Performance of Lactating Dairy Cows Fed Alfalfa or Red Clover Silage as the Sole Forage[1]

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ABSTRACT

Three Latin square trials, with 20 (two trials) or 24 (one trial) multiparous lactating Holstein cows (four in each trial with ruminal cannulae), compared the feeding value of red clover and alfalfa silages harvested over 3 yr. Overall, the forages contained similar amounts of neutral detergent fiber and acid detergent fiber; however, red clover silage contained more hemicellulose, less ash and crude protein (CP), and only 67% as much nonprotein N, as a proportion of total N, as did alfalfa silage. Diets were formulated with equal dry matter (DM) from alfalfa or red clover silage and contained on average 65% forage, 33 or 30% ground high moisture ear corn, and 0 or 3% low soluble fishmeal (DM basis). Diets fed in the Latin squares contained (mean dietary CP): 1) alfalfa (17.8% CP); 2) red clover (15.1% CP); 3) alfalfa plus fishmeal (19.6% CP); and 4) red clover plus fishmeal (16.9% CP). Although performance varied somewhat among trials, overall statistical analysis showed that replacing alfalfa with red clover reduced yields of milk, fat-corrected milk, fat, protein, lactose, and SNF; these effects were related to the 1.2 kg/d lower DM intake for cows fed red clover. Replacing alfalfa with red clover improved body weight gain and reduced concentrations of milk and blood urea and ruminal NH_3. Apparent digestibility of DM, organic matter, neutral detergent fiber, acid detergent fiber, and hemicellulose all were greater when red clover was fed. There were no significant forage × fishmeal interactions for DM intake and yield of milk and milk components, indicating that supplementation with rumen undegradable protein gave similar increases in production on both forages. Net energy of lactation (NE_L), estimated from maintenance, mean milk yield, and body weight change, in alfalfa and red clover silage were, respectively, 1.25 and 1.38 Mcal NE_L/kg of DM, indicating 10% greater NE_L in red clover.

(**Key words:** alfalfa silage, red clover silage, nonprotein N)

Abbreviation key: AS = alfalfa silage, **HMEC** = high moisture ear corn, **MUN** = milk urea N, **RCS** = red clover silage.

INTRODUCTION

Alfalfa silage (**AS**) is a major forage fed to lactating dairy cows in the Midwestern United States. During fermentation, a large proportion of the CP in alfalfa is broken down to NPN; typically, 50 to 60% (8, 17), to more than 80% (18), of the total N in alfalfa is converted to NPN in the silo. Breakdown of true protein in AS to NPN substantially reduces efficiency of CP utilization in lactating cows (5, 19). Red clover silage (**RCS**) contains significantly less NPN than AS (1, 23). Polyphenol oxidase, an enzyme system in red clover, appears to convert phenols normally present in red clover into reactive *o*-quinones (16). These *o*-quinones interact rapidly with proteins in red clover foliage and probably reduce NPN formation principally by inhibiting the plant proteases involved in breaking down foliage proteins in the silo (12). Lower NPN may result in improved utilization of RCS versus AS. Hoffman et al. (15) found greater milk yield on equivalent amounts of RCS than on AS in one of two years; however, milk protein yield was lower on RCS during one of the years.

The objective of this trial was to compare the nutritional value of AS and RCS when fed as the sole source of forage to lactating dairy cows and to assess whether any differences between the forages could be explained by protein status, as indicated by the relative response to supplemental fishmeal protein.

MATERIALS AND METHODS

Forage Harvest and Composition

In trial 1, AS was harvested from two cuttings taken on July 11, 1991 (second cutting), and August 27, 1991

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(third cutting); RCS was harvested from two cuttings taken on July 11, 1991 (seeding year, first cutting), and on August 27, 1991 (seeding year, second cutting). Forages were field-wilted, chopped, and ensiled in separate plastic bags (Ag-Bag International Ltd; Warrenton, OR). In trial 2, AS was harvested on July 2, 1993 (second cutting), and RCS was harvested from two cuttings taken on June 23, 1993 (first cutting), and August 3, 1993 (second cutting). Forages were field-wilted and chopped; alfalfa was ensiled in a bunker silo, and red clover was ensiled in two separate, upright concrete stave-tower silos. In trial 3, AS was harvested on September 1, 1994 (third cutting); RCS was harvested on July 29, 1994 (second cutting). Forages were field-wilted, chopped, and ensiled in separate upright concrete stave tower silos. All forages were cut using a conventional mower conditioner, wilted to about 45% DM (range 35 to 55% DM), chopped to a theoretical length of 2.9 cm and ensiled without additives. Weekly composite samples were prepared for all AS and RCS from daily 0.5-kg samples collected during feed-out throughout the three trials and stored at –20°C until analyzed. At the end of each feeding trial, weekly composites were thawed, water extracts were prepared (18), and pH was measured. Extracts were deproteinized (18) and then analyzed for total AA and NH_3 (7) and for NPN (18) using combustion assays (trial 1: Carlo Erba, NA 1500 Nitrogen Analyzer; Fisons Instruments, Inc., Beverly, MA; trials 2 and 3: Mitsubishi TN-05 Nitrogen Analyzer; Mitsubishi Chemical Corp., Tokyo). A single mean result was computed for each variable for each 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 3-wk period in each trial. These samples then were analyzed for DM at 105°C, ash and OM (2), total N by Kjeldahl with a copper digestion catalyst [Kjeltabs; Tecator Inc., Herndon, VA (2)] in trial 1 and by combustion assay (Leco 2000; Leco Instruments, Inc., St. Joseph, MI) in trials 2 and 3, and NDF and ADF using heat stable α-amylase (24) and Na_2SO_3 (13). When more than one source of AS and RCS was fed within a trial, variables were weighted by the proportion of total DM contributed from each silage. Mean, weighted composition data for the AS and RCS fed over the three trials are in Table 1.

Trial 1

Twenty multiparous Holstein cows (mean ± SD) of 609 ± 64 kg of BW, parity 3.3 ± 1.4, 57 ± 29 DIM, and 39 ± 5 kg/d of milk were blocked into five groups by DIM; one block of four cows was ruminally cannulated. Cows within blocks were assigned randomly to five balanced 4 × 4 Latin squares; the four diets fed in the Latin squares contained (DM basis; Table 2): 1) AS plus ground high moisture ear corn (**HMEC**); 2) RCS and HMEC; 3) AS, HMEC, and fishmeal; and 4) RCS, HMEC, and fishmeal. Second cutting provided 66% and third cutting provided 34% of the AS; first cutting provided 73% and second cutting provided 27% of the RCS. Low-soluble menhaden fishmeal (Sea Lac; Zapata-Haynie Co., Hammond, LA) was fed in the trial. Diets were fed for 3-wk periods (total of 9 wk); the last 3-wk period (wk 10 to 12) was not completed because the supply of RCS was exhausted. The first week of each period was allowed for adaptation to diet; mean yields, and intakes 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 2 and 3 of each period and analyzed for fat, protein, lactose, and SNF contents by infrared analysis (AgSource, Madison, WI); and for milk urea N (**MUN**) by a colorimetric assay (11). 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 (27). Efficiency of feed DM conversion was computed for each cow over the last 2 wk of each period by dividing mean milk yield by mean DMI. Body weights were measured on 3 consecutive d at the start and end of each period to compute BW change.

Cows were housed in tie stalls and had free access to water throughout the trial. The TMR were offered once daily at 1000 h; orts were collected and recorded once daily. The feeding rate was adjusted daily to yield orts of 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 fishmeal 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) for fishmeal. 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 21 of each period. Blood was heparinized and stored at 2°C for about 4 h when plasma was prepared by centrifugation; plasma was deproteinized by mixing 4 volumes of plasma with 1 volume of 15% (wt/vol) 5-sulfosalicylic acid and then

Table 1. Nutrient composition of alfalfa and red clover silages.[1]

Component	Alfalfa	SE[2]	Red clover	SE[2]	$P > F$[3]
DM, %	39.2	2.2	44.8	1.5	0.001
CP, % of DM	21.3	0.5	17.7	0.4	<0.001
Ash, % of DM	11.8	0.4	10.7	0.2	0.014
NDF, % of DM	42.1	0.7	42.9	0.5	0.388
ADF, % of DM	32.7	0.8	31.6	0.6	0.166
In vitro ADF digestibility,[4]					
% of total ADF	83.8	0.6	86.0	0.3	<0.001
Hemicellulose, % of DM	9.43	0.30	11.3	0.5	<0.001
pH	4.65	0.05	4.47	0.12	0.190
NPN, % of total N	50.9	1.2	34.1	1.1	<0.001
Total AA-N, % of total N	29.5	0.9	15.7	0.5	<0.001
NH_3-N, % of total N	8.65	0.75	5.93	0.34	<0.001

[1]Means from three lactation studies.

[2]SE = Standard error.

[3]Probability of a significant difference between forages.

[4]144-h incubation.

Table 2. Composition of diets fed in trials 1, 2, and 3.

Item	Alfalfa silage	Red clover silage	Alfalfa silage + fish meal	Red clover silage + fish meal
	(% of DM)			
Trial 1				
Alfalfa silage	71.1	. . .	71.1	. . .
Red clover silage	. . .	70.5	. . .	70.5
Ground high moisture ear corn	27.7	28.3	24.9	25.5
Fish meal	. . .	. . .	2.8	2.8
Dicalcium phosphate	0.7	0.7	0.7	0.7
Trace-mineralized salt[1]	0.4	0.4	0.4	0.4
Vitamin premix[2]	0.1	0.1	0.1	0.1
Chemical composition				
CP	18.0	15.3	19.8	17.0
NDF	32	33	33	34
Trial 2				
Alfalfa silage	64.3	. . .	64.3	. . .
Red clover silage	. . .	64.3	. . .	64.3
Ground high moisture ear corn	34.5	34.5	31.4	31.4
Fish meal	. . .	. . .	3.1	3.1
Dicalcium phosphate	0.7	0.7	0.7	0.7
Trace-mineralized salt[1]	0.4	0.4	0.4	0.4
Vitamin premix[2]	0.1	0.1	0.1	0.1
Chemical composition				
CP	16.6	15.1	18.4	16.9
NDF	32	30	32	30
Trial 3				
Alfalfa silage	60.3	. . .	60.3	. . .
Red clover silage	. . .	59.9	. . .	59.9
Ground high moisture ear corn	36.2	36.6	33.2	33.6
Solvent soybean meal (44% CP)	2.4	2.4	2.4	2.4
Fish meal	. . .	. . .	3.0	3.0
Dicalcium phosphate	0.6	0.6	0.6	0.6
Trace-mineralized salt[1]	0.4	0.4	0.4	0.4
Vitamin premix[2]	0.1	0.1	0.1	0.1
Chemical composition				
CP	18.9	14.9	20.7	16.7
NDF	33	34	33	34

[1]Provided (per kg of DM) 36 mg of Mn, 36 mg of Zn, 23 mg of Fe, 9 mg of Cu, 0.53 mg of I, 0.40 mg of Se, and 0.13 mg of Co.

[2]Provided (per kg of DM) 3880 IU of vitamin A, 730 IU of vitamin D, and 7.3 IU of vitamin E.

stored at –20°C. Deproteinized blood plasma later was thawed and analyzed for glucose and urea (3). Also on d 21, a single fecal grab sample was collected from each cow; fecal samples were dried in a forced draft oven (60°C; 72 h) then ground through a 1-mm screen (Wiley mill) for later analysis. Samples of strained ruminal fluid, taken on d 21 from the ventral sac of each of the four 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 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 (7). Dried, ground fecal samples and period composites of the TMR were analyzed as described earlier for DM, ash, OM, NDF, and ADF, for total N by Kjeldahl using a copper digestion catalyst [Kjeltabs (2)], and for indigestible ADF (the ADF remaining after 144 h of in vitro ruminal incubations; 10). In vitro ADF digestibility was defined as 100 minus indigestible ADF. Indigestible ADF was used as an internal marker to estimate apparent digestibility of nutrients (9).

Trial 2

This trial replicated trial 1 with the following modifications. The AS was from only second cutting and the RCS was a blend of (DM basis) 55% first and 45% second cutting forage. Diets contained 64% forage DM and somewhat greater amounts of HMEC then were fed in trial 1 (Table 2). The 20 multiparous Holstein cows used in the lactation phase of this trial had means (± SD) of 593 ± 43 kg of BW, parity 3.2 ± 1.3, 42 ± 14 DIM, and 40 ± 5 kg/d of milk. Blocking and assignment to the balanced 4 × 4 Latin squares were as in trial 1; the full 12-wk 4 × 4 Latin square trial was completed. The four cannulated cows used in the square for ruminal sampling were in later lactation (mean 196 DIM); milk yield, intake, and BW data from these cows were not analyzed. Milk sampling and analyses, feeding protocol, feed sampling and analyses, and blood, fecal, and ruminal sampling and analyses all were as described for trial 1 except total N determinations were by combustion assay (Leco 2000; Leco Instruments, Inc.).

Trial 3

This trial was a repeat of the other two trials with the following modifications. Forages fed in this trial were either third-cutting AS or second-cutting RCS. Forage comprised 60% of dietary DM with correspondingly greater amounts of HMEC being fed in trial 3 (Table 2). There were 16 multiparous [BW, 557 ± 47 kg; milk yield, 41 ± 4 kg/d; parity, 2.7 ± 1.0; and DIM, 49 ± 7 (mean ± SD)] and eight primiparous [BW, 500 ± 49 kg; milk yield, 31 ± 4 kg/d; and DIM, 78 ± 12 (mean ± SD)] Holstein cows used in the lactation phase of this trial. Blocking was by parity (primiparous or multiparous cows) and DIM. Otherwise, experimental design was as in the other two trials, and the full 12-wk 4 × 4 Latin square trial was completed. The four cannulated cows used in the square for ruminal sampling were in later lactation (mean 141 DIM); milk yield, intake and BW data from these cows were not analyzed. All other sampling and analyses were as described for trials 1 and 2, except that blood plasma was deproteinized using 4 volumes of plasma: 1 volume of 25% (wt/vol) TCA before storing at –20°C.

Statistical Analysis

Statistical analyses of silage composition data were done using the general linear model procedure of SAS (26) and significance was declared at $P \leq 0.05$. Analysis of overall silage composition from the three trials was done on period means (data from water extracts) or period composites (DM, CP, ash, NDF, ADF, digestible ADF, and hemicellulose); the model used included silage source, trial, period, and trial × 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. The trial × silage interaction was significant ($P < 0.01$) for CP and ash content; other trial × silage interactions were not significant ($P \geq 0.30$). Overall data for production, ruminal metabolite concentrations, and apparent digestibilities from the three trials were analyzed using the MIXED procedures of SAS (26) with significance again declared at $P \leq 0.05$. Squares were replicated five times for production data and once for ruminal data in trial 1; however, data were available only from the first three periods of this trial. Data were from all four periods of the 4 × 4 Latin squares of the other two trials; for production data, squares were replicated five (trial 2) and six (trial 3) times and replicated once for ruminal data in both trials. Apparent digestibilities were estimated using a single fecal grab sample from each cow in each period of each trial; however, digestibilities on each diet are means from 66 observations over the three trials and should be reliable overall estimates despite limited sampling within trial. The overall model included diet, trial, square-within-trial, cow-within-trial and square, period-within-trial, and period × diet and trial × diet interactions. Period × diet interactions were not significant for any variable ($P \geq 0.19$). Orthogonal contrasts were used to compare effects of: 1) forage source

(AS versus RCS), 2) fishmeal addition, and 3) forage × fishmeal interaction.

We used the general linear model procedure of SAS (26) to conduct statistical analyses on results from individual trials on the seven production variables (DMI, milk yield, milk yield/DMI, milk protein content, protein yield, MUN, and blood urea) for which there were significant trial × diet interactions in the overall model. Results from trial 1 were analyzed as a 4 × 4 Latin square, replicated five times, using a model that included diet, square, cow-within-square, period, and period × diet interaction. Orthogonal contrasts were used to compare effects of: 1) forage source (AS versus RCS), 2) fishmeal addition, and 3) forage × fishmeal interaction. The period × diet interaction approached significance for BW change ($P = 0.08$); no other period × diet interactions were significant ($P \geq 0.15$). The same statistical model was used for trials 2 and 3 except data were from all four periods of the 4 × 4 Latin squares; squares were replicated five (trial 2) and six (trial 3) times in the two trials. No period × diet interactions were significant ($P \geq 0.12$) in trial 2; period × diet interaction was significant for milk yield/DMI ($P = 0.03$), but no other period × diet interactions were significant ($P \geq 0.13$) in trial 3.

RESULTS AND DISCUSSION

The AS and RCS were intended to be fed at similar fiber contents during these comparative studies; this objective was achieved because the two silages were not different in NDF and ADF over all three trials (Table 1). However, AS contained more CP and ash, less hemicellulose (NDF – ADF), and had lower digestible ADF (ADF disappearing after 144-h in vitro ruminal incubations; 10). Early bloom alfalfa and full bloom red clover containing, respectively, 42 and 43% NDF were reported to contain 18.0 and 14.6% CP (21). Although overall CP was greater, a similar mean CP differential was found between the AS and RCS fed in our trials (Table 1). Lower ash and greater in vitro digestible ADF (an estimate of potentially digestible ADF) may have contributed to the greater energy values in RCS that will be discussed later. Among the most striking differences between AS and RCS were the lower proportions of total N as NH_3, total free AA and NPN in RCS; NPN content of RCS was only 67% of that in AS (Table 1). Lower NPN content in RCS has been found consistently (1, 23). Although inherent proteolytic activity of alfalfa and red clover extracts is similar, red clover contains an active polyphenol oxidase enzyme system (16). This system reacts with O_2 and phenols (principally caffeic acid) normally present in red clover to form *o*-quinones (16); the *o*-quinones react rapidly with proteins in red clover foliage. The mode of action for reducing NPN formation appears to be via *o*-quinones binding with AA residues present at the active sites of plant proteases involved in breaking down red clover proteins in the silo (12). Decreasing NPN content of hay-crop silages improves CP utilization in lactating cows (19).

Results from the overall statistical analysis of animal performance from all three trials are summarized in Table 3. The forage × fishmeal interaction was significant ($P < 0.01$) only for blood glucose concentration. Blood glucose was significantly influenced by forage source ($P = 0.04$) and diet ($P < 0.01$); however, mean concentrations ranged from only 51 to 53 mg/dl and differences likely were not biologically important. Milk lactose and SNF content, milk yield/DMI, and ruminal pH were the only variables that were not influenced ($P > 0.13$) by forage source. Dry matter intake, yields of milk, FCM, fat, protein, and SNF, and milk fat and protein content, all were greater ($P < 0.001$) when cows were fed AS; lactose yield also was increased ($P = 0.002$). Positive responses ($P < 0.001$) to feeding RCS were increased BW gain, reduced concentrations of MUN, blood urea, and ruminal NH_3 and total AA, and greater apparent digestibility of all nutrients measured except N. Body weight changes were measured over short periods of only 3-wk in these Latin square studies; however, each value was the mean of 58 observations. Net BW gain averaged 0.31 kg/d less on AS. A relative BW loss of 0.31 kg/d would have mobilized 1.53 Mcal NE_L/d in cows fed AS, sufficient NE_L for about 2.2 kg/d of milk with 3.5% fat (21). Yield of 3.5% FCM averaged 1.7 kg/d more in cows fed AS rather than RCS. This computation, plus the greater apparent digestibilities of nutrients on RCS, suggested that the higher yields of milk and milk components on AS were driven by the greater CP content and the 1.2 kg/d greater DMI on those diets. Had DMI been the same on both forages, performance actually may have been better on RCS. When AS and RCS harvested in two seasons at both early and late maturities were fed to lactating cows (15), greater milk yield for cows fed RCS than for cows fed equivalent DMI from AS were found in the first of two feeding years; however, milk protein yield was lower on RCS during the second feeding year. Overall DMI was not affected by silage source in Hoffman's trials (15); however, unlike our studies, RCS on average had similar CP, and lower NDF and ADF, than AS. Because equal DM was added from a lower CP forage (Table 1), our RCS diets averaged 16.0% CP and 33% NDF versus 18.7% CP and 33% NDF for the AS diets (Table 2). Lower DMI on RCS may have resulted from these substantial differences in CP content (28). Thomas et al. (30) found that replacing ryegrass silage with RCS increased DMI and yields of milk, protein, and lactose.

Table 3. Effect of feeding alfalfa silage (AS) or red clover silage (RCS), with or without supplemental fishmeal (FM), on cow performance over all three lactation trials.

						Probabilities				
						Orthogonal contrasts				
Item	AS	RCS	AS + FM	RCS + FM	SE[1]	Forage	FM	Forage × FM	Diet	Trial × diet
DMI, kg/d	21.9	20.7	22.5	21.4	0.2	<0.001	<0.001	0.861	<0.001	<0.001
BW change, kg/d	–0.13	0.20	0.06	0.35	0.08	<0.001	0.007	0.981	<0.001	0.289
Milk, kg/d	32.5	31.2	34.5	33.0	0.2	<0.001	<0.001	0.556	<0.001	0.001
Milk/DMI	1.50	1.52	1.55	1.56	0.01	0.364	0.017	0.795	0.090	0.006
3.5% FCM, kg/d	32.5	30.8	34.0	32.4	0.4	<0.001	<0.001	0.577	<0.001	0.291
Fat, %	3.60	3.38	3.49	3.36	0.06	0.005	0.236	0.663	0.022	0.233
Fat, kg/d	1.14	1.06	1.19	1.12	0.02	<0.001	0.006	0.620	<0.001	0.512
Protein, %	2.91	2.88	2.99	2.94	0.01	<0.001	<0.001	0.337	<0.001	0.014
Protein, kg/d	0.93	0.89	1.01	0.97	0.01	<0.001	<0.001	0.351	<0.001	0.014
Lactose, %	4.82	4.83	4.78	4.79	0.01	0.128	<0.001	0.569	<0.001	0.055
Lactose, kg/d	1.55	1.51	1.63	1.58	0.01	0.002	<0.001	0.609	<0.001	0.241
SNF, %	8.38	8.43	8.49	8.45	0.04	0.979	0.068	0.427	0.261	0.778
SNF, kg/d	2.72	2.63	2.88	2.78	0.02	<0.001	<0.001	0.478	<0.001	0.080
Milk urea, mg N/dl	14.8	8.7	17.1	10.9	0.4	<0.001	<0.001	0.547	<0.001	<0.001
Blood urea, mg N/dl	14.8	8.2	17.7	10.5	0.3	<0.001	<0.001	0.244	<0.001	<0.001
Blood glucose, mg/dl	52.0	51.7	50.9	52.7	0.5	0.038	0.790	0.004	0.006	0.384
Ruminal pH	6.10	6.20	6.18	6.21	0.06	0.294	0.452	0.577	0.587	0.902
Rumnial NH_3, mg N/dl	19.6	6.5	21.3	7.2	0.8	<0.001	0.270	0.731	<0.001	0.142
Ruminal total AA, m*M*	2.86	1.24	2.79	1.06	0.36	0.001	0.800	0.761	0.005	0.326
Digestibility, %										
DM	61.6	64.6	63.2	66.8	0.5	<0.001	<0.001	0.272	<0.001	<0.001
OM	63.0	65.8	65.2	68.3	0.5	<0.001	<0.001	0.414	<0.001	<0.001
NDF	43.0	51.7	46.4	55.1	0.5	<0.001	<0.001	0.381	<0.001	0.063
ADF	44.3	51.6	46.7	55.0	0.5	<0.001	<0.001	0.052	<0.001	<0.001
Hemicellulose	38.8	50.4	45.3	54.4	0.7	<0.001	<0.001	0.326	<0.001	0.499
N	62.1	52.3	66.3	58.4	0.8	<0.001	<0.001	0.067	<0.001	<0.001

[1]SE = Standard error.

Surprisingly, milk fat content increased 0.18 percentage units and fat secretion increased 80 g/d when cows were fed AS rather than RCS. Depressed milk fat often is coupled with increased BW gain. Cows fed AS without fishmeal lost 0.13 kg/d of BW, while cows fed AS plus fishmeal gained 0.06 kg/d of BW. Cows fed AS may have been mobilizing more stored fat than was indicated by BW change alone. Using the equations of Rotz et al. (25), we estimated that minimum BW would be reached at wk 10 after calving while maximum NDF intake would occur at wk 18 after calving. This suggested that NDF intake would continue to increase for 8 wk longer and that gut fill may account for some of the apparent BW gain up to wk 18 after calving. Overall, cows averaged 93 DIM in our trials—about 13 wk postcalving. Increasing gut fill may have contributed to some of the apparent BW gain in all cows in these trials and, despite being close to BW equilibrium, cows fed AS actually may have been in negative energy balance. Cows mobilizing greater amounts of body fat were reported to maintain higher fat tests and to secrete larger quantities of milk fat (22). This may explain the greater fat yields on AS in our trials.

Increases in apparent digestibility with feeding RCS versus AS averaged 3 percentage units for DM and OM, about 8 percentage units for NDF and ADF, and 10 percentage units for hemicellulose; all were highly significant ($P < 0.001$; Table 3). Hoffman et al. (14) tested a number of oven-dried (55°C) forages and found that in situ DM digestibility ranged from 5 to 9 percentage units higher for red clover than alfalfa in samples ranging in maturity from late vegetative to midbloom. Hoffman et al. (15) also observed greater in situ NDF digestibility and in vitro DM digestibility for RCS than AS for one harvest year, but no differences during a second harvest year. Increases in apparent N digestibility, due to dilution of metabolic fecal N (29), and in MUN and blood urea concentrations (6) on the AS diets were expected because of greater CP intake. Higher ruminal concentrations of NH_3 and total AA probably also reflected differences in dietary CP as well as greater amounts of NPN in AS than RCS (Table 1). Similar responses on both silages to supplemental RUP from fishmeal indicated that protein status was, in fact, not better on RCS than on AS. Fishmeal feeding increased ($P < 0.01$) DMI, BW gain, milk protein content, yields

of milk, FCM, fat, protein, lactose, and SNF as well as milk yield/DMI ($P = 0.02$). Mean responses to fishmeal supplementation were 1.9 kg/d of milk and 80 g/d of protein. Previously, we observed that milk protein yield increased by 90 (4), 120 (4), and 100 g/d (31) when 3% low-soluble fishmeal was added to diets containing concentrations of CP and AS that were similar to those fed in the present studies. As in the earlier trials (4), including fishmeal in the diet resulted in a small but consistent decrease ($P < 0.001$) in milk lactose content and significantly increased MUN (4, 5, 31). Feeding fishmeal improved ($P < 0.001$) apparent digestibility of nutrients on both AS and RCS by 2 to 5 percentage units. Previously, no change in DM digestibility was reported in two trials, but increased NDF digestibility was found on one trial, when 3% fishmeal was added to AS diets (5).

Significant ($P \leq 0.014$) trial × diet interactions were observed for seven production and four digestibility variables (Table 3). Statistical analysis of apparent digestibilities was restricted to pooled data because these values were estimated using a single fecal grab sample per period from each animal. Statistical analyses from individual trials for the seven production variables are in Table 4. In trials 1 and 2, DMI was not affected by forage source; fishmeal feeding increased DMI in trial 2 and tended to increase DMI in trial 1. The reverse pattern was observed in trial 3: DMI was greater on AS than RCS and was not affected by fishmeal supplementation. Milk yield response to forage source varied among trials, ranging from about 3 kg/d greater on AS (trial 1) to no difference between forage sources (trial 2). However, in two of the three trials, yields of milk and protein, and milk protein content, all were greater ($P \leq 0.05$) with AS than RCS (trials 1 and 3) and with fishmeal supplementation (trials 2 and 3). Except for MUN in trial 1, both MUN and blood urea concentrations were much greater ($P < 0.001$) with feeding of AS. As expected, MUN was similar to blood urea concentrations (6) and, as discussed earlier (Table 3), urea concentrations were influenced mainly by dietary CP (Table 2). A surprising finding was the small apparent reduction in MUN with the feeding of fishmeal in trial 1, while blood urea was elevated by fishmeal in all three trials. However, trial 1 was the study in which only three of four periods were completed in the Latin square. Generally, increasing dietary CP concentration from any source, regardless of ruminal degradability, will increase MUN (6). The significant trial × diet interactions observed for these seven variables likely reflected: 1) the variation in response that is often observed in short-term trials; and 2) the variation that would be expected among forages harvested at various cuttings over three crop years. Also, trial may be consid-

Table 4. Effects during individual trials of feeding alfalfa silage (AS) or red clover silage (RCS), with or without supplemental fishmeal (FM), on variables for which there were significant ($P < 0.05$) trial-by-diet interactions in the overall statistical analysis (Table 3).

							Contrasts		
Item	Trial	AS	RCS	AS + FM	RCS + FM	SE[1]	Forage	FM	Forage × FM
DMI, kg/d	1	20.9	21.1	22.7	21.3	0.5	0.272	0.060	0.289
	2	20.0	19.3	20.3	20.2	0.2	0.157	0.009	0.070
	3	24.2	21.6	23.2	22.4	0.6	0.009	0.782	0.160
Milk, kg/d	1	35.3	31.9	35.8	32.7	0.5	<0.001	0.131	0.922
	2	29.4	29.9	31.4	32.3	0.3	0.894	<0.001	0.164
	3	33.4	31.8	34.9	33.7	0.3	<0.001	<0.001	0.595
Milk/DMI	1	1.70	1.52	1.59	1.56	0.04	0.023	0.323	0.083
	2	1.49	1.58	1.65	1.62	0.02	0.134	<0.001	0.001
	3	1.38	1.47	1.45	1.51	0.02	<0.001	0.007	0.600
Protein, %	1	2.90	2.81	2.96	2.85	0.03	0.005	0.086	0.709
	2	2.85	2.87	2.98	2.96	0.02	0.576	<0.001	0.407
	3	2.96	2.93	3.02	2.98	0.01	0.009	<0.001	0.699
Protein, kg/d	1	1.02	0.89	1.06	0.93	0.01	<0.001	0.024	0.864
	2	0.84	0.86	0.96	0.96	0.01	0.214	<0.001	0.590
	3	0.95	0.92	1.02	0.99	0.01	0.049	<0.001	0.854
Milk urea, mg N/dl	1	12.6	10.8	11.7	10.0	0.1	0.172	0.005	0.665
	2	12.8	9.0	15.7	12.0	0.2	<0.001	<0.001	0.726
	3	17.7	7.2	21.3	10.7	0.3	<0.001	<0.001	0.853
Blood urea, mg N/dl	1	14.8	8.0	17.3	10.2	0.3	<0.001	<0.001	0.585
	2	11.4	9.1	13.8	10.8	0.2	<0.001	<0.001	0.261
	3	17.8	7.9	20.9	10.7	0.3	<0.001	<0.001	0.558

[1]SE = Standard error.

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

Component	Diet			
	Alfalfa silage	Red clover silage	Alfalfa silage + fishmeal	Red clover silage + fishmeal
Dietary silage, % of DM	65.2	64.9	65.2	64.9
Dietary concentrate, % of DM	32.8	33.1	32.8	33.1
NE_L requirement[2]				
Maintenance, Mcal/d	9.5	9.5	9.5	9.5
BW change, Mcal/d	–0.6	1.0	0.3	1.8
Milk, Mcal/d	22.7	21.1	23.7	22.3
Total requirement, Mcal/d	31.6	31.7	33.5	33.6
Total DMI, kg/d	21.9	20.7	22.5	21.4
Concentrate DMI, kg/d	7.6	7.3	7.8	7.5
Concentrate NE_L,[3] Mcal/kg DM	1.88	1.88	1.86	1.86
Concentrate NE_L, Mcal/d	14.3	13.7	14.5	14.0
NE_L from Silage,[4] Mcal/d	17.2	18.0	19.0	19.6
Silage DMI, kg/d	14.3	13.4	14.7	13.9
Silage NE_L, Mcal/kg DM	1.21	1.34	1.29	1.41
Red clover/Alfala, %		111.0		109.1

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

[2]NE_L (Mcal/d) required for maintenance = $0.08 \times BW^{0.75}$ (21); BW = 638 kg. NE_L (Mcal/d) contributed by BW loss = $4.92 \times$ BW loss (kg/d) and required for gain = $5.12 \times$ BW gain (kg/d) (21). NE_L (Mcal/d) required for milk output = Milk $\times$ (0.09464 $\times$ % fat + 0.049 $\times$ % SNF – 0.0564) (20).

[3]Mean NE_L contents of dietary concentrate in the four diets computed from NRC (21) tables.

[4]Total NE_L requirement minus concentrate NE_L.

ered a random effect (M. K. Clayton, personal communication). Thus, the trial $\times$ diet interactions did not detract from the statistical analyses of pooled data (Table 3) and this approach was the more powerful and reliable way to analyze overall animal response in these studies.

The NE_L inputs and outputs were accounted for with the overall means from the three trials to obtain estimates of NE_L content of the AS and RCS (Table 5). The NE_L requirements for maintenance, BW gain, and milk output (based on observed fat and SNF contents) were computed using NRC (20, 21) equations. The NE_L requirements for mean performance were about equal for cows on AS and RCS diets both without (31.7 Mcal/d) and with fishmeal supplementation (33.6 Mcal/d). Subtracting the NE_L contribution computed for ration concentrate (1.88 and 1.86 Mcal/kg of DM without and with fishmeal; 21) yielded an estimate of the NE_L supplied from AS and RCS. Per unit of DM, AS was computed to contain 1.21 or 1.29 Mcal/kg without and with added fishmeal, versus 1.34 or 1.41 Mcal/kg for RCS without and with added fishmeal; this indicated RCS contained 11 or 9% more NE_L per unit DM. Thus, RCS had about 110% of the NE_L of AS. Mean apparent digestibility of dietary OM was 4.6% (67.1/64.1) greater on RCS than on AS (Table 4), and the forages averaged 65% of ration DM during the three trials (Table 2). The NRC (21) tables indicated that AS (42% NDF) and RCS (43% NDF) contained, respectively, 1.35 and 1.45 Mcal/kg DM of NE_L. Greater nutrient digestibilities and estimated NE_L content and lower NPN content suggested that feeding RCS may result in reduced N losses to the environment than feeding AS.

SUMMARY

Three short-term lactation trials compared the feeding value of AS and RCS harvested over 3 yr. Forages had similar amounts of NDF and ADF; however, RCS contained more hemicellulose, less ash and CP, and only 67% as much NPN (proportion of total N) as AS. Performance varied somewhat among individual trials; however, overall, DMI was reduced 1.2 kg/d and yields of milk, FCM, fat, protein, lactose, and SNF were lower when RCS replaced AS as the dietary forage. Replacing AS with RCS improved BW gain and reduced concentrations of MUN and blood urea and ruminal NH_3. Apparent digestibility of DM, OM, NDF, ADF, and hemicellulose were greater for RCS than AS diets. Estimated NE_L contents were 1.25 Mcal of NE_L/kg of DM in AS and 1.38 Mcal of NE_L/kg of DM in RCS, indicating that RCS contained 10% greater NE_L.

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