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Strategies to Improve Milk Yield of Lactating Dairy Cows Fed Red Clover Silage[1]

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Abstract

Two *trials were implemented to explore nutritional strategies to improve lactation performance of dairy cows fed red clover silage (**RCS**)-based diets. In ial 1, 18 early- to midlactation dairy cows were assigned to a replicated 3 × 3 Latin square trial design and fed diets containing forage bases of all **RCS**, a mixture of RCS and brown midrib corn silage (**BMR**), and a mixture of RCS and normal corn silage (**NCS**). Treatment diets were fed for 28 d and contained similar amounts of protein; treatments were formulated to be high in forage fiber so that utilization differences by the lactating cows could be expressed readily between the forage feeding schemes. Cows fed diets containing BMR and NCS consumed significantly more DM and produced approximately 1.5 kg more milk than cows fed diets containing all RCS. Cows fed diets containing all RCS also produced less true protein (**TP**) and nonprotein nitrogen (**NPN**) in milk. Cows fed BMR ate more DM and NDF than did cows fed diets containing NCS, but the increased DMI did not result in improved lactation performance. In Trial 2, 16 early- to midlactation dairy cows were assigned to a replicated 4 × 4 Latin square trial design and fed RCS-based diets containing moderate (**MCP**; 17.9%) or high (**HCP**; 19.5%) levels of CP with or without 4.2% of DM as supplemental dextrose. Cows fed diets containing HCP had increased DMI and milk production when compared with cows fed MCP. In addition, cows fed diets containing HCP significantly increased the CP, TP, NPN, and solids-no-fat (**SNF**) content of their milk. Yield of all milk components measured was increased by diets containing HCP. Supplementing the diets of cows with 4.2% dextrose had some minor negative effects on milk casein and TP yields, but results were biologically inconclusive.*

Data from Trial 1 suggest that replacing RCS with BMR or NCS can improve DMI and milk yield of lactating dairy cows. Supplementing RCS-based diets with greater levels of protein (Trial 2) similarly improves DMI and milk yield of lactating dairy cows.

(Key words: lactation , dairy, red clover, corn silage, sugar)

Introduction

Red clover is grown widely as forage for dairy cattle in regions with poorly drained or low pH soils that are not suited for alfalfa production. In the past (Undersander et al., 1990), it was commonly assumed that red clover and alfalfa had similar nutritional characteristics, but recent research has demonstrated that lactating dairy cows fed red clover silage (RCS) as compared with alfalfa silage do not perform similarly. In our laboratory (Hoffman et al., 1997), we observed reduced DMI and milk yield when cows were fed RCS that contained the same level of ADF as alfalfa silage. In a series of five lactation trials, Broderick et al. (2000b, 2001) observed DMI reductions from 2.5 to 1.2 kg/d when lactating cows were fed RCS as compared with alfalfa silage or alfalfa-corn silage combinations. In three of the five trials (Broderick et al., 2000b, 2001), cows fed red clover-based diets had reduced milk yields. Observations of decreased DMI and trends toward decreased milk yield when lactating dairy cows are fed RCS is concerning, especially in the light of the fact that RCS has the potential for improved N efficiency because RCS does not undergo extensive proteolysis (Jones et al.,

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1995) during fermentation. A common theme in recent research (Hoffman et al., 1997; Broderick et al., 2000b, 2001) has been to evaluate performance of lactating cows fed RCS as compared with alfalfa silage, leaving nutritional enhancement strategies largely unexplored. For example, the nutritional strategy of feeding a high NDF digestibility forage (Oba and Allen, 1999), such as brown midrib corn silage (BMR), in combination with RCS to help alleviate observed feed intake depression when cows are fed RCS has not been explored. In addition, Broderick et al. (2000a) observed increased DMI in lactating cows when sugar replaced starch in alfalfa-based diets; similarly, this nutritional strategy has not been investigated with RCS-based diets. Finally, red clover contains polyphenol oxidases, which have been shown to beneficially reduce nonprotein nitrogen (NPN) content in RCS (Albrecht and Muck, 1991; Jones et al., 1995), but enhanced utilization of red clover protein by lactating cows has not been demonstrated (Broderick et al., 2000b, 2001). Polyphenol oxidases are known to form orthoquinounes in damaged plant tissue, which can alkylate NH_2 and SH groups of amino acids and can significantly alter amino acid digestibility (Ludlum et al., 1991). If the presence of polyphenol oxidases exerts a negative nutritional effect (Ludlum et al., 1991) on protein utilization by lactating cows, as opposed to a positive effect (Jones et al., 1995), then dietary protein manipulation in RCS-based diets may similarly enhance lactation performance of dairy cows. The following studies were conducted to assess whether performance of lactating dairy cows fed RCS could be enhanced by these nutritional modifications.

Materials and Methods

Trial 1. Treatment forages were grown at the Marshfield Agricultural Research Station (Marshfield, WI; 44° 39′ N, 90° 08′ W) on a Withee silt loam soil. A 7.5-ha field of first-cutting red clover (*Trifolium pratense* L.) was cut at the one-half bloom stage of maturity and swathed. The herbage was allowed to wilt for approximately 40 h and was then chopped with a forage harvester to a theoretical length of 1 cm. Herbage was conserved as low moisture RCS in a 4.9- × 18.3-m concrete-stave silo. Similarly, two 11-ha fields were spring-planted with either a normal corn silage variety (NCS; 38W36Bt®; Pioneer Hi-Bred International, Inc., Des Moines, IA) or a brown midrib mutant 3 variety (BMR; Cargil 657®; Mycogen Seeds, Egan, MN) and were harvested at the one-half milk line stage of maturity. Whole-plant corn was chopped with a forage harvester not fitted with a kernel processor at a 0.64-cm theoretical length of cut and conserved as silage in one-half of a 2.6- × 46.8-m plastic silage bag. Treatment silages (RCS, NCS, and BMR) were stored for 182, 104, and 105 d, respectively, before feedout.

Eighteen multiparous (n = 9) and primiparous (n = 9) Holstein cows in early- to midlactation [64 ± 21 d (X ± SD)] were assigned to a replicated 3 × 3 Latin square lactation trial. Cows were randomly assigned to squares and treatment sequences within squares. Experimental periods were 28 d. The first 21 d served as the adaptation period, and all data collection occurred during d 22 to 28.

Treatments consisted of diets containing forage bases of RCS, a mixture of RCS and NCS, or a mixture of RCS and BMR. The mixtures contained 50% of the forage DM as NCS or BMR. Concepts of treatment diet formulation were as follows. Pretrial forage analysis revealed a high NDF (>54.0% DM) content in RCS. The RCS treatment diet was formulated to contain 45% forage and 55.0% concentrate (DM basis) using some fibrous byproducts in the concentrate mix to avoid high starch intakes. As a result, the RCS treatment diet still contained 41% NDF despite the high concentrate level. For treatment diets containing NCS and BMR, 50.0% of the DM supplied by RCS was replaced with NCS or BMR. Concentrate mixtures were then adjusted to provide isonitrogenous diets that contained similar levels of NDF as compared with the RCS-based diet. Treatment diets were formulated to contain less energy than required for early- to midlactation cows (NRC, 2001) to challenge the ability of the forage source to alter milk production and DMI.

Treatment diets were mixed and fed as a total mixed ration (**TMR**) at 0800 h. Amount of the TMR offered was recorded, and treatment diets were sampled daily for the last 7 d of each period. Orts were weighed, recorded, and sampled according to the same procedures followed for the treatment diets. Treatment silages were sampled weekly throughout the experiment.

Silage, treatment TMR, and ort samples were immediately analyzed for DM by oven-drying for 48 h at 55° C. Silage, treatment TMR, and ort samples were ground through a Wiley mill (Arthur H. Thomas, Philadelphia, PA) fit with a 1-mm screen and retained for chemical analysis. All samples were analyzed for CP, ash, fat, and absolute DM according to AOAC (1990) procedures. Acid detergent fiber and lignin were determined sequentially according to the procedures of Goering and Van Soest (1970), and NDF was determined by the modified procedures of Mertens (1992). Nonfiber carbohydrate (**NFC**) content was calculated by difference [NFC = 100 – (CP + NDF + ash + fat – NDF CP)]. In vitro (48 h) DM and NDF digestibility of silages were determined by the procedures of Goering and Van Soest (1970). The NEl content of treatment diets was determined by procedures described by the NRC (2001) with in vitro NDF digestibility as given previously replacing the lignin matrix calculation to determine caloric contribution from NDF. Minerals (Ca, Mg, and K) were determined by atomic absorption spectroscopy, and P was determined by colorimetric methods (Coleman Instruments, Inc., Maywood IL).

Cows were housed in a free-stall barn equipped with Calan gates (American Calan, Inc., Northwood, NH) and milked twice daily at 0230 and 1430 h. Milk weights were recorded daily, and milk was sampled twice daily on d 23, 25, and 27 of each period. Milk fat, solids-not-fat (SNF), lactose, and milk urea nitrogen (**MUN**) were determined by infrared techniques on individual milk samples (AgSource, Colby, WI). Milk protein, casein, and NPN were determined by the procedures of Rowland (1938). Whey protein and true protein in milk were calculated by difference. All experimental procedures conducted in the trial were approved by the Research Animal Resource Center at the University of Wisconsin-Madison.

Trial 2. A 9.2-ha field of red clover (*Trifolium pratense* L.) was cut at the first flower stage of maturity and swathed. The herbage was allowed to wilt for approximately 48 and was then chopped with a forage harvester and conserved as previously described for Trial 1. Trial 2 RCS was allowed to ferment for 181 d before feedout. In Trial 2, 16 multiparous (n = 8) and primiparous (n = 8) Holstein cows in early- to midlactation [57 ± 37 d (X ± SD)] were assigned to a replicated 4 × 4 Latin square lactation trial. Cows were randomly assigned to squares and treatment sequences within squares. Experimental periods were 21 d. The first 14 d served as the adaptation period, and all data collection occurred during d 15 to 21.

Treatments were arranged in a 2 × 2 factorial and consisted of RCS-based diets with and without 4.2% of the dietary DM as supplemental sugar (dextrose). Treatment diets were also formulated to contain 17.5% (moderate; MCP) or 19.5% (high; HCP) CP to complete the factorial arrangement of treatments. Concentrate mixtures were adjusted slightly to provide isonitrogenous diets that contained similar levels of NDF. As with Trial 1, Trial 2 treatment diets were formulated to contain less energy than required for early- to midlactation cows (NRC, 2001) milking 37.5 kg/d to challenge the ability of sugar and different dietary proteins to alter milk production and DMI. Treatment diets, forage, and orts were mixed, fed, offered, sampled, and analyzed by methods previously described for Trial 1. Similarly, production data were collected and analyzed by methods previously described for Trial 1.

Statistics. Data from Trials 1 and 2 were analyzed using the General Linear Models procedures of SAS® (1985) with the model

$$\gamma = \mu + S_i + C_j(S)_i + P_k + T_l + e_{ijkl}$$

where γ = dependent variable, μ = overall mean of the population, S_i = mean effect of square i, $C_j(S)_i$ = mean effect of cow j nested within square i, P_k = mean effect of period k, T_l = mean effect of treatment l, and eijkl = unexplained residual element assumed to be independent and identically distributed. Exceptions to the statistical model as described previously are as follows. For Trial 2, the effect of treatment was divided, with the main effects of sugar, protein, and their interaction evaluated. Treatment differences in Trial 1 were determined by least significant difference when a significant treatment effect was elicited by the statistical model. For both trials, an initial statistical model was run with parity replacing square in the model. There were no treatment × parity interactions for any measurement; therefore, data from mulitparous and primiparous cows were combined and are presented as such.

Results and Discussion

Experimental Silages. Nutrient composition and in vitro DM and NDF digestion of silages fed to cows in Trials 1 and 2 are presented in Table 1. Because no statistical analysis was conducted on silage nutrient compositions or in vitro digestion characteristics, only a brief general discussion of silage quality in Trials 1 and 2 will be offered. Silages fed in both trials appeared to be well fermented, showed no signs of visible mold, and were aerobically stable (no heating) at feedout. In Trial 1, RCS contained more CP than NCS or BMR. The CP values were typical of values described in feed composition tables (NRC, 2001). The NDF of RCS was abnormally high for red clover (Hoffman et al., 1993) and was notably greater than NCS or BMR. Although NCS and BMR contained similar amounts of NDF, the lignin content of BMR was less, and the in vitro NDF digestibility was greater, as compared with NCS, which is consistent with the previously described nutrient composition of BMR (Oba and Allen, 1999). Mineral compositions of the silages in Trials 1 and 2 differed, but appeared within the normal bounds for forages (NRC, 2001), with the exception of ash contents for RCS in Trials 1 and 2, which were abnormally high (>10% of DM). We believe the high ash contents of RCS in Trials 1 and 2 were due to exogenous ash because at harvest red clover in both fields was lodged with a greater percentage of plant material in direct contact with the soil. The high ash contents of RCS in Trials 1 and 2 resulted in low energy predictions based on the NRC (2001) model. Finally, the in vitro DM digestibility of BMR was numerically greater than that of NCS or RCS in Trial 1. Similarly, the in vitro NDF digestibility of BMR and NCS was 21.3 and 12.5 percentage units greater than that of RCS, respectively. Based on nutrient composition and in vitro digestion measurements, treatment silages appeared to be of normal quality for their respective species (NRC, 2001), with the exception that the RCS in Trials 1 and 2 contained greater levels of ash.

Trial 1. The ingredient and nutrient compositions of treatment diets in Trial 1 are presented in Table 2. Diets were not isonitrogenous, but all treatment diets supplied CP at concentrations to support the production of approximately 42 kg/d of 4.0% fat-corrected milk (FCM) by a

Table 1. Nutrient composition and in vitro digestion of experimental silages.

	Trial 1			Trial 2
Item[a]	Red clover silage	Brown midrib corn silage	Normal corn silage	Red clover silage
Nutrient				
DM, % as fed	46.20	30.50	30.20	40.70
CP	20.90	8.68	10.10	20.96
Soluble CP, % CP	30.10	52.30	45.30	33.34
ADF	38.90	23.90	26.28	32.90
ADF-CP	3.21	0.51	0.73	1.68
NDF	54.70	39.00	39.76	44.80
NDF-CP	9.54	1.62	1.91	7.41
Lignin	6.22	2.27	2.55	5.50
Starch	0.54	28.14	34.80	>.01
NFC[b]	18.36	47.58	44.90	24.95
Fat	2.98	2.52	2.61	2.30
Ca	0.98	0.17	0.25	1.16
P	0.33	0.22	0.23	0.34
K	2.80	1.07	1.26	2.67
Mg	0.44	0.17	0.19	0.45
Ash	12.60	3.84	4.54	14.40
IVTDMD[c]	71.22	87.30	82.10	77.10
IVNDFD[d], % NDF	46.23	67.50	55.04	48.80
NE_L[e], Mcal/kg	1.09	1.67	1.65	1.09

[a]All nutrients expressed on a dry matter basis unless specified otherwise.
[b]NFC= nonfiber carbohydrate; NFC = 100-(CP+NDF+Ash+Fat-NDFCP).
[c]IVTDMD= in vitro true DM digestibility.
[d]IVNDFD= in vitro NDF digestibility.
[e]NE_L = net energy of lactation at 3x maintenance.

590-kg cow (NRC, 2001), which is greater than actual milk production observed in Trial 1; therefore, CP was theoretically not limiting in Trial 1 treatment diets. Treatment diets contained greater levels of NDF (40.0%) and lesser levels of dietary energy (1.5 Mcal/kg), making energy the first-limiting nutrient in the experimental diets (NRC, 2001). Mineral and vitamin concentrations were similar between treatment diets and were at or above NRC (2001) model requirements.

Lactation performance, milk composition, milk component yield, and nutrient intakes of lactating cows fed diets containing RCS, BMR, or NCS are presented in Table 3. Milk yield of cows was increased (P<0.04) approximately 1.5 kg/d when BMR or NCS replaced 50% of the RCS in the treatment diets. Our results are similar to the observation of Broderick et al. (2001), who observed an increase in milk yield when cows were fed a forage-based diet containing 25% NCS and 75% RCS compared with a forage base solely of RCS. Broderick et al. (2001) attributed increased milk yield to increases in DMI when NCS replaced RCS in the diet, which is similar to our Trial 1 observations. Specifically, we observed (P<0.001) a 2.2-kg increase in DMI when either BMR or NCS replaced 50% of RCS in the diet (Table 3). Our data are also similar to Dhiman and Satter (1997), who observed increased DMI and milk yields in lactating dairy cows when NCS replaced alfalfa silage in the diet. Replacing RCS with BMR as compared with NCS, however, did not result in an additional increased milk yield. This result is in contrast to recent lactation trials (Oba and Allen, 1999, 2000; Tine et al., 2000), which showed 2- to 4-kg/d milk increases when BMR replaced isogenic corn varieties in the diet of lactating cows. Greenfield et al. (2001) and Tine et al. (2000) both concluded that milk yield responses attributable to BMR are the result of increased DMI and more nutrient flow because calculated NE_L values of BMR and isogenic corn silage are not significantly different. The conclusions of Greenfield et al. (2001) and Tine et al. (2000) explain our data because we observed no calculated (NRC, 2001) NE_L difference between BMR and NCS (Table 1), but observed (P<0.01) a 1.2-kg/d increase in DMI (Table 3) when diets contained BMR as compared with NCS. In contrast to the aforementioned studies, we did not, however, observe an increase in milk yield when cows were fed the BMR as compared with cows fed NCS. We can present no reasonable hypothesis as to why the increased DMI of cows fed BMR did not result in increased milk yield as compared with cows fed NCS.

There were no differences (P>0.10) in the percentage of fat, CP, casein, whey protein, SNF, and lactose in milk of cows fed diets containing RCS, BMR, or NCS. Similar milk fat percentage in cows fed RCS, BMR, and NCS is logical because all diets contained high levels of NDF (40.0% DM). In addition, we did periodically evaluate (Heinrichs and Lammers, 1997; data not shown) our diets for particle length and found no appreciable differences. Therefore, differences in milk fat percentage between cows fed diets containing RCS, BMR, and NCS would not be expected because of physical and chemical fiber adequacy (NRC, 2001). The uniform milk CP and milk protein fractions among cows fed RCS, BMR, and NCS were similar to the findings of Broderick et al. (2001) who did not observe differences in milk CP or TP contents when corn silage replaced RCS in the diet of ctating dairy cows. These data do not confirm our hypothesis and previous observations (Hoffman et al., 1997) that RCS may depress production of milk protein. Our data do, however, suggest that protein utilization was different between cows fed diets containing all RCS as compared with diets containing BMR or NCS. The NPN in milk was significantly less (P<0.03) for cows fed diets containing all RCS as compared with milk of cows fed diets containing BMR or NCS, indicating that ruminal degradation of protein in the RCS diet was less extensive than ruminal protein degradation in diets containing BMR or NCS (NRC, 2001).

There were no significant differences (P>0.05) in the production of milk fat, milk CP, casein, whey protein, SNF, and lactose between cows fed RCS and cows fed diets containing BMR or NCS (Table 3). Cows fed diets containing BMR or NCS did produce more (P<0.02) milk TP and NPN (P<0.001), which is the product of increased milk yield and differences in TP and NPN percentage in the milk; these issues have been previously discussed. There were also no significant (P>0.05) differences in milk component percentages between cows fed diets containing BMR or NCS. No major differences in milk component percentage would be expected because treatment diets were high in NDF, supporting milk fat production potential. Previous data (Oba and Allen, 2000; Tine et al., 2000) have not demonstrated milk protein, SNF, and lactose percentage differences in milk from cows fed BMR as compared with isogenic corn hybrids.

TABLE 2. Ingredient and nutrient composition of treatment diets (Trial 1).

Item[b]	Treatment TMR[a] RCS	BMR	NCS
Ingredient		(% of DM)	
RCS	44.63	24.67	24.67
BMR	—	24.67	—
NCS	—	—	24.67
Shelled corn	39.89	28.46	28.46
Soybean meal	5.70	9.11	9.11
Corn gluten meal	0.95	1.52	1.52
Soyhulls	3.23	3.23	3.23
Brewers grains	1.90	3.04	3.04
Linseed meal	1.90	3.04	3.04
Calcium carbonate	0.38	0.85	0.85
Dicalcium phosphate	0.57	0.57	0.57
Magnesium oxide	0.19	0.19	0.19
Salt	0.47	0.47	0.47
Vitamin premix[c]	0.19	0.19	0.19
Nutrient			
DM, % as fed	54.00	49.62	49.57
CP	19.25	18.20	18.60
ADF	29.83	25.77	25.25
ADF CP	1.92	1.24	1.41
NDF	41.54	42.19	38.99
NDF CP	6.76	4.72	5.23
Lignin	4.34	3.10	3.41
NFC[d]	31.44	31.54	35.36
Fat	3.67	3.70	3.80
Ca	0.96	0.89	0.83
P	0.44	0.44	0.42
K	2.08	1.65	1.61
Mg	0.44	0.39	0.38
Ash	10.86	9.09	8.48
NE_L[e], Mcal/kg	1.47	1.54	1.57

[a]Treatment total mixed rations (TMR) containing forage bases of red clover silage (RCS), RCS + brown midrib corn silage (BMR), or RCS + normal corn silage (NCS).

[b]All ingredients and nutrients are expressed on a DM basis unless otherwise specified.

[c]Contained 2,645,500 IU/kg of vitamin A, 881,800 IU/kg of vitamin D, and 880 IU/kg of vitamin E.

[d]NFC = nonfiber carbohydrate; NFC = 100 – (CP + NDF + Fat + Ash – NDF CP).

[e]NE_L = net energy of lactation at 3x maintenance.

The DM and nutrient intakes of cows fed treatment diets are also presented in Table 3. Differences in DMI between cows fed diets containing all RCS as compared with BMR or NCS appear to fit current theories of NDF-mediated intake regulation. Oba and Allen (1999) suggested that one unit of enhanced NDF digestibility in forage is positively associated with 0.17 kg of DMI, assuming equal NDF content of the diet. In our study, BMR and NCS contained 21.3 and 8.8 percentage units more digestible NDF (% of NDF) as compared with RCS. Using the data of Oba and Allen (1999), and considering that BMR and NCS replaced only 50% of the forage base in our study, differences in NDF digestibility among RCS, BMR, and NCS should have accounted for differences of 1.8 (BMR) and 0.75 kg/d per cow (NCS) in DMI as compared with cows fed solely RCS. Actual DMI enhancement observed when BMR or NCS

Table 3. Lactation performance, milk components, milk component yield, and nutrient intakes of lactating cows fed treatment total mixed rations (TMR) (Trial 1).

	Treatment TMR[a]				Comparison[b]		
Item	RCS	BMR	NCS	SE	Treatment	RCS *vs* BMR + NCS	BMR *vs* NCS
Milk yield, kg/d	31.9	33.4	33.2	0.46	0.08	0.04	NS
Milk component, %							
Fat	3.91	3.78	3.74	0.069	NS	NS	NS
Total CP	3.13	3.17	3.18	0.020	NS	NS	NS
True protein	2.99	3.03	3.03	0.019	NS	NS	NS
Casein	2.51	2.49	2.59	0.053	NS	NS	NS
Whey protein	0.48	0.54	0.44	0.021	NS	NS	NS
NPN[c]	0.139	0.147	0.151	0.0027	0.02	0.03	NS
MUN[d], mg/dL	12.9	13.2	13.5	0.27	NS	NS	NS
Solids-not-fat	8.57	8.63	8.79	0.101	NS	NS	NS
Lactose	4.83	4.83	4.81	0.016	NS	NS	NS
Milk component yield, kg/d							
Fat	1.25	1.23	1.21	0.036	NS	NS	NS
Total CP	0.98	1.02	1.01	0.018	NS	NS	NS
True protein	0.95	1.00	1.00	0.014	0.02	0.02	NS
Casein	0.80	0.83	0.86	0.020	0.10	NS	NS
Whey protein	0.15	0.17	0.15	0.016	NS	NS	NS
NPN	0.04	0.05	0.05	0.001	0.001	0.001	NS
Solids-not-fat	2.75	2.75	2.81	0.061	NS	NS	NS
Lactose	1.52	1.56	1.54	0.026	NS	NS	NS
Intake, kg/d							
DM	20.5	23.3	22.1	0.30	0.001	0.001	0.01
OM	18.3	21.2	20.3	0.28	0.001	0.001	0.03
CP	3.94	4.23	4.11	0.057	0.01	0.02	NS
ADF	6.11	5.99	5.58	0.082	0.001	NS	0.01
NDF	8.51	9.82	8.62	0.122	0.001	NS	0.001
NE_L,[c] Mcal/d	30.1	35.9	34.8	0.44	0.001	0.00	NS

[a]Contained forage bases of red clover silage (RCS), RCS + brown midrib corn silage (BMR), or RCS + normal corn silage (NCS).
[b]NS = $P>0.10$.
[c]NPN = Nonprotein nitrogen.
[d]MUN = Milk urea nitrogen.
[e]NE_L = net energy of lactation at 3x maintenance.

replaced RCS was 2.8 and 1.6 kg/d per cow, respectively, which is in general agreement with the NDF digestibility models of Oba and Allen (1999). Similarly, cows fed diets containing BMR or NCS had significantly greater OM ($P<0.001$), CP ($P<0.02$), and NE_L ($P<0.001$) intakes, which is reflective of DMI differences among cows fed these diets and diets containing all RCS. Cows fed diets containing BMR, as compared with cows fed diets containing NCS, had greater DM, OM, ADF, and NDF intake, which is consistent with previous reports (Oba and Allen, 2000; Tine et al., 2000) comparing brown midrib corn hybrids with isogenic varieties. In conclusion, replacing RCS with BMR or NCS resulted in greater DM and energy intakes, which resulted in improved milk yield as compared with the milk yield of cows fed RCS alone. Using summative energy prediction techniques (NRC, 2001) clearly defines that RCS diets were less in caloric density and had less intake potential than diets containing BMR or NCS. Therefore, results of this study did not elicit any nutritional aberration associated with RCS; rather, results suggested that using NDF as a primary component of RCS

Table 4. Ingredient and nutrient composition of treatment total mixed rations (TMR) (Trial 2).

	Treatment TMR[a]			
Item[b]	MCP	MCP + Sugar	HCP	HCP + Sugar
Ingredient				
Red clover silage	52.05	52.05	52.05	52.05
Shelled corn	41.64	36.75	33.83	28.63
Expellers soybean meal	4.68	5.41	6.25	6.25
Soybean meal	—	—	6.25	7.29
Dextrose	—	4.16	—	4.16
Dicalcium phosphate	0.62	0.62	0.62	0.62
Magnesium oxide	0.31	0.31	0.31	0.31
Salt	0.46	0.46	0.46	0.46
Vitamin premix[c]	0.23	0.23	0.23	0.23
Nutrient				
DM, % as fed	49.90	49.20	49.50	49.40
CP	17.90	17.80	19.30	19.70
ADF	23.90	23.60	23.80	23.20
ADF CP	1.68	1.30	1.29	1.44
NDF	33.80	34.70	34.40	33.90
NDF CP	2.64	2.60	2.78	2.75
Lignin	3.65	3.54	3.73	3.58
NFC[d]	33.50	32.70	31.20	31.20
Fat	3.67	3.70	3.80	3.72
Ca	1.01	0.99	1.01	1.01
P	0.46	0.45	0.49	0.49
K	1.95	1.89	1.96	2.02
Mg	0.41	0.44	0.43	0.44
Ash	11.73	11.81	12.02	12.21
NE_L[e], Mcal/kg	1.45	1.45	1.44	1.44

aContained moderate CP (MCP) or, high CP (HCP) with (+) and without sugar.
bAll ingredients and nutrients are expressed on a DM basis unless otherwise specified.
cContained 2,645,500 IU/kg of vitamin A, 881,800 IU/kg of vitamin D, and 880 IU/kg of vitamin E.
dNFC = Nonfiber carbohydrate; NFC = 100 – (CP + NDF + Fat + Ash – NDF CP).
eNE_L = net energy of lactation at 3x maintenance.

forage quality and diet composition has serious shortcomings.

Trial 2. The ingredient and nutrient compositions of diets fed in Trial 2 are presented in Table 4. Diets vere isocaloric at 1.45 Mcal/kg as defined by summative energy prediction systems (NRC, 2001). Treatment diets were, however, less in dietary energy than planned because of an extremely high and unexpected ash content of RCS in Trial 2 (Table 1). Treatment diets containing MCP supplied CP at concentrations (17.9% DM) theoretically supporting the production of approximately 37 kg/d of 4.0% FCM by a 590-kg cow (NRC, 2001), which is greater than actual milk production observed in Trial 2; therefore, CP was theoretically not limiting in MCP treatment diets. This was a planned element of the experimental design. Treatment diets containing HCP supplied CP at concentrations (19.5% DM) theoretically in excess of requirements (NRC, 2001) for the aforementioned cows. Mineral and vitamin concentrations were similar between treatment diets and were at or above NRC (2001) model requirements.

Lactation performance, milk composition, milk component yield, and nutrient intakes of lactating cows fed red clover-based diets containing different levels of dietary CP and supplemental sugar are presented in Table 5. Milk yield of cows was increased ($P<0.001$) approximately 1.8 kg/d when cows were fed diets containing HCP *vs* MCP. Similarly, CP, TP, NPN, urea N, and SNF content of milk were increased when cows were fed HCP diets *vs* MCP diets. Numeric trends in greater milk casein ($P<0.18$) and whey protein ($P<0.11$) content were also observed when cows were fed HCP diets. There were no differences ($P>0.10$) in fat or lactose content of milk from cows fed MCP or HCP. Yield of all milk components was increased ($P<0.10$) for cows fed HCP diets as compared with cows fed MCP. In addition, cows fed HCP consumed more ($P<0.05$) DM, OM, CP, NDF, and NEl than cows fed MCP. Cows fed sugar (4.2% of dietary DM) produced milk with less ($P<0.10$) casein concentration and produced less milk casein ($P<0.08$) and true protein ($P<0.10$) than cows not supplemented with sugar, although differences were biologically very small. Sugar in the diets of cows did not increase ($P>0.10$) milk production or DMI or any other key production component in red clover-based diets, which is in contrast to the observations of Broderick et al. (2000a). There are subtle differences in the trial of Broderick et al. (2000a) and our study, as Broderick et al. (2000a) supplemented sucrose to cows fed alfalfa-based diets and we supplemented dextrose to cows fed red clover-based diets. We can find no literature to construct a hypothesis of

TABLE 5. Lactation performance, milk components, milk component yield and nutrient intakes of lactating cows fed treatment total mixed rations (TMR) (Trial 2).

	Treatment TMR[a]					Comparison[b]		
Item	MCP	MCP + Sugar	HCP	HCP + Sugar	SE	CP	Sugar	CP × Sugar
Milk yield, kg/d	33.2	32.5	34.7	34.5	0.47	0.001	NS	NS
Milk component, %								
Fat	3.59	3.64	3.61	3.61	0.068	NS	NS	NS
Total CP	2.77	2.73	2.82	2.83	0.015	0.01	NS	NS
True protein	2.65	2.61	2.68	2.68	0.015	0.07	NS	NS
Casein	2.07	2.01	2.07	2.04	0.025	NS	0.10	NS
Whey protein	0.58	0.60	0.61	0.64	0.021	NS	NS	NS
NPN[c]	0.124	0.121	0.142	0.146	0.002	0.001	NS	NS
MUN[d], mg/dL	10.5	10.4	13.4	13.5	0.13	0.001	NS	NS
Solids-not-fat	8.50	8.47	8.54	8.54	0.022	0.01	NS	NS
Lactose	4.89	4.89	4.90	4.90	0.013	NS	NS	NS
Milk component yield, kg/d								
Fat	1.12	1.13	1.17	1.17	0.029	0.10	NS	NS
Total CP	0.92	0.89	0.98	0.98	0.010	0.001	NS	NS
True protein	0.88	0.85	0.93	0.93	0.011	0.001	0.10	NS
Casein	0.68	0.65	0.71	0.70	0.012	0.01	0.08	NS
Whey protein	0.19	0.20	0.21	0.22	0.008	0.03	NS	NS
NPN	0.04	0.04	0.05	0.05	0.001	0.001	NS	NS
Solids-not-fat	2.64	2.60	2.78	2.75	0.033	0.001	NS	NS
Lactose	1.51	1.50	1.59	1.58	0.019	0.001	NS	NS
Intake, kg/d								
DM	21.1	20.6	21.7	21.6	0.33	0.03	NS	NS
OM	18.7	18.2	19.1	19.0	0.29	0.05	NS	NS
CP 3.8	3.7	4.2	4.3	0.06		.001	NS	NS
ADF	5.1	4.9	5.2	5.0	0.08	NS	0.03	NS
NDF	7.2	7.2	7.4	7.3	0.12	0.04	NS	NS
NE_L[e], Mcal/d	30.7	30.0	31.2	31.2	0.42	0.04	NS	NS

[a]Contained moderate CP (MCP) or, high CP (HCP) with (+) and without sugar.
[b]NS = P>0.10.
[c]NPN = Nonprotein nitrogen.
[d]MUN = Milk urea nitrogen.
[e]NE_L = net energy of lactation at 3x maintenance.

nutritional difference between dextrose and sucrose when fed to lactating dairy cows. In addition, we observed no interaction between dietary protein level and sugar supplementation in any production or intake criteria measured.

It is clear from Trial 2 data that we observed a modest production and intake response by increasing the dietary protein above theoretical requirements of cows fed red clover-based diets. Ex post facto, we evaluated Trial 2 RCS in situ to estimate A, B, and C (NRC, 2001) protein fractions to facilitate evaluation of our MCP diet for theoretical protein adequacy. Dried RCS ground through a 2-mm Wiley screen Arthur H. Thomas, Philadelphia, PA was incubated for 0 and 72 h in duplicate Dacron bags Ankom, Fairport, NY in two lactating dairy cows fit with ruminal cannula according to the procedures of Hoffman et al. (1997). The 72-h in situ residues were estimated for microbial protein contamination by the procedures of Brehm et al. (1997). The A, B, and C protein fractions for Trial 2 RCS were calculated to be 54.8, 40.6, and 4.6% of CP, respectively. We then evaluated (NRC, 2001) our treatment diets (data not shown) using 21-kg intakes at 35 kg of 3.6% FCM using the in situ-derived protein fractions with a standard (NRC, 2001) protein digestion rate of the B fraction of 0.15%/h. Results from this post-trial review of dietary protein supply and requirements similarly suggested that ruminal degradable protein, ruminal undegradable protein, and metabolizable protein supplies were adequate in the MCP diet. Thus, traditional evaluation of dietary protein supply and requirement did not explain our observations of a protein-mediated production response in cows fed HCP, red clover-based diets. In addition, we are unsure whether the first-limiting protein fraction in the MCP diet was RDP, RUP, or a specific amino acid. We observed a low soluble protein content of Trial 2 RCS as compared with other legume silages (Hoffman et al., 1999), which was likely attributable to previously defined proteolysis inhibition (Jones et al., 1995). This result would suggest that ruminal degradable protein might have been limiting in the MCP diets, which is partially supported by our observations of significantly greater milk NPN and MUN levels in milk of cows fed HCP; however, increasing dietary CP from any source of CP has been demonstrated to increase milk NPN and MUN (Broderick and Clayton, 1997). Our results agree with the conclusions of Broderick et al. (2000b), who suggested that theorized benefits of RCS-protein fractions have not resulted in improved protein utilization by lactating dairy cows.

A biological mechanism that may explain our trial observations and observations of others (Broderick et al., 2000b) regarding red clover protein utilization in lactating dairy cows is anti-nutritional factors associated with polyphenol oxidases. Polyphenol oxidases are known to form orthoquinounes that alkylate NH2 and SH groups of proteins and amino acids, altering protein degradation (Jones et al., 1995) and digestion (Felton et al., 1992). Although reduction in protein degradation in a legume silage is assumed to be of nutritional benefit (NRC, 1985), alkylation of selected proteins and amino acids is not of benefit. Felton et al. (1992) observed dramatic reductions in insect larvae growth rate when larvae were fed polyphenol oxidase-treated casein, soy protein, gluten, and zein proteins. Other studies (Felton et al., 1989; Ludlum et al., 1991) have also shown protein utilization changes in insects fed polyphenol oxidase-treated proteins. Unfortunately, no trials have been conducted specifically examining the possible negative effects of polyphenol oxidases on ruminal protein and amino acid utilization. Based on our observed and unexplainable dietary protein-mediated production response in HCP diets and the findings of Broderick et al. (2000b), who did not observe enhanced protein utilization in lactating cows fed RCS despite classic laboratory inferences (lower NPN, RDP, etc.) of enhanced protein utilization, we believe studies examining possible negative effects of protein utilization in lactating dairy cows fed RCS diets is warranted.

Implications

These studies were implemented to seek nutritional strategies to improve red clover utilization by lactating dairy cows, because recent research has demonstrated that feeding lactating dairy cows RCS presented nutritional challenges. In Trial 1, NDF alone did not adequately represent the nutritional nuances of RCS. Full evaluation of RCS using summative energy systems (NRC, 2001) was required to adequately define the energy content of RCS-based diets. Replacing RCS with BMR or NCS was a beneficial nutritional strategy because DMI and milk yield of lactating dairy cows was increased. In Trial 2, lactating dairy cows ate more feed and produced more milk containing more protein when diets were supplemented with protein above NRC (2001) requirements. Supplementing red clover-based diets with sugar had no significant biological effect on lactating cow performance and was not a beneficial nutritional strategy. The results of these studies as well as results of other studies (Hoffman et al., 1997; Broderick et al., 2000b, 2001) that have examined various aspects of red clover utilization by lactating dairy cows suggest utilization does not fit classic nutritional dogma. Although anecdotal, we question whether polyphenol oxidases present in red clover are of nutritional benefit or detriment.

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Literature Cited

1. Albrecht, K. A., and R. E. Muck. 1991. Proteolysis in ensiled forage legumes that vary in tannin concentration. Crop Sci. 31:464.

2. Association of Official Analytical Chemists. 1990. Official Methods of Analysis. (15th ed.). AOAC, Arlington, VA.

3. Brehm, N. M., P. C. Hoffman, J. B. Peters, D. K. Combs, and D. J. Undersander. 1997. Estimation of crude protein and microbial protein contamination of in situ residues by near infrared spectroscopy. J. Dairy Sci. 80 (Suppl. 1):219(Abs.).

4. Broderick, G. A., and M. K. Clayton. 1997. A statistical evaluation of animal and nutritional factors influencing concentrations of milk urea nitrogen. J. Dairy Sci. 80:2964.

5. Broderick, G. A., N. D. Luchini, W. J. Smith, S. Reynal, G. A. Varga, and V. A. Ishler. 2000a. Effect of replacing dietary starch with sucrose on milk production in lactating dairy cows. J. Dairy Sci. 83 (Suppl. 1):248(Abs.).

6. Broderick, G. A., R. P. Walgenbach, and S. Maignan. 2001. Production of lactating dairy cows fed alfalfa or red clover silage at equal dry matter or crude protein contents in the diet. J. Dairy Sci. 84:1728.

7. Broderick, G. A., R. P. Walgenbach, and E. Sterrenburg. 2000b. Performance of lactating dairy cows fed alfalfa or red clover silage as the sole forage. J. Dairy Sci. 83:1543.

8. Dhiman, T. R., and L. D. Satter. 1997. Yield response of dairy cows fed different proportions of alfalfa silage and corn silage. J. Dairy Sci. 80:2069.

9. Felton, G. W., K. Donato, R. J. Del Vecchio, and S. S. Duffey. 1989. Activation of plant foliar oxidases by insect feeding reduces nutritive quality of foliage for noctuid herbivores. J. Chem. Ecol. 15(12):2667.

10. Felton, G. W., K. K. Donato, R. M. Broadway, and S. S. Duffey. 1992. Impact of oxidized plant phenolics on the nutritional quality of dietary protein to a noctuid herbivore, Spodoptera exigua. J. Insect Physiol. 38(4):277.

11. Goering, H. K., and P. J. Van Soest. 1970. Fiber Analyses (Apparatus, Reagents, Procedures, and Some Applications). Agric. Handbook No. 379. ARS-USDA, Washington, DC.

12. Greenfield, T. L., R. L. Baldwin, R. A. Erdman, and K. R. McLeod. 2001. Ruminal fermentation and intestinal flow of nutrients by lactating cows consuming brown midrib corn silages. J. Dairy Sci. 84:2469.

13. Heinrichs, A. J., and B. P. Lammers. 1997. Particle size recommendations for dairy cattle. In Silage: Field to Feed Bunk. p 268. Northeast Region Agricultural Engineers Society, Hershey, PA.

14. Hoffman, P. C., N. M. Brehm, L. M. Bauman, J. B. Peters, and D. J. Undersander. 1999. Prediction of laboratory and in situ protein fractions in legume and grass silages using near infrared reflectance spectroscopy. J. Dairy Sci. 82:764.17.

15. Hoffman, P. C., D. K. Combs, N. M. Brehm, and D. A. Welch. 1997. Performance of lactating dairy cows fed red clover or alfalfa silage. J. Dairy Sci. 80:3308.

16. Hoffman, P. C., S. J. Sievert, R. D. Shaver, D. A. Welch, and D. K. Combs. 1993. In situ dry matter, protein and fiber degradation of perennial forages. J. Dairy Sci. 76:2632.

17. Jones, B. A., R. E. Muck, and R. D. Hatfield. 1995. Red clover extracts inhibit legume proteolysis. J. Sci. Food Agric. 67:329.

18. Ludlum, C. T., G. W. Felton, and S. S. Duffey. 1991. Plant defenses: Chlorogenic acid and polyphenol oxidase enhance toxicity of Bacillus thuringiensis subsp. kurstaki to Heliothis Zea. J. Chem. Ecol. 17:217.

19. Mertens, D. R. 1992. Critical conditions in determining detergent fibers. In Proc. Natl. Forage Testing Assoc. Forge Analysis Workshop, Denver, CO. p C-1. Natl. Forage Testing Assoc., Omaha, NE.

20. National Research Council. 1985. Ruminant Nitrogen Usage. Natl. Acad. Sci., Washington, DC.

21. National Research Council. 2001. Nutrient Requirements of Dairy Cattle. (7th Rev. Ed.). Natl. Acad. Sci., Washington, DC.

22. Oba, M., and M. S. Allen. 1999. Effects of brown midrib 3 mutation in corn silage on dry matter intake and productivity of high yielding dairy cows. J. Dairy Sci. 82:135.

23. Oba, M., and M. S. Allen. 2000. Effects of midrib 3 mutation in corn silage on productivity of dairy cows fed two concentrations of dietary neutral detergent fiber: 1. Feeding behavior and nutrient utilization. J. Dairy Sci. 82:589.

24. Rowland, S. J. 1938. The determination of the nitrogen distribution in milk. J. Dairy Res. 9:42.

25. SAS® User's Guide: Statistics, Version 5 Edition. 1985. SAS Inst., Inc., Cary, NC.

26. Tine, M. A., K. R. Mcleod, R. A. Erdman, and R. L. Baldwin, VI. 2001. Effects of brown midrib corn silage on the energy balance of dairy cattle. J. Dairy Sci. 84:885.

27. Undersander, D., R. R. Smith, K. Kelling, J. Doll, G. Worf, J. Wedberg, J. Peters, P. Hoffman, and R. Shaver. 1990. Red Clover: Establishment, Management and Utilization. Publ. A3492. Univ. Wisconsin-Extension, Madison,WI.