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Production of Lactating Dairy Cows Fed Alfalfa or Red Clover Silage at Equal Dry Matter or Crude Protein Contents in the Diet[1]

G. A. Broderick,* R. P. Walgenbach,* and S. Maignan†
*Agricultural Research Service, USDA
US Dairy Forage Research Center
1925 Linden Drive West, Madison 53706,
†Ecole Superieure D'Agriculture, 55 Rue Rabelais, 49007 Angers, France

ABSTRACT

Two Latin square trials, using 21 or 24 multiparous lactating Holstein cows, compared the feeding value of red clover and alfalfa silages harvested over 2 yr. Red clover silages averaged 2 percentage units lower in crude protein (CP) and more than 2 percentage units lower in neutral detergent fiber and acid detergent fiber than did alfalfa silage. In trial 1, diets were formulated to 60% dry matter (DM) from alfalfa, red clover silage, or alfalfa plus red clover silage (grown together); CP was adjusted to about 16.5% by adding soybean meal, and the balance of dietary DM was from ground high moisture ear corn. Nonprotein N in red clover and alfalfa-red clover silages was 80% of that in alfalfa silage. Although DM intake was 2.5 and 1.3 kg/d lower on red clover and alfalfa plus red clover, yield of milk and milk components was not different among diets. In trial 2, four diets containing rolled high moisture shelled corn were formulated to 60% DM from alfalfa or red clover silage, or 48% DM from alfalfa or red clover silage plus 12% DM from corn silage. The first three diets contained 2.9% soybean meal, and the red clover-corn silage diet contained 5.6% soybean meal; the 60% alfalfa diet contained 18.4% CP, and the other three diets averaged 16.5% CP. Nonprotein N in red clover silage was 62% of that in alfalfa silage. Intake of DM was about 2 (no corn silage) and 1 kg/d (plus corn silage) lower on red clover. Yield of milk and milk components was not different among the first three diets; however, yields of milk, total protein, and true protein were higher on red clover-corn silage with added soybean meal. Replacing alfalfa with red clover improved feed and N efficiency and apparent digestibility of DM, organic matter, neutral detergent fiber, acid detergent fiber, and hemicellulose in both trials. Net energy of lactation computed from animal performance data was 18% greater in red clover than alfalfa. Data on milk and blood urea and N efficiency suggested better N utilization on red clover. (**Key words:** alfalfa silage, red clover silage, nonprotein N)

Abbreviation key: ARCS = alfalfa plus red clover silage, **AS** = alfalfa silage, **HMEC** = high moisture ear corn, **HMSC** = high moisture shelled corn, **MUN** = milk urea N, **RCS** = red clover silage, **SBM** = soybean meal, **CS** = corn silage.

INTRODUCTION

The action of the polyphenol oxidase enzyme system in red clover (Jones et al., 1995) results in silage (**RCS**) containing 30 to 40% less NPN, as a proportion of total N, than is present in alfalfa silage (**AS**; Albrecht and Muck, 1991). Large amounts of NPN substantially reduce the efficiency of utilization of AS protein by lactating dairy cows (Nagel and Broderick, 1992). In previous feeding studies, Broderick et al. (2000) found that, although DM and NDF digestibility, feed efficiency (milk yield/DMI), and estimated NE_L all were substantially greater on RCS, DMI and yield of milk, fat, and protein all were significantly lower than on diets containing equal DM from AS. Depressed performance was not corrected by supplementation with ruminant-grade fish meal; however, these results were confounded by the fact that the RCS and AS fed in these diets averaged, respectively, 17.7 and 21.3% CP (Broderick et al., 2000). Thus, it was not possible to determine whether the substantially smaller milk and blood urea concentrations on the RCS diets were due to improved N efficiency or merely reflected lower CP intake.

Therefore, two additional trials were conducted to compare lactation performance of cows fed diets with equal DM from AS or RCS, except that solvent-extracted soybean meal (**SBM**) was used, in most cases, to equalize dietary CP content. It was of particular interest in the present work to explore whether similar

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Corresponding author: G. A. Broderick; e-mail: glenb@dfrc.wisc.edu.
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production would occur on lower DM and CP intakes on RCS to capture any advantage of improved digestibility and utilization. The objective of both trials was to compare the nutritional value of AS and RCS and to assess whether differences between the forages could be explained by differences in nutrient efficiency.

MATERIALS AND METHODS

Forage Harvest and Composition

In trial 1, AS was harvested from first cutting taken on June 12, 1995; RCS was harvested from first cutting taken on June 19, 1995. Equal weights of alfalfa and red clover seed were thoroughly mixed and underseeded with barley in April 1994 at a rate of 13.5 kg/ha. The barley was harvested (combined), and the straw was removed from the field in July 1994; one cutting of alfalfa plus red clover forage was removed in late August 1994. This seeding produced a mixed stand that was visually estimated in June 1995 to be about 50% alfalfa and 50% red clover. Forage was harvested from first cutting on June 15, 1995, to produce a mixture of alfalfa and red clover silage (**ARCS**). All forages were field-wilted, chopped, and ensiled into three separate upright concrete stave silos. In trial 2, AS was harvested from first cutting beginning on May 20 and ending on May 28, 1998; RCS was harvested from two cuttings taken on June 8 (first cutting) and July 14, 1998 (second cutting). Forages were field-wilted, chopped, and ensiled in two bunker silos (AS) or in two separate plastic bags (RCS; Ag-Bag International Ltd; Warrenton, OR). In both trials, forages were cut using a conventional mower conditioner, wilted to about 40% DM (range 29 to 51% DM), chopped to a theoretical length of 2.9 cm, and ensiled without additives. No forage was rained on during any harvest. Weekly composite samples were prepared for all silages fed from daily 0.5-kg samples collected during feed-out throughout both trials and stored at –20°C until analyzed. At the end of each feeding trial, 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 NH_3 (Broderick and Kang, 1980) and for NPN (Muck, 1987) using combustion assays (trial 1, Leco 2000; Leco Instruments, Inc., St. Joseph, MI; trial 2, Mitsubishi TN-05 Nitrogen Analyzer; Mitsubishi Chemical Corp., Tokyo, Japan). A single mean result was computed for each silage for each variable for each 4-wk period in each trial. 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 composited by mixing equal amounts of DM to obtain samples corresponding to each 4-wk period in each trial. These samples then were analyzed for DM at 105°C, ash, and OM (AOAC, 1980), total N by combustion assay (Leco 2000; Leco Instruments, Inc., St. Joseph, MI), and NDF, ADF, and ADIN using heat stable α-amylase (Robertson and Van Soest, 1981) and Na_2SO_3 (Hintz et al., 1995). In trial 2, where more than one cutting of RCS was fed, variables were weighted by the proportion of total DM contributed from each cutting. Mean, weighted composition data for the silages fed over both trials are in Table 1.

Trial 1

Twenty-one multiparous Holstein cows (mean ± SD) of 603 ± 50 kg of BW, parity 3.7 ± 1.8, 65 ± 15 DIM, and 38 ± 4 kg of milk per day were blocked into seven groups by DIM; one block of three cows was ruminally cannulated. Cows within blocks were assigned randomly to seven 3 × 3 Latin squares. The three diets, fed in the Latin squares as TMR, differed mainly in forage source (Table 2): 1) AS, 2) RCS, and 3) ARCS. High moisture ear corn (**HMEC**) that had been ground through a 9.5-mm screen (Ekinci and Broderick, 1997) was the principal concentrate component. Geometric mean particle size of the ground HMEC determined by a sieving technique (ASAE, 1995) was 1.6 mm. Diets were formulated to have equal CP based on analyses of two initial samples of each feed ingredient. Diets were fed for three 4-wk periods (total of 12 wk). The first 2 wk of each period was allowed for adaptation to diet; individual means from each cow for production traits from the last 2 wk of each period were analyzed statistically. Cows were milked twice daily, and individual milk yields were recorded at each milking. Milk samples were collected at two consecutive (p.m. and a.m.) milkings midway through wk 3 and 4 of each period and analyzed for fat, protein, lactose, and SNF contents by infrared methods (AgSource, Verona, WI); and for milk urea N (**MUN**) by a colorimetric assay (Ekinci and Broderick, 1997). Concentrations and yields of fat, protein, lactose, and SNF were computed as the weighted means from p.m. and a.m. milk yields on each test day. Yield of 3.5% FCM also was computed (Sklan et al., 1992). 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; efficiency of utilization of feed N similarly was computed for each cow by dividing mean milk N output (total milk protein/6.38) by mean N intake. Body weights were measured on 3 consecutive d at the start and end of each period to compute BW change.

All cows were injected with 500 mg/d of bST (Posilac; Monsanto, St. Louis, MO) beginning on d 1 of the trial, and then injected at 14-d intervals throughout. Cows

Table 1. Effect of forage source on composition of silages fed in trials 1 and 2.[1]

Component	Alfalfa	Red clover	Alfalfa + red clover	SE[2]	$P > F$[3]
Trial 1					
DM, %	52.0[a]	35.8[b]	49.0[a]	2.3	0.02
CP, % of DM	19.1	17.9	17.4	0.4	0.12
Ash, % of DM	10.1	10.6	11.0	0.5	0.53
NDF, % of DM	46.3[a]	43.9[b]	41.8[c]	0.1	<0.01
ADF, % of DM	36.2[a]	33.6[b]	31.7[b]	0.5	0.01
Hemicellulose, % of DM	10.0	10.2	10.1	0.5	0.95
pH	4.46	4.37	4.43	0.04	0.34
NPN, % of total N	50.3[a]	40.3[b]	38.7[b]	1.1	<0.01
NH_3-N, % of total N	8.4	9.8	8.0	0.5	0.10
Total AA-N, % of total N	32.5[a]	25.6[b]	26.2[b]	0.9	0.01
ADIN, % of total N	4.0	4.9	4.2	0.3	0.26
Trial 2					
DM, %	36.6	28.9	. . .	0.4	<0.01
CP, % of DM	21.7	19.1	. . .	0.7	0.08
Ash, % of DM	10.5	11.5	. . .	0.3	0.13
NDF, % of DM	44.6	42.6	. . .	0.4	0.04
ADF, % of DM	35.6	32.9	. . .	0.4	0.02
Hemicellulose, % of DM	9.0	9.7	. . .	0.4	0.40
pH	4.70	4.54	. . .	0.09	0.35
NPN, % of total N	64.0	39.6	. . .	1.6	<0.01
NH_3-N, % of total N	13.2	11.1	. . .	0.3	0.01
Total AA-N, % of total N	26.7	16.6	. . .	0.6	<0.01
ADIN, % of total N	3.1	5.2	. . .	0.2	<0.01

[1]Means from three lactation studies.

[2]SE = Standard error.

[3]Probability of a significant difference due to silage source.

Table 2. Composition of diets fed in trials 1 and 2.[1]

	Trial 1			Trial 2			
Item	AS	RCS	ARCS[2]	AS	RCS	AS+CS	RCS+CS
	(% of DM)						
Alfalfa silage	60.5	. . .	. . .	60.5	. . .	48.3	. . .
Red clover silage	. . .	59.8	. . .	. . .	60.7	. . .	48.5
Mixed alfalfa-red clover silage[2]	. . .	. . .	60.1	. . .	. . .	. . .	. . .
Corn silage	. . .	. . .	. . .	. . .	. . .	12.3	12.3
Ground high moisture ear corn	36.3	32.9	32.7	. . .	. . .	. . .	. . .
Rolled high moisture shelled corn	. . .	. . .	. . .	35.7	35.6	35.6	32.8
Solvent soybean meal	2.1	6.1	6.1	2.9	2.9	2.9	5.6
Dicalcium phosphate	0.6	0.6	0.6	0.5	0.5	0.5	0.5
Potassium magnesium sulfate[3]	0.1	0.1	0.1	. . .	. . .	. . .	. . .
Trace-mineralized salt[4]	0.3	0.3	0.3	. . .	. . .	. . .	. . .
Salt	. . .	. . .	. . .	0.3	0.3	0.3	0.3
Vitamin premix[5]	0.1	0.1	0.1	. . .	. . .	. . .	. . .
Mineral and vitamin premix[6]	. . .	. . .	. . .	0.1	0.1	0.1	0.1
Chemical composition							
CP	16.3	16.8	16.5	18.4	16.6	16.5	16.3
Ash	7.5	8.2	8.7	7.6	8.2	6.8	7.7
NDF	35.0	32.5	30.9	32.0	31.3	32.1	31.7
ADF	25.7	22.5	21.7	24.1	22.7	22.7	21.8

[1]ARCS = Alfalfa plus red clover silage; AS = alfalfa silage; RCS = red clover silage; CS = corn silage.

[2]Silage harvested from alfalfa and red clover planted and grown as a mixture.

[3]Provided 110 mg of Mg, 180 mg of K, and 220 mg of S/kg of DM.

[4]Provided 27 mg of Mn, 27 mg of Zn, 17 mg of Fe, 7 mg of Cu, 0.40 mg of I, 0.30 mg of Se, and 0.10 mg of Co/kg of DM.

[5]Provided 3880 IU of vitamin A, 730 IU of vitamin D, and 0.73 IU of vitamin E/kg of DM.

[6]Provided 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/kg of DM.

were housed in tie stalls and had free access to water throughout the trial. The TMR was offered once daily at about 1000 h; orts were collected and recorded once daily at about 0900 h. The feeding rate was adjusted daily to yield orts of about 5 to 10% of intake. Weekly composites of each TMR, orts, AS, RCS, and HMEC were collected from daily samples of about 0.5 kg and stored at –20°C. Weekly samples of soybean meal were stored at 21 to 24°C. Proportions of each ration ingredient on an as-fed basis were adjusted weekly based on DM determined by drying weekly composites at 60°C (48 h) for AS, RCS, and HMEC and at 105°C (2 h) for soybean meal. Intake of DM was computed based on these DM determinations for the TMR and orts. After drying, ingredients and TMR were ground through a 1-mm screen (Wiley mill). Period composites of the major diet ingredients and TMR were prepared by mixing equal amounts of DM from weekly composites.

Blood was sampled from the coccygeal artery or vein of each cow at 4 h after feeding on d 28 of each period. Blood was heparinized and held at 2°C for about 2 h and then deproteinized by mixing 4 volumes of whole blood with 1 volume of 25% (wt/vol) TCA and centrifuging (15,000 × *g*, 4°C, 15 min). Supernatants were stored at –20°C; deproteinized blood later was thawed and analyzed for glucose and urea (Broderick, 1986). Also on d 28, two fecal grab samples were collected from each cow at about 4 and 20 h after feeding; fecal samples were dried in a forced draft oven (60°C; 72 h), then ground through a 1-mm screen (Wiley mill). Equal DM from each fecal subsample was mixed to obtain a single composite for each cow during each period. Period fecal and TMR composites were analyzed as described earlier for DM, ash, OM, NDF, ADF, and total N, and for indigestible ADF (the ADF remaining after 144 h of in vitro ruminal incubations; Craig et al., 1984). In vitro ADF digestibility was defined as the one minus indigestible ADF. Indigestible ADF was used as an internal marker to estimate apparent digestibility of nutrients (Cochran et al., 1986). Samples of strained ruminal fluid, taken on d 28 from the ventral sac of each of the three ruminally cannulated cows at 0 (just prior to feeding), 1, 2, 3, 4, and 6 h after feeding, were prepared by straining ruminal contents through two layers of cheesecloth. After pH was measured, ruminal fluid was preserved by the addition of 1 ml of 50% (vol/vol) H_2SO_4 to 50 ml of ruminal fluid and stored at –20°C. Samples later were thawed and centrifuged at 30,000 × *g* for 15 min at 2°C; supernatants were analyzed for NH_3 and total AA (Broderick and Kang, 1980).

Trial 2

Twenty-four multiparous Holstein cows (mean ± SD) of 637 ± 60 kg of BW, parity 2.4 ± 0.9, 146 ± 36 DIM, and 38 ± 3 kg/d of milk were blocked into six groups by DIM. Cows within blocks were assigned randomly to six balanced 4 × 4 Latin squares. The four diets, fed in the Latin squares as TMR, contained about 60% forage from (Table 2): 1) AS, 2) RCS, 3) AS plus corn silage (**CS**), and 4) RCS plus CS. Sufficient SBM was added to diet 4 to equal the CP content of diets 2) and 3). High moisture shelled corn (**HMSC**) that was rolled when removed from the silo to a geometric mean particle size of 2.0 mm (ASAE, 1995) was fed as the principal concentrate component in this trial. Diets were fed for 4-wk periods (total of 16 wk). Proportions of dietary DM from each ration ingredient were adjusted weekly as described in trial 1 except that diets also were reformulated weekly to obtain target CP concentrations based on weekly CP analyses of dried (60°C; 48 h) and ground (1-mm screen; Wiley mill) samples of the AS and both RCS and the initial CP contents of HMSC, soybean meal, and CS. All of the AS was from first cutting; RCS DM was 51% from first cutting and 49% from second cutting. Although neither blood nor ruminal sampling were done in this study, other aspects of the trial, including milk sampling and analyses, feeding protocol, feed sampling and analyses, and fecal sampling and analyses were as described for trial 1. Additionally, milk TCA supernatants prepared for MUN determination (Ekinci and Broderick, 1997) also were analyzed for milk NPN using a combustion assay (Mitsubishi TN-05 Nitrogen Analyzer; Mitsubishi Chemical Corp., Tokyo, Japan). Milk true protein was computed by subtracting NPN from total protein N (from infrared analysis; AgSource, Verona, WI) using the equation: [(total protein/6.38) – NPN] × 6.38.

Ruminal in vitro incubations were conducted with the rolled HMSC to determine whether its larger particle size would result in slower fermentation rates relative to that of the ground HMEC fed in trial 1. To duplicate spinner flasks with nominal volumes of 125 ml (actual volumes 250 ml; Bellco, Vineland, NJ) were added samples of 0 (blank) or 5 g of DM from control, ground (9.5 mm) or rolled HMSC; geometric mean particle sizes (ASAE, 1995) of these HMSC were 5.5 (control), 1.0 (ground), and 2.0 mm (rolled). McDougall's buffer (50 ml) was added to each flask and flasks were held at 39°C for 1 h before adding the inoculum. An inoculum of strained ruminal fluid enriched with particle-associated organisms was prepared as described by Craig et al. (1984); 2 m*M* cysteine HCl and 10 m*M* NH_3 (from $(NH_4)_2SO_4$) were added and temperature was brought up to 39°C. Incubations were begun by adding 100 ml of inoculum to each flask. Immediately after inoculating (0 h) and every hour up to 6 h, pH was measured, duplicate 2-ml aliquots were taken from each flask, vortexed with 0.5 ml of 25% wt/vol of TCA, held on ice

for 30 min, and then centrifuged. Supernatants are analyzed for NH_3 (Broderick and Kang, 1980). Incubations were replicated twice.

Statistical Analysis

Statistical analyses of silage composition within trial were done using the general linear models procedure of SAS (1989) with significance declared at $P \leq 0.05$. The model included silage source, period, and period × silage interaction and was weighted for the proportion of DM from each cutting when more than one cutting of a silage was fed within a trial. In trial 2, period × silage interactions approached significance for NDF ($P = 0.07$) and ADF ($P = 0.09$); however, no other period × silage interactions were significant ($P \geq 0.30$) in either trial. The general linear models procedure of SAS (1989) was used to conduct statistical analyses on results from individual trials on all variables. Results from trial 1 were analyzed as a 3×3 Latin square, replicated seven times, using a model that included diet, square, cow-within-square, period, and period × diet interaction. The period × diet interaction approached significance for BW change ($P = 0.08$); no other period × diet interaction was significant ($P \geq 0.15$). The same statistical model was used for trial 2 except data were analyzed as a 4×4 Latin square, replicated six times. The period × diet interaction was significant for the milk N fractions total protein and true protein yields ($P = 0.06$), and for milk NPN and MUN contents ($P = 0.02$); no other period × diet interaction was significant ($P \geq 0.11$). Where there were significant F-tests for diet ($P \leq 0.05$) in each trial, mean separation was conducted by least significant difference at $\alpha = 5\%$. A simple statistical model was used to analyze pH and NH_3 concentrations in in vitro incubations with unprocessed and processed samples of HMSC. Comparisons at each incubation time were made using a model that included treatment, replicate within incubation (n = 2), and incubation (n = 2).

RESULTS AND DISCUSSION

Forage Composition

Red clover silages contained less NDF and ADF than the AS in both trials, but CP content in AS was numerically greater; CP in AS tended to be higher than RCS only in trial 2 (Table 1). The AS and RCS fed in our previous trials had similar fiber contents, but CP content was always higher in AS (Broderick et al., 2000). Silage NPN was about 40% NPN of total N in ARCS (trial 1) and RCS (trials 1 and 2) and was, as expected, lower than in AS (Table 1): NPN in RCS and ARCS averaged 79% of that in AS (trial 1) and NPN in RCS averaged 62% of that in AS (trial 2). The proportions of total N present as free AA were lower in RCS than AS in both trials, but NH_3 content was lower in RCS only in trial 2. The NH_3 and free AA content of RCS was lower than AS in all of our earlier trials (Broderick et al., 2000).

The lower NPN that has been found consistently in RCS (Albrecht and Muck, 1991; Papadopoulos and McKersie, 1983) results from the action of polyphenol oxidase. This enzyme system reacts with O_2 and phenols normally present in red clover to produce quinones that inhibit the plant proteases that break down forage proteins in the silo (Hatfield and Muck, 1999; Jones et al., 1995). Although there was no difference in ADIN content of forages in trial 1, RCS contained 2.1 percentage units more ADIN than AS in trial 2 (Table 1). Reaction of quinones with red clover proteins may have accounted for this small but significant increase in the proportion of total N present as ADIN. Although the inherent proteolytic activity of alfalfa and red clover macerates was similar, Jones et al. (1995) found that adding red clover extracts containing polyphenol oxidase to alfalfa macerates depressed proteolysis to the point that it was similar to that of red clover alone. The NPN contents of ARCS and RCS were about equal (Table 1), suggesting that quinones produced in red clover tissue cross-reacted with both alfalfa and red clover proteins. That decreased NPN content in RCS would improve CP utilization in lactating cows (Nagel and Broderick, 1992) was our hypothesis.

Trial 1

Although formulated to 60% DM from forage and equal CP, the diets with RCS and ARCS contained slightly more CP (Table 2); RCS and ARCS diets also were 2.5 and 4.1 percentage units lower in NDF. Results from the lactation study in which the AS, RCS, and ARCS diets were fed are summarized in Table 3. Cows ate significantly more AS; DMI was 2.5 and 1.3 kg/d less on diets containing, respectively, RCS and ARCS. However, despite these differences in feed intake, there were no differences ($P \geq 0.28$) in BW gain, milk composition, or in yield of milk and milk components due to forage source. Similar milk yield on lower intake resulted in greater ($P = 0.05$) feed efficiency (milk/DMI) on both the RCS and ARCS diets; N efficiency [(milk total protein/6.38)/N intake] also was greater ($P = 0.04$) than on the AS diet (Table 3). Blood urea was affected by diet ($P = 0.05$) and was lower on ARCS than on AS, despite the numerically greater CP in the ARCS diet (Table 2). Improved N efficiency suggested that lower silage NPN improved N utilization on RCS and ARCS. There were no significant effects of forage source on

Table 3. Effect of feeding diets containing alfalfa silage (AS), red clover silage (RCS), or silage from a mixture of alfalfa and red clover grown together (ARCS), on performance of lactating dairy cows (Trial 1).

	Diets				
Item	AS	RCS	ARCS	SE[1]	$P > F^2$
DMI, kg/d	25.5[a]	23.0[b]	24.2[b]	0.4	0.01
BW gain, kg/d	0.38	0.03	0.29	0.15	0.29
Milk, kg/d	32.0	32.7	33.6	0.6	0.74
3.5% FCM, kg/d	31.4	32.4	34.3	0.8	0.90
Fat, %	3.36	3.42	3.59	0.13	0.65
Fat, kg/d	1.08	1.12	1.21	0.04	0.82
Protein, %	3.04	3.02	3.03	0.02	0.28
Protein, kg/d	0.98	0.99	1.02	0.02	0.73
Lactose, %	4.77	4.81	4.81	0.03	0.86
Lactose, kg/d	1.54	1.58	1.63	0.03	0.82
SNF, %	8.52	8.55	8.56	0.03	0.51
SNF, kg/d	2.75	2.81	2.90	0.06	0.75
Milk/DMI	1.27[b]	1.43[a]	1.40[a]	0.04	0.05
Milk N/N intake	0.233[b]	0.250[a]	0.253[a]	0.006	0.04
Milk urea, mg N/dl	9.9	10.5	9.6	0.4	0.29
Blood urea, mg N/dl	13.1[a]	12.9[ab]	12.4[b]	0.4	0.05
Blood glucose, mg/dl	55.0	55.9	54.5	0.5	0.09
Ruminal pH	6.15	6.05	6.15	0.05	0.23
Ruminal NH_3, mg/dl	13.0	12.0	9.7	1.3	0.22
Ruminal total AA, mM	1.75	1.06	1.49	0.33	0.35
Apparent digestibility, %					
DM	56.3[c]	64.1[b]	67.6[a]	0.9	<0.01
OM	58.0[c]	65.4[b]	68.3[a]	0.9	<0.01
NDF	42.7[b]	49.9[a]	51.3[a]	0.5	<0.01
ADF	45.8[c]	49.6[b]	51.9[a]	0.5	<0.01
Hemicellulose	34.0[b]	50.4[a]	49.8[a]	1.3	<0.01
N	46.2[c]	56.2[b]	61.5[a]	1.7	<0.01

[a,b,c]Means within the same row without a common superscript differ ($P < 0.05$).

[1]SE = Standard error.

[2]Probability of a significant effect of diet.

MUN, ruminal pH, or ruminal concentrations of NH_3 and total AA.

Differences in feed efficiency were associated with large, significant effects ($P < 0.01$) of forage source on apparent nutrient digestibility (estimated using indigestible ADF as internal marker; Table 3). Apparent digestibility of DM and ADF were highest on ARCS, intermediate on RCS, and lowest on AS; apparent digestibility of OM, NDF, and hemicellulose were similar on RCS and ARCS and higher than on AS. Relative improvement in digestibility was smallest for ADF (8 and 13% on RCS and ARCS) and greatest for hemicellulose (48 and 46% on RCS and ARCS). Increased hemicellulose digestibility accounted for 13 (ARCS diet) and 24% (RCS diet) of the overall improvement in apparent DM digestibility. Previously, similar improvement (20% increase) in apparent NDF digestibility, but smaller difference (5% increase) in apparent DM digestibility, were found when RCS replaced AS (Broderick et al., 2000); increased NDF digestibility appeared to account for nearly all of that improvement in DM digestibility. However, unlike the current study in which RCS and ARCS had lower NDF contents, AS and RCS fed in the earlier trials were nearly equal in NDF concentration and the RCS likely was more mature. Increased digestibility of the NDF in the present study may have been due to greater immaturity of RCS. 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 maturities from late vegetative to midbloom. Hoffman et al. (1997) reported greater in situ NDF digestibility and in vitro DM digestibility for RCS than AS in one out of two harvest years. The differences in apparent N digestibility, although interesting, are confounded by the slightly greater CP intake and the replacement of CP from AS with CP from soybean meal on the RCS and ARCS containing diets.

Although there were substantial differences in DMI and OM digestibility, lower intakes on RCS and ARCS resulted in similar consumption of digestible OM among diets: 13.5 (AS), 13.8 (RCS), and 14.9 kg/d (ARCS). There was a trend for intake of digestible OM to differ with forage source ($P = 0.07$), but these results suggested that lower DMI on RCS and ARCS occurred because cows ate to about equal energy supply. In our earlier work, replacing AS with RCS had no significant effect on feed efficiency but lowered both DMI and yield of milk and milk components (Broderick et al., 2000).

Part of the difference in lactation performance between the present and earlier studies may have been due to a relative BW loss in cows fed AS in previous trials that would have mobilized more than enough NE_L to account for observed increases in yield of 3.5% FCM.

Trial 2

In this experiment, diets were formulated with 60% DM from AS or RCS alone, or with one-fifth of those forages being replaced by CS (Table 2); variation among diets in NDF content was not as great as in trial 1. Other major differences in diet composition in trial 2 were that rolled HMSC replaced ground HMEC and that enough SBM was added to the RCS + CS diet to make its CP content equal to that in the RCS and AS + CS diets (Table 2). Geometric mean particle size was reduced from 5.0 mm in unprocessed HMSC to 2.0 and 1.0 mm by rolling and grinding, respectively. To determine whether rolling would give rise to microbial fermentability similar to grinding, despite larger particle size, ruminal in vitro NH_3 and pH were followed over time using unprocessed, rolled, and ground HMSC (Figure 1). Both NH_3 concentration (Figure 1a) and pH (Figure 1b) declined more rapidly in incubations conducted with rolled and ground HMSC; NH_3 and pH were lower ($P < 0.05$) than control at, respectively, incubation times greater than 4 and 5 h. Furthermore, in vitro decline of NH_3 and pH for rolled and ground HMSC were virtually identical. These results indicated that rolling HMSC was as effective as grinding HMSC, and that rolled HMSC would have similar effects on ruminal fermentation and NPN uptake for microbial protein synthesis as the ground HMEC fed in trial 1. In vivo results have shown increased fermentation rates with processed grains (Ekinci and Broderick, 1997; McAllister et al., 1993). Galyean et al. (1979) found that digestibility of whole corn was lower than that of ground corn, but observed no differences in digestibility in steers among corn particle sizes of 8, 5, and 3 mm.

Results from the lactation study in which the four forage sources were fed are summarized in Table 4. As in trial 1, cows ate significantly more when fed AS than when fed RCS, with or without CS or supplemental SBM added to the diet. Yields of milk, total protein, true protein, and SNF were not different among cows fed diets containing forage from AS, RCS, or AS + CS, regardless of CP level or DMI. Unlike trial 1, cows fed forage as RCS + CS (with additional SBM) actually yielded more milk, protein, true protein, and SNF than when fed the other three diets, despite lower feed intake than on either AS diet. This observation was at variance with the reduced yields that accompanied lower intakes on RCS diets in our earlier work (Broderick et al., 2000). The RCS diets fed in those previous trials, although containing forage DM equal to that of the AS diets, always contained less CP; without supplemental protein, diets averaged 18.3% CP (AS) and 15.2% CP (RCS). In those trials, production on RCS may have been limited by absorbable protein supply; however, supplementing with ruminant-grade fish meal gave similar yield increases on both AS and RCS. The additional SBM in the RCS + CS diet provided both RDP and RUP and the greater yields of total and true protein may have reflected improved protein status on this diet. As in trial 1, feed efficiency and N efficiency were greater on both RCS diets than on the AS diets (Table 4). Although confounded by dietary CP level, MUN concentration and proportion of milk NPN from MUN were

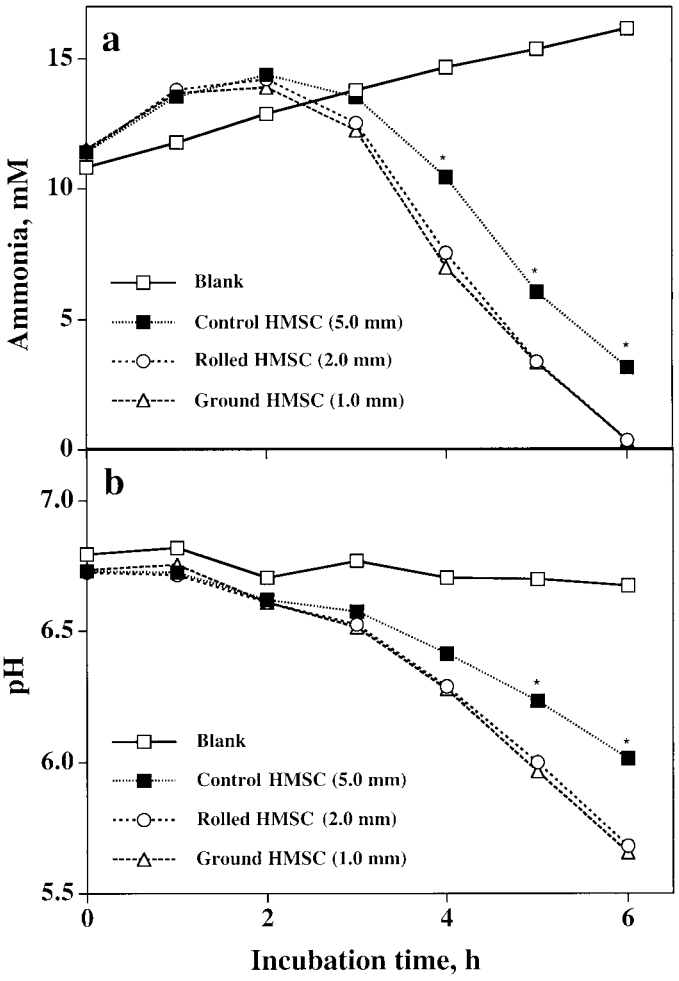

Figure 1. Concentration of NH_3 (a) and pH (b) in ruminal in vitro incubations with no added substrate (Blank) or with added high moisture shelled corn (HMSC) that was unprocessed control (5.0 mm mean particle size), rolled (2.0 mm mean particle size), or ground (1.0 mm mean particle size). Times at which there were differences ($P < 0.05$) between control HMSC and rolled and ground HMSC are denoted with asterisks.

Table 4. Effect of feeding diets containing alfalfa silage (AS), red clover silage (RCS), or AS or RCS plus corn silage (CS), on performance of lactating dairy cows (Trial 2).

	Diets					
Item	AS	RCS	AS+CS	RCS+CS	SE[1]	$P > F^2$
DMI, kg/d	23.5[a]	21.8[c]	23.8[a]	22.8[b]	0.2	<0.01
BW gain, kg/d	0.30	0.56	0.46	0.60	0.12	0.27
Milk, kg/d	30.4[b]	30.4[b]	30.3[b]	31.7[a]	0.3	0.02
3.5% FCM, kg/d	31.6	31.1	32.0	32.4	0.5	0.27
Fat, %	4.30[a]	4.06[b]	4.33[a]	4.08[b]	0.07	<0.01
Fat, kg/d	1.15	1.11	1.16	1.15	0.02	0.32
Protein, %	3.33	3.30	3.39	3.36	0.03	0.29
True protein, %	3.13	3.14	3.20	3.18	0.03	0.41
Protein, kg/d	0.89[b]	0.90[b]	0.91[b]	0.95[a]	0.01	<0.01
True protein, kg/d	0.83[b]	0.86[b]	0.86[b]	0.90[a]	0.01	<0.01
Lactose, %	4.67	4.76	4.69	4.78	0.04	0.17
Lactose, kg/d	1.25[c]	1.31[ab]	1.26[bc]	1.36[a]	0.02	<0.01
SNF, %	8.81	8.87	8.89	8.95	0.08	0.64
SNF, kg/d	2.35[b]	2.43[b]	2.39[b]	2.54[a]	0.03	<0.01
Milk/DMI	1.30[b]	1.40[a]	1.28[b]	1.40[a]	0.01	<0.01
Milk N/N intake	0.204[c]	0.249[a]	0.228[b]	0.256[a]	0.003	<0.01
Milk urea, mg N/dl	13.6[a]	9.2[d]	11.2[b]	10.1[c]	0.3	<0.01
Urea, % of milk NPN	41.8[a]	35.4[b]	37.0[b]	36.9[b]	0.3	<0.01
Apparent digestibility, %						
DM	64.5[c]	71.5[a]	65.1[c]	68.4[b]	0.7	<0.01
OM	66.7[c]	73.3[a]	67.4[c]	70.0[b]	0.6	<0.01
NDF	44.1[c]	55.3[a]	44.3[c]	49.8[b]	0.7	<0.01
ADF	44.2[c]	54.2[a]	43.4[c]	49.1[b]	0.7	<0.01
Hemicellulose	43.9[c]	58.0[a]	46.3[c]	51.3[b]	1.1	<0.01
N	62.3	59.7	60.7	58.2	1.1	0.06

[a,b,c]Means within the same row without a common superscript ($P < 0.05$).

[1]SE = Standard error.

[2]Probability of a significant effect of diet.

higher on the diet containing only AS forage than on the other three diets. Note that MUN was lower on RCS than on AS + CS and RCS + CS, despite its numerically greater dietary CP (Table 2). However, DMI and N intake also were lowest on the RCS diet and excess N intake (total N intake – milk N secretion) was found to be nearly as well correlated to MUN as dietary CP concentration (Broderick and Clayton, 1997).

Additional significant effects detected were lower milk fat concentration and greater milk lactose yield on the two RCS diets (Table 4). Previously, we observed lower milk fat content and yield when RCS replaced AS as dietary forage (Broderick et al., 2000); that effect appeared to be related to a relative weight loss on the AS diets, versus net weight gain on RCS. As discussed earlier, there were no differences due to silage source in milk fat content or yield, or in weight gain, in trial 1. In trial 2, there were no effects on relative weight change, but milk fat content averaged about 0.25 units lower on the two RCS diets. However, milk fat content was in excess of 4%, quite high for Holsteins, and there was no effect of forage source on fat yield ($P = 0.32$). Generally, lactose yield paralleled milk yield but was greater on RCS than on AS.

As in trial 1, differences in feed efficiency were associated with large effects ($P < 0.01$) of diet on nutrient digestibility (Table 4). Apparent digestibility of DM, OM, NDF, ADF, and hemicellulose were highest on RCS, intermediate on RCS + CS, and lowest on the two AS diets. Replacing RCS with CS reduced apparent digestibilities, but there was no effect when CS replaced dietary AS. This suggested that apparent digestibility of RCS nutrients exceeded that of CS but that digestibilities of AS and CS were comparable in this trial. Relative to the AS diet, DM, OM, and hemicellulose digestibilities were increased 11, 10, and 33% on RCS, about equal to that observed on the comparable diets fed in trial 1 (Table 3). However, effects on apparent fiber digestibility were greater in trial 2: relative NDF and ADF digestibilities were 25 and 23% greater on RCS than on AS. Despite differences in DMI, intake of digestible OM was approximately equal across diets, ranging from 14.5 to 14.9 kg/d, and was not different due to forage source ($P = 0.55$). This finding indicated that the cause of lower DMI on the RCS diets was greater energy digestibility and, as in trial 1, the cows ate to constant energy supply.

Relative Energy and Protein Utilization

The NE_L requirements for maintenance, BW gain, and milk output (based on observed fat and SNF contents) were estimated using NRC (1981, 1989) equations (Table 5). The NE_L requirements for mean performance were about equal on AS and RCS diets (average 34.1 Mcal/d). Subtracting the NE_L contributed from the concentrate portion of the diet (1.88 Mcal/kg of DM; NRC, 1989) yielded an estimate of NE_L supplied by AS and RCS. Per unit of DM, AS was estimated to contain 1.08 Mcal/kg versus 1.28 Mcal/kg for RCS, indicating that RCS contained 118% of the NE_L of AS. Thus, RCS with 43.3% NDF had 18% greater NE_L than AS with 45.5% NDF. Mean relative digestibility of dietary OM (Tables 3 and 4) was $100 \times (73.3 + 71.5)/(58.0 + 66.7) = 116\%$, or 16% greater on the RCS diets. Previously, we estimated that RCS contained about 10% more NE_L than AS (Broderick et al., 2000); however, NDF contents of those forages were 42% in AS and 43% in RCS. The NRC (1989) tables indicated that AS (46% NDF) and RCS (43% NDF) contained, respectively, 1.30 and 1.45 Mcal/kg DM of NE_L.

Direct comparison of N utilization on the AS and RCS diets was confounded in these experiments by the shortfall in CP on RCS (relative to AS) being made up by SBM in trial 1 and the greater dietary CP content of the AS diet than the RCS diet in trial 2. Thus, the improved N efficiencies on RCS cannot be attributed solely to its lower NPN content. However, significantly greater N efficiency on RCS was observed in both trials; omitting data obtained on the ARCS and CS diets, weighted means for N efficiencies were 21.6% on AS and 25.0% on RCS from the two studies. Weighted mean milk and protein yields were 31.5 and 0.93 kg/d (AS) and 31.6 and 0.94 kg/d (RCS). Lactations in which production was 10,000 kg/cow of milk would last 320 d if this represented average daily yield; average milk N secretion would be $320 \times 0.935/6.38 = 47$ kg/lactation. Weighted mean N intakes were 681 and 595 g/d (218 and 190 kg/320 d). Under these assumptions, net N excretions would have been 171 and 143 kg/cow-lactation, a savings of 28 kg/cow-lactation on RCS. If only half this amount, 14 kg of N/lactation, were conserved, then N output in excreta would still be reduced by 1400 kg per lactation-year for a 100-cow dairy. Most of this reduction likely would be in lower excretion of the more labile urinary N. Although clearly speculative, these computations suggest that feeding RCS may reduce N losses to the environment.

Table 5. The NE_L contents of alfalfa and red clover silage estimated from overall mean intake and performance data.[1]

Item	Forage source	
	Alfalfa silage	Red clover silage
Dietary silage, % of DM	60.5	60.2
Dietary concentrate, % of DM	39.5	39.8
NE_L requirement[2]		
Maintenance, Mcal/d	10.0	10.0
BW gain, Mcal/d	1.7	1.6
Milk yield, Mcal/d	22.5	22.4
Total requirement, Mcal/d	34.2	34.0
Total DMI, kg/d	24.4	22.4
Concentrate DMI, kg/d	9.7	8.9
Concentrate NE_L,[3] Mcal/kg DM	1.88	1.88
Concentrate NE_L, Mcal/d	18.2	16.7
NE_L from Silage,[4] Mcal/d	16.0	17.2
Silage DMI, kg/d	14.8	13.5
Silage NE_L, Mcal/kg DM	1.08	1.28
Red clover/Alfalfa, %		118.1

[1]Mean performance data from both lactation trials weighted for the number of cows in each.

[2]NE_L (Mcal/d) required for maintenance = $0.08 \times BW^{0.75}$ (mean BW = 621 kg) and NE_L (Mcal/d) required for gain = $5.12 \times$ BW gain (NRC, 1989). NE_L (Mcal/d) required for milk output = Milk $\times$ (0.09464 $\times$ % fat + 0.049 $\times$ % SNF − 0.0564) (NRC, 1981).

[3]Mean NE_L contents of dietary concentrate in the four diets computed from NRC (1989) tables.

[4]Total NE_L requirement minus concentrate NE_L intake.

CONCLUSIONS

Two Latin square lactation trials compared the feeding value of AS and RCS harvested over 2 yr. Relative to AS, RCS fed in these trials averaged two percentage units lower in CP, NDF, and ADF and had only 70% as much NPN as a proportion of total N. When these forages were fed at equal proportions of dietary DM, cows consumed less DM on RCS but similar digestible OM. Yields of milk, FCM, protein, and SNF were equal when RCS replaced AS, despite 2.0 kg/d less DMI. Supplementing a RCS + CS diet (in which CS replaced 20% of the forage) with enough SBM to increase CP to the level of an equivalent AS + CS diet increased yields of milk, total protein, true protein, and SNF. Replacing AS with RCS increased apparent digestibility of dietary DM, OM, NDF, ADF and hemicellulose and increased feed efficiency and N efficiency. Estimated NE_L of RCS was 18% greater than that of AS. Utilization of both energy and CP in RCS clearly exceeded that of AS. Greater nutrient digestibilities and estimated NE_L content and lower NPN content suggest that feeding RCS may result in lower environmental N losses than feeding AS.

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