Effects of Feeding Formate-Treated Alfalfa Silage or Red Clover Silage on the Production of Lactating Dairy Cows¹

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ABSTRACT

In trial 1, 15 Holsteins were fed 3 total mixed rations (TMR) with 33% neutral detergent fiber in 3 × 3 Latin squares (28-d periods). Two TMR contained (dry matter basis): 40% control alfalfa silage (CAS) or 40% ammonium tetraformate-treated alfalfa silage (TAS), 20% corn silage (CS), 33% high-moisture shelled corn (HMSC), 6% solvent soybean meal (SSBM), and 18% crude protein (CP); the third TMR contained 54% red clover silage (RCS), 6% dried molasses, 33% HMSC, 6% SSBM, and 16.3% CP. Silages differed in nonprotein N (NPN) and acid detergent insoluble N (ADIN; % of total N): 50 and 4% (CAS); 45 and 3% (TAS); 27 and 8% (RCS). Replacing CAS with TAS increased intake, yields of milk, fat-corrected milk, protein, and solids-not-fat, and apparent dry matter and N efficiency. Replacing CAS with RCS increased intake and N efficiency but not milk yield. Replacing CAS or TAS with RCS lowered milk urea N, increased apparent nutrient digestibility, and diverted N excretion from urine to feces. In trial 2, 24 Holsteins (8 ruminally cannulated) were fed 4 TMR in 4 × 4 Latin squares (28-d periods). Diets included the CAS, TAS, and RCS (RCS1) fed in trial 1 plus an immature RCS (RCS2; 29% NPN, 4% ADIN). The CAS, TAS, and RCS2 diets contained 36% HMSC and 3% SSBM and the RCS1 diet contained 31% HMSC and 9% SSBM. All TMR had 50% legume silage, 10% CS, 27% neutral detergent fiber, and 17 to 18% CP. Little difference was observed between cows fed CAS and TAS. Intakes of DM and yields of milk, fat-corrected milk, fat, protein, lactose, and solids-not-fat, and milk fat and protein content were greater on alfalfa silage

vs. RCS. Blood urea N, milk urea N, ruminal ammonia, and total urinary N excretion were reduced on RCS, suggesting better N utilization on the lower NPN silage. Apparent N efficiency tended to be higher for cows fed RCS but there was no difference when N efficiency was expressed as kilograms of milk yield per kilogram of total N excreted.

Key words: production, silage, nitrogen utilization, dairy cow

INTRODUCTION

Between 44 and 87% of the CP in alfalfa silage (AS) was found to be degraded to NPN in the silo whereas the comparable value for red clover silage (RCS) was only 7 to 40% (Papadopoulos and McKersie, 1983; Muck, 1987). Extensive protein degradation in the silo may lead to both reduced DMI and N efficiency in ruminants (Waldo, 1985), raising environmental concerns regarding excess urinary N excretion. Lower NPN in RCS results from the action of polyphenol oxidase, which converts *o*-diphenols, present at high concentration in red clover, into reactive *o*-quinones (Jones et al., 1995c). These compounds react rapidly with both proteases and substrate proteins, reducing the extent of proteolysis in the silage mass (Hatfield and Muck, 1999). Lower proportions of NPN in RCS may account for its better protein utilization than AS when fed as the sole forage to lactating dairy cows (Broderick, 2002). However, production results have been inconsistent with RCS. When RCS replaced AS in the diet, Hoffman et al. (1997) observed increased milk yield and Broderick et al. (2001) found greater apparent total tract digestibility of DM and NDF and better feed efficiency. Conversely, Broderick et al. (2000) reported lower milk yield in 2 out of 3 experiments when cows were fed RCS rather than AS, despite higher energy digestibility. Results from feeding studies comparing these 2 forages have nearly always been confounded by greater CP content in AS.

There is extensive European evidence that formic acid treatment reduces NPN formation in direct-cut grass silages and improves their nutritive value for ruminants (McDonald et al., 1991). Nagel and Broder-

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ick (1992) showed that formic acid treatment of wilted AS decreased NPN formation and substantially improved N utilization when fed to lactating dairy cows. Ammonium tetraformate (**ATF**) is a buffered form of formic acid containing 1 mol of ammonia for every 4 mols of formate. This compound is less corrosive and easier to handle than formic acid and has been shown to reduce proteolysis in direct-cut grass silages (Randby, 2000). It seemed likely that ATF also would be effective for reducing proteolysis in AS.

Two feeding trials were conducted to investigate the effects of silage NPN content on production, ruminal metabolism and nutrient utilization in lactating dairy cows fed AS or RCS as their principal forage. In both trials, ATF was added to alfalfa at ensiling to reduce proteolysis and the RCS that was fed had similar NDF content (and lower CP) compared with the AS. An earlier maturity RCS with comparable CP to the AS also was fed in the second study.

MATERIALS AND METHODS

Harvest and Composition of AS and RCS

Alfalfa silage was harvested from third cutting. Red clover was seeding-year forage and was judged visually to be a pure stand. The first RCS (**RCS1**) was harvested from first cutting when some flowers were present, at a later maturity intended to produce forage with NDF content similar to the AS, as had been done in earlier feeding trials (Broderick et al., 2000, 2001). The second RCS (**RCS2**) was harvested from second cutting when no flowers were present, at an earlier maturity intended to produce forage with CP content similar to the AS. All forages were cut using a conventional mower conditioner and field-wilted to about 40% DM. This DM was attained in 1 d with the AS but tedding plus an additional drying day was required for the 2 RCS. Forages were chopped to a theoretical length of 2.9 cm. The control alfalfa and both red clovers were ensiled without additives. Alternate windrows of alfalfa were harvested either as the control AS (**CAS**) or treated using field application while chopping with ATF (GrasAAT; Norsk Hydro ASA, Porsgrunn, Norway) to reduce NPN formation in the silo (**TAS**). Approximately 7 L of ATF was applied per ton of wet silage based on the weight of silage harvested and the volume of GrasAAT utilized. Three forages (CAS, TAS, and RCS1) were ensiled in upright concrete stave silos; RCS2 was ensiled in a plastic bag (Ag-Bag International Ltd., Warrenton, OR). No forage was rained on during harvest.

Weekly composite samples were prepared for all silages from daily 0.5-kg samples collected during feed-out throughout both trials and stored at -20°C until analyzed. At the end of the trials, weekly composites

were thawed, water extracts were prepared (Muck, 1987), and extract pH was measured. Extracts were deproteinized (Muck, 1987) and then analyzed for total AA and ammonia (Broderick et al., 2004) using flow-injection (Lachat Quik-Chem 8000 FIA, Lachat Instruments, Milwaukee, WI) and for NPN (Muck, 1987) using a combustion assay (Mitsubishi TN-05 Nitrogen Analyzer; Mitsubishi Chemical Corp., Tokyo, Japan). Thawed weekly composites also were dried at 60°C (48 h), ground through a 1-mm screen (Wiley mill, Arthur H. Thomas, Philadelphia, PA), and analyzed for DM at 105°C , ash, and OM (AOAC, 1980), total N by combustion assay (Leco 2000, Leco Instruments, Inc., St. Joseph, MI), sequentially (Van Soest et al., 1991) for NDF and ADF using heat stable α -amylase and Na_2SO_3 (Hintz et al., 1995), and for neutral detergent insoluble N (**NDIN**) and ADIN. Mean composition data for the silages fed during both trials are in Table 1.

Trial 1

Twelve multiparous (average parity 2.8, SD 1.0) and 3 primiparous Holstein cows averaging (mean $\pm$ SD) 33 ± 5 kg of milk/d, 256 ± 48 DIM, and 643 ± 72 kg of BW at the beginning of the trial were blocked into 5 squares by parity and DIM and, within each square, randomly assigned to treatment sequences in replicated 3×3 Latin squares. Each experimental period lasted 28 d and consisted of 14 d for diet adaptation and 14 d for data and sample collection. All cows were injected with bST (500 mg of Posilac, Monsanto, St. Louis, MO) beginning on d 1 of the trial and at 14-d intervals thereafter until completion of the study. Cows were housed in tie stalls and had free access to water throughout the experiment. Care and handling of the animals was conducted as outlined by the guidelines of the University of Wisconsin institutional animal care and use committee.

Diets were fed as TMR and were formulated from CAS, rolled corn silage, rolled high-moisture shelled corn (**HMSC**), and solvent-extracted soybean meal (**SSBM**) (diet CAS); TAS, rolled corn silage, HMSC, and SSBM (diet TAS); and RCS1, dried molasses, HMSC, and SSBM (diet RCS). Rolled corn silage was added to the AS diets to equalize NDF; dried molasses was added to the RCS diet to improve intake. Vitamin and mineral supplements also were fed. Table 2 gives the composition and chemical analyses of these 3 diets. Diets were offered once daily at 1000 h, and orts were collected daily at 0900 h. The amount of feed offered to the animals was adjusted daily to yield refusals of about 5 to 10% of intake.

Daily samples of approximately 0.5 kg of silages, HMSC, TMR, and orts were collected, stored at -20°C ,

Table 1. Chemical composition of alfalfa and red clover silages¹

Item	CAS	TAS	RCS1	RCS2	SE	<i>P</i> > <i>F</i>
DM, %	41.8	42.2	43.4	41.8	0.7	0.41
pH	4.94	4.71	4.86	4.95	0.09	0.10
CP, % of DM	24.6 ^b	25.8 ^a	18.1 ^d	23.3 ^c	0.3	<0.01
Ash, % of DM	11.3 ^c	10.8 ^d	12.3 ^b	12.6 ^a	0.1	<0.01
NDF, % of DM	39.4 ^b	39.0 ^b	41.4 ^a	40.0 ^b	0.5	<0.01
ADF, % of DM	29.6 ^a	29.1 ^a	27.1 ^b	24.9 ^c	0.4	<0.01
Hemicellulose, % of DM	8.5 ^b	8.9 ^b	12.6 ^a	12.8 ^a	0.2	<0.01
N-fractions (% of total N)						
Neutral detergent insoluble N (NDIN)	7.4 ^c	8.4 ^c	28.2 ^a	22.8 ^b	0.8	<0.01
ADIN	3.5 ^{bc}	3.2 ^c	7.5 ^a	4.4 ^b	0.5	<0.01
Fraction B ₃ ²	3.9 ^c	5.2 ^c	20.7 ^a	18.4 ^b	0.6	<0.01
NPN ³	49.9 ^a	45.3 ^b	27.1 ^c	29.4 ^c	1.0	<0.01
Ammonia N	4.1 ^a	3.4 ^b	2.6 ^c	3.5 ^b	0.2	<0.01
Free AA N ⁴	39.3 ^a	30.1 ^b	12.5 ^d	17.1 ^c	0.9	<0.01
Unidentified N ⁵	6.4 ^b	11.8 ^a	12.0 ^a	8.9 ^b	1.1	<0.01

^{a-d}Least squares means in the same row with different superscripts differ ($P < 0.05$).

¹CAS = Control alfalfa silage; TAS = ammonium tetraformate-treated alfalfa silage; RCS1 = late maturity red clover silage (fed in trials 1 and 2); RCS2 = early maturity red clover silage (fed in trial 2).

²NDIN – ADIN (Sniffen et al., 1992).

³Proportion of total N soluble in 10% (wt/vol) TCA (Muck, 1987).

⁴Free AA N = total free AA, mmol $\times$ (40.3 mg of N/mmol of total free AA); Broderick, 1987.

⁵NPN not accounted for as N in ammonia and total free AA.

and used to make weekly composites; weekly samples of SSBM and dried molasses also were taken.

Dry matter contents of representative samples of weekly composites of all feeds were determined by drying at 60°C (forced-air oven) for 48 h. Dried samples were ground to pass a 1-mm screen (Wiley mill) and saved for later analysis. The 60°C DM contents of dietary ingredients were used weekly to adjust as-fed compositions of the TMR. Dry matter intake was determined based on the 60°C DM contents of the TMR andorts. Pooled weekly composites of TMR and feed ingredients were later analyzed for total N (Leco 2000), absolute DM, ash, and OM (AOAC, 1980), sequentially (Van Soest et al., 1991) for NDF and ADF using heat stable α -amylase and Na₂SO₃ (Hintz et al., 1995), and for NDIN and ADIN. Weekly TMR composites were analyzed for indigestible ADF (ADF remaining after 12 d of in situ incubation; Huhtanen et al., 1994). The TMR composites also were analyzed for total fat (method 920.39, AOAC, 1997; Dairyland Laboratories, Arcadia, WI) to compute NFC and for soluble sugars using sucrose as the standard and for starch (Hall et al., 1999; T. K. M. Webster, West Virginia Univ., Morgantown). Chemical compositions of diets are in Table 2.

Cows were milked twice daily and milk yield was recorded at each milking in all experimental periods. Milk samples from a.m. and p.m. milkings were collected on d 17 and 24 of each period and analyzed for fat, true protein, lactose, and SNF by infrared analysis (AgSource, Verona, WI) with a Foss FT6000 (Foss North America Inc., Eden Prairie, MN) using AOAC (1990;

method 972.16). For determination of MUN, 5-mL milk samples from both milkings were treated with 5 mL of 25% (wt/vol) TCA. Samples were vortexed and allowed to stand for 30 min at room temperature before filtering through Whatman #1 filter paper. Filtrates were stored at –20°C until MUN analysis by an automated colorimetric assay (Broderick and Clayton, 1997) adapted to flow-injection (Lachat Quik-Chem 8000 FIA). Concentrations and yields of fat, true protein, lactose, and SNF, and MUN concentration, were all computed as the weighted means from a.m. and p.m. milk yields on each test day. Efficiency of conversion of feed DM was computed for each cow over the last 2 wk of each period by dividing mean milk yield by mean DMI; similarly, apparent efficiency of utilization of feed N (assuming no retention or mobilization of body N) was calculated for each cow by dividing mean milk N output (milk true protein/6.38) by mean N intake. For computation of BW change, BW was measured on 3 consecutive days at the beginning of the experiment and at the end of each period.

Fecal grab samples were collected approximately 6 h prefeeding (a.m. sampling) and 6 h postfeeding (p.m. sampling) on d 26 or 27, transferred to aluminum pans, and held at 60°C in a forced-air oven until completely dried. Fecal samples then were ground to pass a 1-mm Wiley mill screen and a single composite was prepared for each cow in each period by mixing equal DM from both samples. Fecal samples were analyzed for DM, OM, NDF, ADF, total N, and indigestible ADF as described earlier. Indigestible ADF was used as an inter-

Table 2. Composition of diets¹

Item	Trial 1			Trial 2			
	CAS	TAS	RCS	CAS	TAS	RCS1	RCS2
Feed ingredients	(% of DM)						
Control alfalfa silage	40.3	—	—	50.4	—	—	—
ATF-treated alfalfa silage	—	40.1	—	—	50.4	—	—
Red clover silage 1	—	—	54.0	—	—	49.7	—
Red clover silage 2	—	—	—	—	—	—	50.6
Rolled corn silage	20.0	20.0	—	9.9	9.9	10.0	9.8
Rolled HMSC	32.9	33.1	33.4	36.1	36.1	30.6	35.8
Solvent soybean meal	6.3	6.3	6.3	3.2	3.2	9.2	3.4
Dried molasses	—	—	5.9	—	—	—	—
Dicalcium phosphate	0.1	0.1	0.1	—	—	—	—
Salt	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Vitamins and trace minerals ²	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Chemical composition							
CP, % DM	18.0	18.0	16.3	17.7	18.4	17.2	17.2
Ash, % of DM	7.8	7.8	10.0	7.38	7.12	8.15	8.08
NDF, % of DM	33.1	32.5	32.9	27.2	27.2	27.7	27.2
ADF, % of DM	22.5	21.7	21.8	18.2	18.0	16.9	16.1
RDP, ³ % of DM	11.6	11.6	9.85	13.2	13.7	12.3	12.7
RUP, ³ % of DM	6.38	6.41	6.45	4.50	4.59	5.03	4.52
RDP, ⁴ % of DM	—	—	—	14.7	14.2	11.5	12.0
RUP, ⁴ % of DM	—	—	—	3.31	4.22	6.07	5.21
Fat, % of DM	3.05	2.68	3.69	3.44	4.32	3.90	3.63
Sugar, % of DM	2.41	2.66	4.60	2.08	2.13	2.08	2.37
Starch, % of DM	29.4	31.0	24.6	28.0	26.0	27.8	27.1
NFC, ⁵ % of DM	38.7	39.7	37.8	43.1	44.3	45.9	46.8
NE _L , ⁶ Mcal/kg of DM	1.58	1.58	1.53	1.57	1.57	1.56	1.57

¹ATF = ammonium tetraformate; CAS = control alfalfa silage; HMSC = high moisture shelled corn; RCS1 = later maturity red clover silage; RCS2 = earlier maturity red clover silage; TAS = ATF-treated alfalfa silage.

²Provided (/kg of DM): 56 mg of Zn, 46 mg of Mn, 22 mg of Fe, 12 mg of Cu, 0.9 mg of I, 0.4 mg of Co, 0.3 mg of Se, 6440 IU of vitamin A, 2000 IU of vitamin D, and 16 IU of vitamin E.

³Estimated using the NRC (2001) model.

⁴Measured in vivo. See companion paper (Brito et al., 2007) for details.

⁵NFC = 100 – (%NDF + %CP + %Fat + %Ash) + %NDIN × 6.25.

⁶Computed by discounting dietary energy content based on actual DMI (NRC, 2001).

nal marker to estimate apparent nutrient digestibility and fecal output (Cochran et al., 1986). Blood samples were taken 4 h after feeding from the coccygeal artery or vein of each cow on d 27 of each period, transferred to scintillation vials, and stored at –20°C until later analysis. After thawing at room temperature, 5 mL of heparinized blood was transferred to a 15-mL centrifuge tube. Then, 1.25 mL of 25% TCA (wt/vol) was added to each tube, tubes vortexed, held at room temperature for 30 min, and centrifuged (15,000 × g, 15 min, 4°C). The supernatants were stored at –20°C until analyzed for BUN with the flow-injection system used for MUN.

Trial 2

Twenty-four (8 ruminally cannulated) multiparous Holstein cows averaging (mean ± SD) parity 2.7 ± 0.9, 36 ± 6 kg of milk/d, 192 ± 59 DIM, and 616 ± 70 kg of BW at the beginning of the trial were blocked by DIM and, within squares, randomly assigned to treatment sequences in 6 replicated 4 × 4 Latin squares (2 squares

of ruminally cannulated cows). Treatment sequences within each Latin square were organized to balance effects of carryover so each treatment would follow every other treatment one time in each Latin square. Experimental periods lasted 28 d and consisted of 14 d for diet adaptation and 14 d for data and sample collection, except for period 3, in which 14 d were allotted for diet adaptation and 7 d for data and sample collection. This was necessary because there was insufficient RCS2 (early maturity RCS) to complete all 4 periods and only 3 treatments were fed during period 4. This resulted in fewer replicates of the change from the diet containing RCS1 to that containing RCS2. The 4 diets were fed as TMR and formulated to contain (DM basis) about 50% CAS, TAS, RCS1 or RCS2 (Table 1), 10% rolled corn silage, plus minerals and vitamin supplements. Diets with CAS, TAS, and RCS2 contained 36% rolled HMSC and 3% SSBM; the RCS1 diet contained 31% rolled HMSC and 9% SSBM. Proportions of dietary DM from each ration ingredient were adjusted weekly as described in trial 1 except that diets also

were reformulated weekly to make the RCS1 diet about equal in CP to the CAS diet based on weekly CP analyses of dried (60°C; 48 h) and ground (1-mm screen; Wiley mill) samples of the CAS and RCS1 as well as initial CP contents of the corn silage, HMSC, and SSBM. Diet compositions are in Table 2. Otherwise, feeding protocol and feed sampling and analysis were as described for trial 1. Most other aspects of this trial, including milking, milk sampling and analysis, injection with bST, animal housing, BW measurements, and sampling and analysis of blood and feces, were as described for Trial 1. Care and handling of the animals, including ruminal cannulation, was conducted as outlined by the guidelines of the University of Wisconsin institutional animal care and use committee.

Spot urine samples were obtained approximately 6 h prefeeding (a.m. sampling) and 6 h postfeeding (p.m. sampling) on d 26 or 27 of each period (d 19 and 20 in period 3) by mechanical stimulation of the vulva. After collection, 15 mL of urine was pipetted into specimen containers holding 60 mL of 0.072 *N* H₂SO₄ and stored at -20°C until analysis. After thawing at room temperature, urine samples were analyzed for creatinine using a picric acid assay (Oser, 1965) adapted to flow-injection analysis (Lachat Quik-Chem 8000 FIA), for total N (Mitsubishi TN-05 Nitrogen Analyzer), for allantoin using the method of Vogels and van der Grift (1970) adapted to a 96-well plate reader, for uric acid with a commercial kit (ThermoDMA; Arlington, TX), and for urea with the colorimetric method used for MUN. Daily urinary volume and excretion of urea N, total N, and purine derivatives (PD; allantoin plus uric acid) were estimated from urinary creatinine concentrations assuming a creatinine excretion rate of 29 mg/kg of BW (Valadares et al., 1999). Samples of whole ruminal contents (about 200 mL) were taken from the ventral sac of the rumen of 8 ruminally cannulated cows on d 22 and 23 of each period (d 15 and 16 in period 3) at 0 (prefeeding), 1, 2, 4, 8, 12, 18, and 24 h postfeeding, strained through 2 layers of cheesecloth, followed by immediate pH measurement. Two 10-mL samples were then preserved in scintillation vials by addition of 0.2 mL of 50% H₂SO₄ and stored at -20°C until analysis. Samples were thawed at room temperature, centrifuged (15,000 × *g*, 15 min, 4°C), and supernatants analyzed for ammonia and total free AA (Broderick and Kang, 1980) using flow injection (Lachat Quik-Chem 8000 FIA) and for VFA using GLC (Brotz and Schaefer, 1987).

Statistical Analysis

Statistical analysis of chemical composition of silages fed in both trials was done using the GLM procedure

of SAS (SAS Institute, 1999). The model included silage source and sampling week. Data from the lactation trials were analyzed using Proc Mixed in SAS (SAS Institute, 1999) for replicated 3 × 3 (trial 1) and 4 × 4 (trial 2) Latin squares. The following model was fitted to all variables that did not have repeated measures over time:

$$Y_{ijkl} = \mu + S_i + P_j + C_{k(i)} + T_l + ST_{il} + E_{ijkl}$$

where Y_{ijkl} = dependent variable, μ = overall mean, S_i = effect of square i , P_j = effect of period j , $C_{k(i)}$ = effect of cow k (within square i), T_l = effect of treatment l , ST_{il} = interaction between square i and treatment l , and E_{ijkl} = residual error. All terms were considered fixed, except $C_{k(i)}$ and E_{ijkl} , which were considered random. The interaction term was removed from the model when $P \geq 0.25$. In trial 2, preplanned, single degree of freedom orthogonal contrasts were constructed to assess the effects of: ATF treatment of AS (CAS vs. TAS; ATF contrast), RCS maturity (RCS1 vs. RCS2; maturity contrast), and silage source (CAS + TAS vs. RCS1 + RCS2; silage contrast). Significance was declared at $P \leq 0.05$ and trends at $0.05 \leq P \leq 0.10$. All reported values are least squares means. When standard errors of the differences of the least squares means were not equal due to unequal replication in trial 2 the largest standard error was reported.

For ruminal pH, ammonia, free AA, and VFA (trial 2), which had repeated measures over time, the following model was used:

$$Y_{ijklm} = \mu + S_i + P_j + C_{k(i)} + T_l + ST_{il} + E1_{ijkl} + H_m + HT_{ml} + E2_{ijklm}$$

where Y_{ijklm} = dependent variable, μ = overall mean, S_i = effect of square i , P_j = effect of period j , $C_{k(i)}$ = effect of cow k (within square i), T_l = effect of treatment l , ST_{il} = interaction between square i and treatment l , and $E1_{ijkl}$ = whole plot error, H_m = effect of hours postfeeding analyzed as repeated measures, HT_{ml} = interaction between hour m and treatment l , and $E2_{ijklm}$ = subplot error. The spatial covariance structure SP(POW) was used for estimating covariances and the subject of the repeated measurements was defined as cow(square × period × treatment). All terms were considered fixed, except $C_{k(i)}$, $E1_{ijkl}$, and $E2_{ijklm}$, which were considered random. The interaction term was removed from the model when $P \geq 0.25$. Preplanned, single degree of freedom orthogonal contrasts were constructed to assess the effects of: ATF treatment of AS (CAS vs. TAS; ATF contrast), RCS maturity (RCS1 vs. RCS2; maturity contrast), and silage source (CAS + TAS vs. RCS1 + RCS2;

silage contrast). Significance was declared at $P \leq 0.05$ and trends were considered to exist if $0.05 < P \leq 0.10$. All reported values are least squares means.

RESULTS AND DISCUSSION

Forage Composition

Results of chemical analyses of the AS and RCS fed in both trials are in Table 1. As expected with third-cutting alfalfa, CAS was of high quality, averaging (DM basis) greater than 24% CP and less than 30% ADF and 40% NDF. The CP content of TAS was 1.2 percentage units higher than CAS. The GrasAAT ATF applied to AS had a density of 1.18 and contained 64.5% formic acid and 6.0% N wt/wt (I. Selmer-Olsen, Norsk Hydro ASA, Oslo, Norway; personal communication), equivalent to an ammonia:formate ratio of 0.22 rather than 0.25. The ATF product contained 5.5% wt/wt ammonia N by our analysis. Applying 7 L/t of ATF with 6.0% N to AS with 42.2% DM (Table 1) would have added N equivalent to 0.6% rather than 1.2% CP (DM basis). Although TAS was lower in ash than CAS, suggesting reduced OM fermentation in the silo (McDonald et al., 1991), CAS and TAS had similar contents of NDF, ADF, hemicellulose, NDIN, ADIN, and CP fraction B₃ (NDIN – ADIN; Sniffen et al., 1992). The main difference between the 2 AS was that TAS was 9.2% lower in NPN, principally due to reduced free AA. The NPN content of TAS was 45.3% of 25.8 percentage units of CP, equivalent to 11.7 percentage units of CP as NPN. If all of the 1.2 percentage units of extra CP in TAS were from N in ATF, then 10.5 percentage units of NPN would have been formed from the 24.6 percentage units of CP in the original CAS. This was equivalent to ATF-treatment reducing breakdown of AS CP from 12.3 (0.499×24.6) to 10.5 percentage units, a decrease of nearly 15%. Nagel and Broderick (1992) found that applying 8.2 L of formic acid/ton to AS with 41% DM reduced NPN from 43 to 29% of total N, a 33% reduction that was more than twice the effect obtained with 7 L of ATF/ton of AS.

The CP and NDF content of early-cut RCS2 was similar to CAS, and its ADF content was lower than the later-cut RCS1, indicating that it was of substantially higher quality (Table 1). Although ash was greater in RCS than AS, lower ADF indicated that the 2 RCS contained about 4 percentage units more hemicellulose. Thus, the goal of obtaining an RCS with similar CP as the AS was met with the early maturity RCS2. However, late maturity RCS1 was about 2 percentage units higher in NDF than the AS, probably because of the alfalfa fed in the current studies was relatively immature. In 5 previous trials, AS averaged 20.9% CP and

43.3% NDF and RCS averaged 17.9% CP and 43.0% NDF (Broderick, 2002).

The silages were most different in composition of the N fractions. Both RCS were higher in NDIN and contained, on average, 4.2 times more fraction B₃ (NDIN – ADIN) and 41% less NPN than AS (Table 1). Papadopoulos and McKersie (1983) reported that reduced NPN was associated with the browning reaction in RCS. Numerous workers (e.g., Muck, 1987; Albrecht and Muck, 1991; Broderick, 2002) observed lower NPN formation in RCS, which does not result from inherently lower proteolytic activity or more rapid pH decline to a lower final pH (Jones et al., 1995a; Jones et al. 1995c). Rather, polyphenol oxidase activity and *o*-diphenols, both normally present in red clover tissue in high amounts, react with O₂ to form *o*-quinones that rapidly interact with proteins, including the plant proteases, to inhibit proteolysis (Hatfield and Muck, 1999). Jones et al. (1995b) evaluated 38 legumes including alfalfa and found that only red clover had measurable polyphenol oxidase activity and gave rise to extract browning.

Although RCS2 was similar to both AS, RCS1 contained more than twice as much ADIN as CAS and TAS. Means from 5 previous trials were (% of total N) 3.5% ADIN in AS and 5.1% ADIN in RCS (Broderick, 2002). Substantially greater ADIN in RCS1 than RCS2 was surprising and difficult to explain, although overheating during ensiling cannot be ruled out (Yu and Thomas, 1976). Van Soest (1965) indicated that ADIN content of forages not heat-damaged may be as high as 7% of total N; however, greater ADIN in RCS1 relative to RCS2 suggested that the later maturity RCS could have sustained excessive heating, which impaired utilization of its protein.

Trial 1

Diets were formulated to have equal CP but both AS were substantially higher in CP than the assumed value of 22% (Table 1). Therefore, although about equal to each other, the CAS and TAS diets had 1.7 percentage units more CP than the diet containing RCS (Table 2), which likely influenced N efficiency in this trial. All 3 diets had similar amounts of NDF and ADF; computed (NRC, 2001) RUP and NE_L also were similar but NFC (computed from chemical composition) and RDP (computed from NRC, 2001) were lower on RCS (Table 2). Production and nutrient utilization results are summarized in Table 3. Intake of DM was greater on the RCS diet vs. CAS and similar to TAS, indicating that adding dried molasses had the desired effect of counteracting the intake depression previously observed with RCS (Broderick et al., 2000, 2001; Broderick, 2002). Despite similar intake on RCS, yields of milk, FCM, true pro-

Table 3. Effect of feeding control alfalfa silage (CAS) or ammonium tetraformate-treated alfalfa silage (TAS) plus corn silage, or red clover silage (RCS) plus dried molasses, on production, apparent digestibility, and N excretion in lactating cows (trial 1)

Item	CAS	TAS	RCS	SE	<i>P</i> > <i>F</i> ¹
DMI, kg/d	24.0 ^b	25.0 ^a	24.9 ^a	0.8	<0.01
Milk yield, kg/d	27.8 ^b	30.2 ^a	28.4 ^b	1.0	0.01
3.5% FCM, kg/d	30.3 ^b	32.4 ^a	30.0 ^b	1.2	0.04
Milk yield/DMI	1.16 ^{ab}	1.20 ^a	1.15 ^b	0.03	0.05
Milk N/N intake, %	21.1 ^b	22.4 ^a	22.3 ^a	0.4	0.01
Milk fat, %	4.20 ^a	3.96 ^b	3.95 ^b	0.15	<0.01
Milk fat, kg/d	1.14	1.19	1.10	0.06	0.08
Milk true protein, %	3.40 ^a	3.43 ^a	3.29 ^b	0.08	<0.01
Milk true protein, kg/d	0.93 ^b	1.03 ^a	0.92 ^b	0.04	<0.01
Milk lactose, %	4.74	4.78	4.79	0.06	0.21
Milk lactose, kg/d	1.30 ^b	1.44 ^a	1.34 ^b	0.06	0.01
Milk SNF, %	9.05 ^{ab}	9.12 ^a	8.99 ^b	0.12	0.02
Milk SNF, kg/d	2.48 ^b	2.75 ^a	2.52 ^b	0.10	<0.01
MUN, mg/dL	17.8 ^a	18.0 ^a	13.8 ^b	0.4	<0.01
BW gain, kg/d	0.31	0.44	0.64	0.16	0.37
Apparent digestibility, %					
DM	57.0 ^b	56.6 ^b	63.3 ^a	1.2	<0.01
OM	58.1 ^b	57.4 ^b	63.5 ^a	1.3	<0.01
NDF	35.5 ^b	35.6 ^b	53.2 ^a	1.1	<0.01
ADF	40.6 ^b	38.0 ^b	55.2 ^a	1.7	<0.01
Hemicellulose	24.3 ^b	30.7 ^b	49.0 ^a	3.6	<0.01
CP	55.8 ^a	55.3 ^a	46.0 ^b	1.4	<0.01
Excretion, kg/d					
Fecal DM	10.4 ^a	10.9 ^a	9.1 ^b	0.5	<0.01
Fecal N	0.298 ^b	0.318 ^b	0.351 ^a	0.015	0.01
Urinary N (difference) ²	0.232 ^a	0.231 ^a	0.156 ^b	0.011	<0.01

^{a,b}Least squares means in the same row with different superscripts differ (*P* < 0.05).

¹Probability of a significant effect of diet.

²Urinary N = N intake – milk true protein/6.38 – fecal N.

tein, lactose, and SNF, and milk yield/DMI were greater on TAS. Overall, production on CAS and RCS was similar, except that milk content of fat and true protein was greater on CAS (Table 3).

Except for CP, apparent nutrient digestibility (estimated using indigestible ADF as internal marker) was greater on RCS (Table 3). Compared with both AS, digestibility was increased by an average of 10% for DM and OM, 50% for NDF, 40% for ADF, and 78% for hemicellulose on the RCS diet. Differences of this magnitude indicated that the greater energy availability when cows were fed RCS was unaccounted for; apparent BW gain was numerically but not significantly higher than on the 2 AS. Of course, not all of the production differences can be attributed to the legume silages alone because rolled corn silage was fed with AS and dried molasses was fed with RCS. Previously, OM and NDF digestibilities were increased an average of 8 and 22% over 5 trials in which 60% of dietary DM was fed as RCS rather than AS (Broderick, 2002). Hoffman et al. (1993) observed that in situ DM digestibilities were 5 to 9 percentage units higher for red clover than alfalfa in forage samples ranging in maturity from late vegetative to mid bloom. Hoffman et al. (1997) reported greater in situ NDF digestibility and in vitro DM digest-

ibility for RCS than AS in 1 out of 2 harvest years. Moreover, replacing HMSC with dried molasses, which contains about 60% soluble sugars (sucrose, glucose, and fructose) and 27% NDF from soyhulls (D. Caldwell, Westway Feed Products, Cedar Lake, IN; personal communication), also may have influenced fermentation in the rumen. Broderick and Radloff (2004) reported that the major effect of substituting dried molasses for HMSC was increased feed intake and DM digestibility with no alteration of yield of milk or milk components. Although urinary PD excretion was not affected in that study, depressed ruminal ammonia suggested a possible improvement in microbial protein supply with the feeding of dried molasses.

Similar apparent N efficiencies on TAS and RCS were confounded by the lower CP intake on the latter diet. However, lower MUN and urinary N excretion (estimated by difference) suggested improved utilization of absorbed N when RCS was fed. Conversely, the 17% lower apparent CP digestibility and the greater fecal N excretion suggested impaired intestinal protein digestion, possibly related to excessive ADIN formation in RCS. Simple comparison of N utilization data among the 3 dietary treatments could not be made because of the influence of CP on MUN and dilution of metabolic

fecal N. However, Nousiainen et al. (2004) observed a slope of 1.7 mg of MUN/dL per percentage unit of dietary CP, which was equivalent to 2.9 mg of MUN/dL for 1.7% greater CP. The mean difference observed between AS and RCS was 4.1 mg/dL (Table 3), suggesting a relative reduction in MUN due to RCS feeding. Subtracting metabolic fecal CP assumed equal to 9% of fecal DM (NRC, 1989; Table 3) yielded estimates of "true" CP digestibility of 79, 78, and 66% for, respectively, diets containing CAS, TAS, and RCS. This computation, as well as significantly greater estimated fecal N excretion (Table 3), suggested that reduced NPN in RCS did not translate into greater productivity because CP utilization was impaired by excessive protein damage. Depressed CP digestion may have limited the supply of RDP on the RCS diet. However, ruminal contents were not sampled in this trial so the degree to which RDP supply may have been depressed could not be determined.

Feeding TAS improved DMI, yield of milk and milk components, and feed efficiency (milk yield/DMI) compared with the other 2 diets. These responses were consistent with the 9, or estimated 15%, reduction in NPN with ATF-treatment of AS discussed earlier. Nagel and Broderick (1992) observed daily yield improvements of 3.4 kg of milk, 110 g of protein, and 120 g of fat when cows were fed high forage diets containing formic acid-treated AS, in which NPN was reduced from 43 to 29% of total N. Yields were greater by 2 or 1.5 kg of milk/d and 70 or 100 g of protein/d when alfalfa hay (with less than 10% NPN) replaced equal DM from AS (with 49 or 43% NPN) in 2 of 3 trials (Broderick, 1995; Vagnoni and Broderick, 1997). Despite greater dietary CP (due to lower leaf-loss during silage harvest), cows fed AS were more responsive to RUP. Supplementing AS diets with high RUP fish meal increased milk protein yield (average 100 g/d) in 3 of 3 trials but significantly improved milk protein yield (average 30 g/d) in only 1 of 3 trials on alfalfa hay diets. Clearly, NPN formation in hay-crop silages impairs CP utilization; anything reducing NPN without altering intestinal AA availability should improve N efficiency.

Trial 2

The CP contents of diets were more similar in this trial, ranging from 17.2 to 18.4%; NDF averaged 27% and differed only by 0.5 percentage units across diets (Table 2). However, dietary ash, NDIN, and ADIN contents were higher, and ADF lower, on RCS, reflecting the relative composition of the forages fed in this study (Table 1). The NE_L , computed using the NRC (2001) model, and NFC contents were nearly equal across diets.

Effects of diet on DMI and on milk yield and composition are in Table 4. Intake of DM was similar for cows fed CAS and TAS, and for those fed RCS1 and RCS2, but averaged 1.4 kg/d less ($P < 0.01$) for cows consuming RCS vs. AS. Dewhurst et al. (2003a) showed that the ruminal outflow rate of undigested feed residues from AS was more rapid than those from RCS. Therefore, greater DMI on AS in the present trial may have been due to longer retention of undigested RCS residues, resulting in increased ruminal fill. Hoffman et al. (1997) reported similar intakes on these forages; however, Broderick et al. (2001) in 2 trials, and Broderick et al. (2000) in 1 of 3 trials, observed higher DMI when cows were fed AS vs. RCS.

Yields of milk, FCM, fat, true protein, lactose, and SNF and milk contents of protein and SNF were greater ($P < 0.01$), as was milk fat content ($P < 0.03$) when cows were fed AS vs. RCS (Table 4). Although Hoffman et al. (1997) reported higher yields of milk, protein, and fat in cows fed RCS vs. AS in 1 of 2 trials, Broderick et al. (2000) observed greater milk and protein yield in 2 of 3 trials and greater FCM and fat yield in 3 of 3 trials when cows were fed AS instead of RCS. However, equal DM from each silage was fed and, because AS was higher in CP, greater protein in AS (average 17.8% CP) vs. RCS diets (average 15.1% CP) confounded interpretation of the earlier findings (Broderick et al., 2001). When Broderick et al. (2001) fed diets with similar CP, no significant differences in yield of milk, FCM, and milk components were observed. Higher milk true protein content and yield found in the present study probably were related to greater total microbial NAN flow in cows fed AS (Brito et al., 2007), which would have increased AA supply for milk protein synthesis. Unlike trial 1, yields of milk and milk components were not increased on TAS vs. CAS (Table 4). Intake was greater on TAS than CAS in trial 1 (Table 3), which probably accounted for at least part of that response.

Both BUN and MUN were lower on RCS vs. AS, averaging 20 and 23 mg/dL (BUN) and 15 and 19 mg/dL (MUN; Table 4). Greater BUN and MUN are expected on higher CP diets; Broderick (2002) found mean MUN of 12.5 mg/dL on diets containing 63% AS with 17.7% CP and mean MUN of 8.7 mg/dL on diets containing 63% RCS with 15.8% CP. The regression of Nousiainen et al. (2004) indicating that MUN increased 1.7 mg/dL per percentage unit increase in dietary CP would explain most of the difference reported by Broderick (2002). However, the 0.5- and 1.2-percentage-units greater CP in AS diets in trial 2 (Table 2) would account for only 0.9 and 2.0 mg/dL (Nousiainen et al., 2004), not the 4 mg/dL greater MUN that was actually observed. There were no differences in MUN and BUN between CAS and TAS, which suggested that the reduction in

Table 4. Effects of feeding dietary forage as alfalfa silage or red clover silage on production of lactating dairy cows (trial 2)

Item	Diets ¹				SED ²	Contrasts (<i>P</i> -value) ³		
	CAS	TAS	RCS1	RCS2		ATF	RCS	Silage
DMI, kg/d	24.0	24.6	22.8	23.0	0.71	0.33	0.75	<0.01
N intake, g/d	703	743	640	641	21.0	0.02	0.96	<0.01
Milk yield, kg/d	30.4	30.6	29.4	28.7	0.74	0.80	0.31	<0.01
3.5% FCM, kg/d	33.3	33.4	31.1	30.1	0.94	0.88	0.29	<0.01
Milk yield/DMI	1.26	1.24	1.29	1.25	0.03	0.38	0.15	0.38
Milk fat, %	3.99	3.99	3.86	3.78	0.12	0.99	0.49	0.03
Milk fat, kg/d	1.23	1.24	1.13	1.08	0.04	0.75	0.30	<0.01
Milk true protein, %	3.26	3.31	3.18	3.13	0.03	0.12	0.17	<0.01
Milk true protein, kg/d	1.00	1.02	0.93	0.90	0.03	0.44	0.25	<0.01
Lactose, %	4.82	4.82	4.83	4.83	0.04	0.94	0.91	0.67
Lactose, kg/d	1.50	1.49	1.44	1.40	0.04	0.69	0.39	<0.01
SNF, %	9.00	9.04	8.93	8.86	0.07	0.58	0.33	<0.01
SNF, kg/d	2.79	2.79	2.64	2.56	0.08	0.97	0.32	<0.01
MUN, mg/dL	19.0	18.1	15.2	14.8	0.78	0.18	0.62	<0.01
BUN, mg/dL	23.2	23.4	19.6	20.1	0.49	0.60	0.30	<0.01
BW gain, kg/d	0.23	0.12	0.63	0.66	0.22	0.58	0.89	<0.01

¹CAS = control alfalfa silage; TAS = ammonium tetraformate-treated alfalfa silage; RCS1 = red clover silage 1; RCS2 = red clover silage 2.

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

³ATF = CAS vs. TAS; RCS = RCS1 vs. RCS2; Silage = CAS + TAS vs. RCS1 + RCS2.

NPN was insufficient to obtain a detectable improvement in N utilization in the present study.

Cows in this experiment gained an average 0.47 kg/d more BW ($P < 0.01$) on RCS than AS, even though DMI was 1.4 kg/d greater for cows fed AS (Table 4). Average milk fat yield also was reduced 130 g/d ($P < 0.01$) with feeding of RCS. Fat yield in trial 1 was numerically lower (90 g/d) on RCS than on TAS (Table 3). Earlier, we observed a trend over 5 trials for greater BW gain that was accompanied by significantly reduced milk fat content and a trend for reduced fat yield, on RCS vs. AS (Broderick, 2002). Although diets averaged 27% NDF during trial 2 (Table 2), neither the pattern (Figure 1) nor overall means (Table 5) suggested that ruminal pH was a factor in the milk fat depression observed on RCS. Dietary unsaturated fatty acids were not determined and total dietary fat was similar across diets within each trial (Table 2). However, the observed differences in energy partitioning between gain and milk fat secretion suggested that there might have been incomplete ruminal biohydrogenation of dietary linoleic acid on the RCS diets (Bauman and Griinari, 2001). Lee et al. (2003) reported that, compared with grass silage, feeding RCS increased duodenal flow of *cis*-vacenic acid, linoleic acid, α -linolenic acid, and *cis*-9, *trans*-11 conjugated linoleic acid.

Apparent total tract digestibilities of all nutrients except CP were higher in cows fed RCS (Table 5). As discussed above, data from trial 1 and our earlier work (Broderick, 2002) also showed greater total tract apparent digestibilities of DM, OM, NDF, ADF, and hemicel-

lulose when RCS replaced dietary AS. Coblenz et al. (1998) observed higher extent of ruminal degradation of material from whole red clover plant vs. whole alfalfa plant. Hoffman et al. (1993) reported that red clover had greater ruminal DM degradation in situ than alfalfa at all stages of maturity, and speculated that this was caused by differences in phenological growth characteristics between the 2 plants. However, higher nutrient digestibilities did not consistently improve production in the studies cited above and in the present trial because the extra energy derived from RCS was channeled to BW gain rather than milk yield.

Although there were no differences between cows fed either CAS or TAS, cows consuming more mature RCS1 always had lower apparent digestibility than cows fed less mature RCS2 (Table 5). Lower CP digestibility on RCS1 probably was associated with that silage's higher ADIN content as discussed for trial 1. Fraction C of the Cornell Net Carbohydrate and Protein System (ADIN) is composed of proteins associated with tannins and lignin as well as by heat-damaged proteins such as Maillard products and is considered to be indigestible in both the rumen and intestines (Sniffen et al., 1992).

Ruminal pH did not differ and averaged 6.4 across diets (Table 5). In addition, the time $\times$ diet interaction was not significant ($P = 0.26$) for pH in this trial. We did not observe significant differences in ruminal pH when feeding these forages in our earlier work (Broderick et al., 2000, 2001). As expected, ruminal pH declined with time after feeding but did not fall below 6.0 on any diet except at 8 h postfeeding on the CAS treatment

Figure 1. Effects of feeding dietary forage as alfalfa silage or red clover silage on ruminal pH. CAS = control alfalfa silage; TAS = ammonium tetraformate-treated alfalfa silage; RCS1 = red clover silage 1 (late maturity); RCS2 = red clover silage 2 (early maturity). Error bars indicate ± 1 SEM. Time $\times$ diet interaction was not significant ($P = 0.26$).

(pH = 5.99; Figure 1). Ruminal ammonia N and total free AA were higher ($P < 0.01$; Table 5) in cows fed AS diets, reflecting the greater NPN (Table 1) and RDP supply (Brito et al., 2007). Broderick et al. (2000) also observed greater amounts of these metabolites in the rumens of cows fed AS vs. RCS. However, Broderick et al. (2001) found similar ruminal concentrations of ammonia and total free AA on the 2 silages. Ruminal acetate, propionate, acetate:propionate ratio, and total VFA did not differ when AS was replaced by RCS (Table 5). However, cows fed the AS diets had greater ruminal concentrations of butyrate, valerate, isobutyrate, and isovalerate. The branched-chain VFA arise largely from ruminal catabolism of branched-chain AA (Hoover, 1986) and AS diets supplied more RDP (Brito et al., 2007), which probably explains this effect. Within silages, CAS gave rise to higher ruminal isobutyrate ($P = 0.05$) and tended to have higher ($P = 0.07$) isovalerate than TAS, whereas cows fed RCS2 showed increased ($P = 0.04$) isovalerate compared with those fed RCS1. Waghorn (1986) observed greater ruminal concentrations of butyrate, valerate, and isovalerate in Jersey

cows fed fresh alfalfa vs. fresh red clover. Dewhurst et al. (2003b) demonstrated that feeding white clover silage or AS, but not RCS, increased molar proportions of isobutyric, isovaleric, and valeric acids in the rumen.

Urine volume, estimated from urinary creatinine concentration, was lower ($P < 0.01$) on AS than on RCS diets (Table 6). Within pairs of diets, cows fed RCS1 had significantly greater urinary output ($P = 0.02$) than those fed RCS2, but there was no difference between CAS and TAS (Table 6). Urine volume is related to intake of mineral cations, particularly potassium (Kojima et al., 2005), and potassium has been reported to constitute 33 to 48% of forage ash (Anke et al., 1995). Although we observed no difference in ash content over 5 previous studies (Broderick, 2002), RCS was higher in total ash than AS in the present trials (Table 1). Urinary excretion of allantoin was higher ($P = 0.02$) on AS than on RCS diets, whereas the opposite was observed for uric acid (Table 6). Within RCS diets, cows fed RCS1 excreted 14.6 mmol/d more uric acid ($P < 0.01$) than those fed RCS2. No significant differences were found for urinary excretion of PD, which averaged 439

Table 5. Effects of feeding dietary forage as alfalfa silage or red clover silage on total tract apparent digestibility and ruminal metabolism in lactating dairy cows

Item	Diets ¹				SED ²	Contrasts (<i>P</i> -value) ³		
	CAS	TAS	RCS1	RCS2		ATF	RCS	Silage
Apparent digestibility								
DM, %	58.8	57.7	60.9	64.1	1.00	0.20	<0.01	<0.01
OM, %	59.3	57.9	60.8	64.3	1.02	0.13	<0.01	<0.01
NDF, %	37.2	37.0	48.5	54.9	1.11	0.79	<0.01	<0.01
ADF, %	40.2	40.1	50.9	57.4	1.02	0.92	<0.01	<0.01
Hemicellulose, %	30.5	30.0	44.1	50.7	1.57	0.74	<0.01	<0.01
CP, %	56.5	56.4	45.8	51.4	1.19	0.92	<0.01	<0.01
Ruminal metabolism								
pH	6.38	6.41	6.45	6.45	0.05	0.45	0.91	0.09
Ammonia N, mg/dL	14.0	12.7	6.48	7.90	1.00	0.15	0.16	<0.01
Total free AA, mM	3.97	3.44	2.29	2.86	0.40	0.14	0.15	<0.01
Acetate, mM	74.4	73.8	75.7	73.5	2.31	0.77	0.32	0.73
Propionate, mM	21.2	21.1	21.9	21.2	0.91	0.89	0.42	0.58
Butyrate, mM	13.1	13.6	12.7	12.3	0.62	0.38	0.50	0.05
Isobutyrate, mM	1.56	1.45	0.99	1.07	0.06	0.05	0.22	<0.01
Valerate, mM	1.95	1.89	1.66	1.70	0.15	0.67	0.77	0.02
Isovalerate, mM	2.45	2.26	1.14	1.39	0.12	0.07	0.04	<0.01
Total VFA, mM	115	114	114	111	3.85	0.86	0.40	0.48
Acetate:propionate	3.56	3.57	3.55	3.54	0.09	0.92	0.94	0.79

¹CAS = control alfalfa silage; TAS = ammonium tetraformate-treated alfalfa silage; RCS1 = red clover silage 1; RCS2 = red clover silage 2.

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

³ATF = CAS vs. TAS; RCS = RCS1 vs. RCS2; Silage = AS + TAS vs. RCS1 + RCS2.

mmol/d across diets (Table 6). Urinary allantoin accounted for an average 84% of the total PD across diets and was similar to previous reports (Vagnoni and Broderick, 1997; Valadares et al., 1999; Broderick et al., 2000). Using only allantoin to estimate microbial protein synthesis, cows fed AS yielded on average 28 g/d more microbial protein ($P = 0.02$) than those fed RCS diets in the present trial (Table 6). However, no significant difference was observed in microbial N flow when total PD was used as the indirect microbial marker (Table 6). The small difference (11%) between AS and RCS computed from allantoin only, and the apparent contradiction with results derived from total PD, prevented any conclusions being made regarding microbial growth in the rumen. However, omasal sampling indicated that total microbial NAN flow was about 20% greater on AS than RCS diets (Brito et al., 2007).

Total urinary N excretion was greater ($P < 0.01$) for cows fed AS compared with those fed RCS but no differences were observed within silage groups (Table 6). This higher urinary N excretion in cows fed AS diets was probably related to greater dietary CP content (Table 2) and N intake (Tables 4 and 6) compared with RCS diets. Furthermore, higher NPN content in AS has been associated with poor N efficiency (Nagel and Broderick, 1992; Broderick et al., 2001), which also might increase urinary N. When expressed as a percentage of N intake, total urinary N excretion also was greater ($P < 0.01$) for cows fed AS (Table 6). A trend

($P = 0.07$) for improved N efficiency (milk N/N intake) was observed for cows fed RCS diets. A trend ($P = 0.09$) also was detected for greater N efficiency, expressed as kilograms of milk yield per kilogram of N excreted, in cows fed CAS vs. TAS but AS and RCS were not different (Table 6). Urinary urea N excretion paralleled total urinary N and was higher ($P < 0.01$) on AS than RCS (Table 6). Urea N accounted for an average 76% of total urinary N (Table 6), which agreed with previous reports (Sannes et al., 2002; Broderick, 2003). However, only 19% of consumed N was excreted as urinary urea N in cows fed RCS, which was lower ($P < 0.01$) than the 23% observed on AS, indicating reduced potential for N pollution on RCS diets.

Inefficient N utilization in dairy cows has a negative impact on the environment (Tamminga, 1992). In the present trial, it was hypothesized that lower NPN would improve N efficiency and consequently milk production. Feeding RCS diets significantly decreased ruminal ammonia, MUN, BUN, and urinary N excretion; however, DMI and yield of milk, FCM and all milk components also were reduced on RCS. Therefore, better utilization of RCS N was not associated with improved production in the present study. Olmos Colmenero and Broderick (2006) showed that, despite a linear increase in MUN when dietary CP was increased from 13.5 to 19.4%, milk and milk protein yields were sustained by a diet containing 16.5% CP. Increasing dietary RDP from 6.8 (12.3% CP) to 11% (17.1% CP) also

Table 6. Effects of feeding dietary forage as alfalfa silage or red clover silage on urinary nitrogen excretion and efficiency, and fecal excretion in lactating dairy cows

Item	Diets ¹				SED ³	Contrasts (<i>P</i> -value) ²		
	CAS	TAS	RCS1	RCS2		ATF	RCS	Silage
Total volume, L/d	23.2	21.6	28.3	25.5	1.22	0.14	0.02	<0.01
N intake, g/d	703	743	640	641	21.0	0.02	0.96	<0.01
Urinary excretion								
Total N, g/d	204	210	151	159	5.47	0.21	0.16	<0.01
Total N, % of N intake	29.7	29.0	23.8	25.3	1.28	0.57	0.24	<0.01
Urea N, g/d	158	162	116	119	5.34	0.42	0.61	<0.01
Urea N, % of total N	77.4	77.4	76.9	74.1	2.87	0.98	0.33	0.33
Urea N, % of N intake	23.0	22.3	18.3	19.0	1.07	0.46	0.51	<0.01
Allantoin, mmol/d	373	399	344	356	21.2	0.20	0.58	0.02
Uric acid, mmol/d	62.3	60.4	87.0	72.4	4.79	0.66	<0.01	<0.01
Purine derivatives, mmol/d	436	459	431	429	24.6	0.30	0.92	0.29
Microbial N flow, ⁴ g/d	231	252	211	217	17.0	0.18	0.77	0.02
Microbial N flow, ⁵ g/d	248	268	249	243	18.0	0.24	0.76	0.33
Fecal excretion								
DM, kg/d	9.96	10.6	8.99	8.22	0.77	0.08	0.05	<0.01
OM, kg/d	9.10	9.75	8.27	7.50	0.36	0.05	0.04	<0.01
NDF, kg/d	4.15	4.27	3.27	2.79	0.15	0.33	<0.01	<0.01
ADF, kg/d	2.67	2.71	1.90	1.57	0.09	0.61	<0.01	<0.01
N, g/d	306	324	348	313	13.0	0.11	0.01	0.08
N, % of N intake	43.5	43.6	54.2	48.6	1.19	0.92	<0.01	<0.01
Total N excretion								
g/d	509	534	499	472	15.0	0.08	0.09	<0.01
% of N intake	73.0	72.4	77.9	74.0	1.76	0.72	0.03	<0.01
N efficiency								
Milk N, % of N intake	21.7	20.7	22.8	21.5	0.74	0.12	0.09	0.07
kg of milk:kg of N excreted	59.3	56.2	59.3	59.5	1.97	0.09	0.93	0.23

¹CAS = control alfalfa silage; TAS = ammonium tetraformate-treated alfalfa silage; RCS1 = red clover silage 1; RCS2 = red clover silage 2.

²ATF = CAS vs. TAS; RCS = RCS1 vs. RCS2; Silage = AS + TAS vs. RCS1 + RCS2.

³SED = standard error of the least squares means difference.

⁴Estimated from urinary allantoin excretion and ⁵from urinary excretion of purine derivatives according to Vagnoni et al. (1997).

increased milk protein content from 2.95 to 3.11%, milk protein yield from 0.94 to 1.05 kg/d, and MUN from 9.5 to 16.4 mg/dL (Kalscheur et al., 2006); milk protein content and yield were sustained at a dietary RDP level of 9.6% (15.5% total CP). Results from both of these studies suggested that formulating diets for more than 16.5% CP reduced N efficiency without improving production. Nousiainen et al. (2004) reported that grass silage-based diets giving rise to 12 mg of MUN/dL provided sufficient RDP to satisfy the requirements of ruminal microorganisms. However, these authors also stated that production responses were obtained at MUN greater than 16 mg/dL, although at reduced N efficiency. In the present trial, milk and milk true protein yields were decreased at 15 mg/dL of MUN (RCS diets) and urinary N excretion was increased at 19 mg/dL of MUN (AS diets).

Castillo et al. (2001) showed that increasing the dietary CP level from 15.0 to 17.8% increased urinary N excretion by 74 g of N/d; urinary N excretion also was increased with greater RDP supplementation without improving production. These authors reported that, be-

low an intake of about 400 g of N/d, the principal route of N excretion was feces; urinary N excretion increased exponentially above this level. Olmos Colmenero and Broderick (2006) found a linear increase from 23.8 to 36.2% and a linear decrease from 40.3 to 29.6% in, respectively, urinary and fecal N excretion as proportions of dietary N intake when N intake increased from 483 to 711 g/d. In the present trial, cows fed RCS consumed 83 g/d less N than those fed AS, which resulted in a tendency ($P = 0.08$) for increased fecal N excretion on RCS diets. In addition, fecal N as a proportion of N intake was greater ($P < 0.01$) on RCS diets (Table 6), indicating diversion of N from urine to feces when animals ingest lower amounts of N. Because urinary N is less desirable due to its greater tendency to leaching (Pakrou and Dillon, 1995) and ammonia volatilization, a shift in N excretion from urine to feces is desirable. Lines and Weiss (1996) and Castillo et al. (2001) both reported that fecal N excretion increased and urinary N excretion declined when RUP supply was increased. For cows fed RCS, greater RUP flow (Brito et al., 2007) was associated with lower urinary N excretion but

greater fecal N as a proportion of N intake; thus, N excretion was shifted proportionately from urine to feces on RCS diets.

CONCLUSIONS

Treating AS with ATF reduced NPN content and, when fed as the principal forage, increased intake and yield of milk and milk components compared with untreated AS in 1 of 2 trials. Red clover silage of early maturity, with CP content about equal to AS, or of later maturity had only 60% as much NPN as a proportion of total N, and feeding RCS instead of either AS reduced MUN, BUN, ruminal ammonia, and urinary N excretion, and increased fecal N excretion and apparent efficiency of capture of feed N in milk protein. However, replacing AS with RCS did not improve milk production in the first trial and decreased intake and milk production in the second trial. The later maturity RCS had elevated ADIN, which appeared to impair N utilization. Although apparent digestibility of DM, OM, and fiber was always greater on diets containing RCS vs. AS, greater weight gain indicated that the extra energy derived from RCS was channeled into fat storage rather than milk fat secretion. Apparent N efficiency was higher for cows fed RCS; however, there were no differences between RCS and AS when N efficiency was expressed as kilograms of milk yield per kilogram of total N excreted.

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