	0.68	0.04	0.79
C18:2n-6 <i>trans</i>	0.21	0.20	0.01	0.32
C18:2n-6 <i>cis</i>	1.48	1.55	0.05	0.19
C18:3n-3	1.77	1.80	0.05	0.53
<i>Cis</i> -9, <i>trans</i> -11 C18:2	0.44	0.45	0.02	0.74
C20:3n-6	0.078	0.075	0.004	0.55
C20:4n-6	0.11	0.13	0.004	0.03
C20:5n-3	0.085	0.083	0.007	0.74
SFA ⁴	73.5	71.2	0.84	0.10
MUFA ⁵	22.3	23.8	0.84	0.12
PUFA ⁶	4.18	4.28	0.07	0.19

¹Data from the 8 ruminally cannulated cows.²SED = standard error of the least squares means difference.³Probability of treatment effect (PM vs. AM).⁴Saturated fatty acids = C10:0 + C12:0 + C14:0 + C16:0 + C18:0.⁵Monounsaturated fatty acids = C14:1 + C16:1 + C18:1-*trans* + C18:1n-9 *cis* + C18:1n-7 *cis*.⁶Polyunsaturated fatty acids = C18:2n-6 *trans* + C18:2n-6 *cis* + C18:3n-3 + *cis*-9, *trans*-11 C18:2 + C20:3n-6 + C20:4n-6 + C20:5n-3.

ies on AA requirements of forage-fed cattle are scarce (Titgemeyer and Löest, 2001). Feeding sheep with different alfalfa protein concentrates, which comprised 60% of the total dietary N, Lu et al. (1983) estimated that Met and Lys were the first 2 limiting AA in all 4 diets evaluated. Hegsted and Linkswiler (1980), feeding rats a protein-free basal diet supplemented with high- or low-saponin alfalfa protein concentrate plus incremental levels of L-Met, concluded that, using protein efficiency ratio and slope-ratio assay, Met was the first-limiting AA for growth followed by Lys. Therefore, increased plasma concentrations of Lys and Met may have been responsible for the improved yields of milk and milk protein when cows received PM alfalfa. Plasma concentrations of Cys were lower ($P < 0.01$) when feeding PM vs. AM alfalfa baleage, whereas the opposite was observed for the remaining nonessential AA with the exception of Asp, which did not differ between forage treatments (Table 9). Plasma concentration of Asn tended ($P = 0.09$) to increase and that of total AA was increased ($P = 0.02$) when cows were fed PM-cut alfalfa. The dietary concentration of all AA fractions (Table 3) was numerically lower in PM vs. AM alfalfa, suggesting that the increased plasma concentrations of Lys, Met, Thr, and most nonessential AA were caused

by a greater ruminal outflow of microbial protein when cows were fed PM alfalfa.

CONCLUSIONS

Shifting alfalfa mowing from sunup to sundown increased ($P < 0.01$) the TNC concentration of baleage fed to late-lactation dairy cows by an average of 23 g/kg of DM, which significantly enhanced DMI and yields of milk and milk components. Significant increases in digestible OM intake, OM digestibility, and plasma concentration of Lys when cows were fed PM baleage indicate that more nutrients were available for synthesis of milk and milk components. Lower BW gain and retained N as a proportion of N intake by feeding PM vs. AM alfalfa further indicated that nutrients were channeled to milk synthesis rather than to body reserves. Concentration of MUN decreased, whereas milk N efficiency increased when cows were fed PM alfalfa baleage, thus confirming an improvement in N utilization. Overall, cutting alfalfa late in the day and feeding as baleage with no concentrate was effective in increasing milk N efficiency and milk production in late-lactation dairy cows.

Table 9. Plasma amino acid concentration (μM) in dairy cows fed baleages harvested from alfalfa cut at sundown (PM) or sunup (AM)

Item ¹	Time of cutting		SED ²	<i>P</i> > <i>F</i> ³
	PM	AM		
Essential AA				
His	40.8	40.8	1.61	0.98
Ile	133	129	6.33	0.50
Leu	147	147	8.87	0.99
Lys	98.9	82.6	6.20	0.02
Met	27.7	25.4	1.18	0.07
Phe	54.8	52.9	2.59	0.49
Thr	120	104	5.86	0.02
Trp	57.9	57.6	1.65	0.84
Val	256	257	10.7	0.97
BCAA	536	532	25.4	0.88
Total	936	895	39.8	0.33
Nonessential AA				
Ala	200	171	8.94	<0.01
Asn	42.4	38.3	2.25	0.09
Asp	15.1	15.3	0.94	0.85
Cys	100	108	1.86	<0.01
Gln	341	308	13.3	0.02
Glu	46.6	44.0	0.83	<0.01
Gly	235	209	7.98	<0.01
Pro	64.4	58.9	2.49	0.04
Ser	74.2	66.5	2.79	0.02
Tyr	63.0	55.8	3.27	0.04
Total	1,183	1,074	28.0	<0.01
Total AA	2,118	1,970	57.6	0.02

¹BCAA = branched-chain AA (Ile + Leu + Val).²SED = standard error of the least squares means difference.³Probability of treatment effect (PM vs. AM).

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