Replacing alfalfa or red clover silage with birdsfoot trefoil silage in total mixed rations increases production of lactating dairy cows¹

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ABSTRACT

The objective of this study was to compare milk production and nutrient utilization in dairy cattle fed silage made from alfalfa (AL) or red clover (RC) versus birdsfoot trefoil (BFT) selected for low, normal, and high levels of condensed tannins. Condensed tannin contents of the 3 BFT silages were 8, 12, and 16 g/kg of DM by butanol-HCl assay. Twenty-five multiparous Holstein cows (5 fitted with ruminal cannulas) were blocked by days in milk and randomly assigned within blocks to incomplete 5 × 5 Latin squares. Diets contained [dry matter (DM) basis] about 60% AL, 50% RC, or 60% of 1 of the 3 BFT; the balance of dietary DM was largely from high-moisture corn plus supplemental crude protein from soybean meal. Diets were balanced to approximately 17% crude protein and fed for four 3-wk periods; 2 wk were allowed for adaptation and production data were collected during the last week of each period. No differences existed in DM intake or milk composition due to silage source, except that milk protein content was lowest for RC. Yields of milk, energy-corrected milk, fat, protein, lactose, and solids-not-fat were greater for the 3 BFT diets than for diets containing AL or RC. Feeding BFT with the highest condensed tannin content increased yield of milk, protein, and solids-not-fat compared with BFT containing the lowest amount of condensed tannin. Moreover, milk-N/N-intake was higher, and milk urea nitrogen concentration and urinary urea-N excretion were lower for diets with normal levels of BFT than for AL or RC diets. Feeding RC resulted in the highest apparent digestibility of DM, organic matter, neutral detergent fiber, acid detergent fiber, and hemicellulose and lowest ruminal concentrations of ammonia and free amino acids. Ruminal branched-chain volatile fatty acid levels were lowest for RC diets and diets with high

levels of BFT and highest for the AL diet. Overall, diets containing BFT silage supported greater production than diets containing silage from AL or RC. The results indicated that feeding BFT or other legume silages containing condensed tannins can enhance performance and N utilization in lactating dairy cows.

Key words: birdsfoot trefoil, condensed tannins, milk yield

INTRODUCTION

Condensed tannins (CT) are natural plant polyphenolic compounds that bind to proteins via hydrophobic interactions and hydrogen bonding (Mueller-Harvey, 2006). Plant CT can have beneficial or adverse effects on ruminant production, depending on their concentration and nature, animal species involved, physiological state of the animal, and composition of the ration (Waghorn, 2008; Patra and Saxena, 2011). Although birdsfoot trefoil (BFT; *Lotus corniculatus* L.) is not commonly grown on US farms, the feeding of conserved BFT or other forages containing moderate concentrations of CT could improve milk production and N efficiency of lactating dairy cattle. When fed as the sole component of the diet, freshly cut or grazed BFT improved milk production by 14 to 20% at similar DMI in cows (Woodward et al., 1999, 2000), and by 21% in ewes (Wang et al., 1996a), compared with BFT in which CT was inactivated by addition of polyethylene glycol. Improved performance with freshly cut or grazed BFT can be attributed to increased flow of plant protein to the abomasum and greater intestinal absorption of essential AA due to reduced ruminal degradation mediated by CT (Waghorn et al., 1987; Wang et al., 1996b). Condensed tannins may also limit proteolysis during conservation and ruminal fermentation of BFT hays and silages (Albrecht and Muck, 1991; Grabber and Coblenz, 2009; Grabber et al., 2011), but it is not known whether CT in BFT silage can enhance milk production and N efficiency of cows when fed as the forage component of a mixed ration.

Condensed tannins are not present in the foliage of alfalfa (AL) and ruminants make poor use of its high protein content because the NPN formed during ensil-

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ing and in the rumen that is not captured as microbial protein is largely excreted as urinary N and can be readily lost to the environment (Misselbrook et al., 2005). Red clover (**RC**) contains small amounts of floral CT (Sivakumaran et al., 2004), but NPN formation in RC silage is relatively low due to the action of *o*-quinones formed by polyphenol oxidase (**PPO**; Albrecht and Muck, 1991; Jones et al., 1995). Although yield of milk and protein were unchanged, replacing AL silage with RC silage has been reported to improve DM and N efficiency in lactating cows (Broderick et al., 2001).

Our hypothesis was that replacing dietary AL silage with BFT silage would improve N efficiency in lactating cows and result in similar N efficiency as feeding RC silage. Therefore, the objective of this study was to assess milk production and nutrient efficiency of dairy cattle fed mixed rations containing BFT silages with varying levels of CT. Responses to CT-containing BFT silages were compared with responses when feeding silages made from CT-free AL and PPO-containing RC.

MATERIALS AND METHODS

Experimental Procedure

Twenty-five multiparous Holstein cows (5 fitted with ruminal cannulas) were selected with mean (SD) parity 2.6 (0.9), 640 (54) kg of BW, 161 (51) DIM, and producing 47 (8) kg of milk/d at the start of the trial. Cows were housed in tiestalls bedded with straw and had free access to water throughout the trial. The Animal Care and Use Committee for the College of Agriculture and Life Sciences of the University of Wisconsin-Madison approved all animal procedures. Cows were blocked by DIM into 5 groups of 5 cows and randomly assigned within blocks to 5 treatment sequences in replicated, incomplete 5 × 5 Latin squares. Dietary treatments were fed during four 3-wk periods (total 12 wk). Each experimental period had 2 wk for adaptation and 1 wk for collection of production data and samples.

Following a seed increase in 2001, populations of NC-83 BFT with low CT (**LBFT**), normal (**NBFT**), and high (**HBFT**) concentrations of CT (Miller and Ehlke, 1997) were planted on April 17, 2002, at an average seeding rate of 10.3 kg/ha. The RC variety Marathon and the alfalfa varieties Forecast 1001 and Rebound 4.2 were planted around April 11 and April 24, 2002, at average seeding rates of 14.6 and 16.8 kg/ha, respectively. All forages were planted on silt loam soils in adjacent fields of the US Dairy Forage Research Center farm (Prairie du Sac, WI) using a no-till planter with row spacings of 19 cm. Following first-cutting (BFT) and second-cutting (AL) harvests in July, AL and BFT forages were cut with a mower-conditioner on August

19, 2002. Mowed forage was wilted to about 35% DM and chopped with a New Holland FX 58 forage harvester (CNH Global NV, New Holland, PA) to a 2-cm theoretical length of cut on August 20, 2002, and immediately ensiled into bag silos (Ag-Bag model G6000; Ag-Bag Systems Inc., St. Nazianz, WI). Second-cutting RC forage was also mowed, field-wilted, chopped, and ensiled into bag silos using the same equipment. Before ensiling, subsamples of chopped BFT were quick frozen in liquid nitrogen and subsequently freeze dried (Udy Corp., Fort Collins, CO) for the analysis of total CT by a butanol-HCl method (Grabber, 2008).

Diets were fed as TMR and contained about 60% of DM from AL or 1 of the 3 BFT silages plus 40% of DM from corn silage, high-moisture corn, solvent soybean meal, and minerals and vitamins. The RC diet contained about 50% RC silage DM plus additional high-moisture corn and soybean meal to adjust for higher NDF and lower CP content of that forage. Corn silage fed in this trial was a brown midrib variety (F657; Cargill Hybrid Seeds, Minneapolis, MN) harvested at about 50% milk-line. The corn was chopped to a theoretical length of 1.9 cm, with field processing of grain by rolling (roller clearance of 1 to 3 mm), and then ensiled in a large bunker silo without additives. Diets were formulated to 17% CP and 28% NDF. Cows were fed experimental diets once daily at 1000 h and orts were collected at 0900 h. The feeding rate was adjusted daily to yield orts equivalent to about 5 to 10% of intake.

Daily samples of about 0.5 kg of the 5 legume silages, corn silage, high-moisture shelled corn, each TMR, and mixed orts from each diet were collected and stored at -20°C to obtain weekly composites that were used to determine the composition of the diets actually consumed during the trial. Soybean meal was sampled once each week. Weekly legume silage composites were divided into 2 subsamples. One was stored at -20°C for later extract preparation and a second was dried at 55°C (48 h) and retained for chemical analysis. After drying, ingredients and TMR were ground through a 1-mm screen (Wiley mill; Arthur H. Thomas Co., Philadelphia, PA). Proportions of each ration ingredient on an as-fed basis were adjusted weekly based on DM content determined by drying weekly composites at 60°C (48 h). Intake of DM was computed based on the 60°C DM values for TMR and orts. Dried weekly TMR composites, and silage composites from each period, were analyzed for DM at 105°C, for ash and OM (AOAC, 1980), and for total N using a combustion assay (Leco FP-2000 N analyzer; Leco Instruments Inc., St. Joseph, MI). Samples were analyzed sequentially for NDF and ADF including heat-stable α -amylase and Na₂SO₃ added during NDF extraction (Van Soest et

Table 1. Chemical composition of silages¹

Item	Silage					SE	P-value
	AL	RC	LBFT	NBFT	HBFT		
CT, g/kg of DM	0	2.3 ^d	8.3 ^c	12.1 ^b	15.7 ^a	0.20	<0.01
DM, %	41.2 ^b	60.0 ^a	40.3 ^{bc}	34.0 ^d	36.7 ^{cd}	1.19	<0.01
CP, % of DM	22.0 ^a	18.1 ^c	21.7 ^a	20.4 ^b	20.1 ^b	0.31	<0.01
Ash, % of DM	11.2 ^b	13.4 ^a	9.9 ^c	10.1 ^c	10.1 ^c	0.34	<0.01
NDF, % of DM	35.3 ^b	42.7 ^a	32.4 ^c	32.4 ^c	32.2 ^c	0.50	<0.01
ADF, % of DM	26.7 ^b	28.4 ^a	25.8 ^{bc}	25.3 ^c	25.1 ^c	0.46	<0.01
NDIN, % of total N	3.03 ^b	12.49 ^a	2.62 ^{bc}	1.86 ^d	2.41 ^{bcd}	0.221	<0.01
ADIN, % of total N	1.71 ^b	2.65 ^a	1.23 ^b	1.20 ^b	1.38 ^b	0.365	<0.01
B3, ² % of total N	1.33 ^{bc}	9.83 ^a	1.39 ^b	0.66 ^c	1.03 ^{bc}	0.636	<0.01
NH ₃ -N, % of total N	4.4 ^a	2.7 ^c	3.2 ^c	3.8 ^b	2.9 ^c	0.17	<0.01
TAAN, ³ % of total N	37.1 ^a	11.3 ^b	32.1 ^a	38.6 ^a	31.3 ^a	2.38	<0.01
NPN, % of total N	52.1 ^b	25.1 ^c	57.4 ^a	57.7 ^a	55.4 ^{ab}	1.59	<0.01
pH	4.63 ^a	4.30 ^b	4.71 ^a	4.20 ^b	4.17 ^b	0.068	<0.01
Fermentation product, g/kg of DM							
Lactate	5.83 ^c	7.27 ^{ab}	4.15 ^d	6.72 ^b	7.83 ^a	0.254	<0.01
Acetate	1.81 ^a	0.92 ^b	0.81 ^b	0.94 ^b	1.03 ^b	0.087	<0.01
Succinate	0.47 ^a	0.12 ^b	0.13 ^b	0.11 ^b	0.12 ^b	0.010	<0.01
Formate	0.06 ^c	0.20 ^a	0.11 ^{bc}	0.16 ^{ab}	0.23 ^a	0.023	<0.01
1,2-Propanediol	0.13 ^a	0.02 ^b	0.12 ^a	0.00 ^a	0.00 ^a	0.018	<0.01
Propionate	0.00	0.00	0.00	0.02	0.02	0.006	0.06
Ethanol	0.07	0.06	0.04	0.06	0.06	0.006	0.53
Butyrate	0	0	0	0	0	0	—

^{a-d}Least squares means within the same row without a common superscript differ ($P < 0.05$).

¹AL = alfalfa; RC = red clover; LBFT = low condensed tannin (CT) birdsfoot trefoil; NBFT = normal CT birdsfoot trefoil; HBFT = high CT birdsfoot trefoil.

²Fraction B3 = NDIN – ADIN (Sniffen et al., 1992).

³Total AA-N = total free AA, mmol $\times$ (1 mg of N/40.3 μ mol of total free AA) (Broderick, 1987).

al., 1991; Hintz et al., 1996). Combustion N analysis (Leco FP-2000 N analyzer) on this ADF material was used to determine ADIN content. Legume silages were assayed for CT concentrations as described by Grabber (2008). Silages and TMR also were analyzed for NDIN without use of Na₂SO₃ (Van Soest et al., 1991). The TMR composites were analyzed for total fat content (method 920.39, AOAC International, 1997; Dairyland Laboratories Inc., Arcadia, WI), and for water-soluble carbohydrates and starch (Hall et al., 1999; Rumen Fermentation Profiling Laboratory, West Virginia University, Morgantown). The TMR composites also were analyzed for indigestible ADF (ADF remaining after 288 h of in situ incubation) by the method of Huhtanen et al. (1994). Legume silage extracts were prepared by subsampling and extracting period composites with distilled water as described by Muck (1987). Extract pH was determined immediately and extracts were deproteinized (Muck, 1987) and analyzed by flow-injection methods (Lachat QuikChem 8000 FIA; Lachat Instruments, Milwaukee, WI) for NPN (Lachat method 12-107-04-3-b) and ammonia and total free AA using the methods of Broderick et al. (2004). Extracts also were analyzed for succinate, lactate, formate, acetate, propionate, butyrate, 1,2-propanediol, ethanol, and 2,3-butanediol using HPLC methodology (Siegfried et

al., 1984). Chemical compositions of the legume silages are in Table 1. Proportions of each ingredient and the chemical compositions (determined by analyzing weekly TMR composites) of each diet as fed are in Table 2.

Cows were injected with a half dose of recombinant bovine somatotropin (rBST; 250 mg of Posilac; Monsanto, St. Louis, MO) once per week over the course of the trial. Cows were milked daily at 0500 and 1700 h and milk weights recorded at every milking. Milk yields from wk 3 were used to compute mean daily milk yield for each cow in each period. Milk samples were collected from each cow at an a.m. and p.m. milking mid-way through wk 3, preserved with 2-bromo-2-nitropropane-1,3-diol, stored at 4°C, and samples sent to AgSource Inc. (Verona, WI) for analysis of fat, protein, lactose, and SNF by infrared method no. 972.16 (AOAC, 1990) using a Foss FT6000 instrument (Foss North America Inc., Eden Prairie, MN). Milk subsamples also were deproteinized (Shahani and Sommer, 1951) and analyzed for MUN by a colorimetric assay (Broderick and Clayton, 1997), adapted to a flow-injection analyzer (Lachat QuikChem 8000 FIA). Yields of fat, protein, lactose and SNF, and MUN concentrations were computed as the weighted means from p.m. and a.m. milk yields on each test day. Yields of ECM also were computed as described by Krause and Combs (2003).

Table 2. Composition of diets

Item	Silage source ¹				
	AL	RC	LBFT	NBFT	HBFT
Ingredient, % of DM					
Alfalfa silage	59.4	0	0	0	0
Red clover silage	0	50.5	0	0	0
LBFT silage	0	0	60.5	0	0
NBFT silage	0	0	0	60.3	0
HBFT silage	0	0	0	0	60.7
Corn silage	15.6	15.6	15.2	15.2	15.1
High-moisture corn	21.9	24.4	21.2	21.3	21.2
Soybean meal (48%)	2.3	8.9	2.3	2.3	2.3
Sodium bicarbonate	0.3	0.3	0.3	0.3	0.3
Sodium chloride	0.2	0.2	0.2	0.2	0.2
Dicalcium phosphate	0.1	0.1	0.1	0.1	0.1
Vitamin-mineral premix ²	0.1	0.1	0.1	0.1	0.1
Chemical composition					
Dietary DM, %	47.8	61.3	47.1	43.3	45.0
CP, % of DM	18.0	17.8	17.3	16.6	17.0
Ash, % of DM	9.1	10.1	7.9	8.1	8.3
Fat, % of DM	4.0	3.6	3.1	3.6	3.4
Starch, % of DM	23.7	27.3	24.8	25.4	23.0
WSC, ³ % of DM	3.0	3.1	4.2	3.4	3.8
NFC, ⁴ % of DM	40.3	41.5	45.9	45.2	44.7
NDF, % of DM	29.2	28.6	26.2	26.9	27.1
ADF, % of DM	20.0	17.1	18.3	18.3	20.1
NDIN, % of total N	3.2	9.1	2.5	2.7	2.9
ADIN, % of total N	1.7	2.8	1.4	1.3	1.6
Fraction B3, % of total N	1.6	6.3	1.0	1.4	1.4

¹AL = alfalfa; RC = red clover; LBFT = low condensed tannin (CT) birdsfoot trefoil; NBFT = normal CT birdsfoot trefoil; HBFT = high CT birdsfoot trefoil.

²Provided (/kg of DM): 56 mg of Zn, 46 mg of Mn, 22 mg of Fe, 12 mg of Cu, 0.9 mg of I, 0.4 mg of Co, 0.3 mg of Se, 6,440 IU of vitamin A, 2,000 IU of vitamin D, and 16 IU of vitamin E.

³WSC = water soluble carbohydrates.

⁴Nonfiber carbohydrates = $100 - \text{CP} \times (100 - \text{NDIN}/100) - \text{ash} - \text{fat} - \text{NDF}$.

Efficiency of feed conversion was calculated for each cow in each period by dividing mean yield of milk and ECM by mean DMI over the last week of each period. Efficiency of feed N utilization was computed for each cow in each period by dividing mean milk N output (milk protein yield/6.38) by mean N intake. Cows were weighed on 3 consecutive days at the start of the trial and at the end of each period to compute BW change.

At the end of wk 3 of each period, blood samples were collected from the coccygeal vein or artery into heparinized tubes about 4 h after feeding and held on ice for transport to the laboratory. Blood was centrifuged ($1,160 \times g$ for 15 min at 4°C) and the resulting plasma was deproteinized by mixing with 15% wt/vol sulfosalicylic acid (1 vol plus 4 vol of plasma) and centrifuging ($24,700 \times g$ for 15 min at 4°C). The supernatant was stored at -20°C until analyzed for plasma urea-N with the assay used for MUN. Spot urine and fecal samples also were collected at the end of wk 3 of each period at 0600 and 1800 h. Urine samples were immediately diluted by mixing 15 mL of each urine sample with 60 mL of 0.072 *N* H₂SO₄ and storing at -20°C until analy-

sis. Fecal samples were dried for 72 h at 60°C, ground through a 1-mm screen (Wiley mill) and composited on an equal DM basis to obtain 1 fecal sample per cow per period. All fecal samples were analyzed for DM, ash, OM, NDF, ADF, total N, and indigestible ADF contents using the assays described above. Indigestible ADF was used as an internal marker to estimate apparent nutrient digestibility and fecal output (Cochran et al., 1986). Urine samples were thawed and analyzed for total N by elemental analysis (Mitsubishi TN-05 nitrogen analyzer; Mitsubishi Chemical Corp., Tokyo, Japan), for urea with the colorimetric method used for MUN, and for creatinine (Valadares et al., 1999). Daily urine volume and excretion of urea N and total N were estimated from mean urinary concentrations in each period assuming a creatinine excretion rate of 29 mg/kg of BW (Valadares et al., 1999). Ruminal fluid was collected via ruminal cannulas on d 20 of each period at 0, 1, 2, 4, 6, 8, and 12 h after feeding from multiple sites in the ventral rumen. Samples were filtered through 2 layers of cheesecloth, the pH measured immediately by glass electrode, and then preserved by adding 0.2

mL of 50% (vol/vol) H_2SO_4 to 10-mL ruminal fluid and stored at -20°C until analyzed. After thawing and centrifugation ($15,300 \times g$ for 20 min at 4°C), ruminal supernatants were analyzed by flow-injection methods for ammonia by a phenol-hypochlorite method (Lachat method 18-107-060-1-A) and for total free AA by a fluorometric procedure based on *o*-phthalaldehyde (Roth, 1971), using Leu as the standard AA. Individual and total ruminal VFA were determined in supernatants using a modification of the GLC method described in Supelco Bulletin 855B (Supelco Inc., Supelco Park, Bellefonte PA) with flame-ionization detection. Ruminal lactate, formate, succinate, 1,2-propandiol, ethanol, and 2,3-butanediol also were measured in these samples using HPLC methods (Siegfried et al., 1984).

Statistical Analyses

Composition data from analysis of the 5 legume silages were analyzed with GLM in SAS (SAS Institute, 2002–2003) using a model that included period, week, and treatment (silage source). Animal production data were analyzed as an incomplete 5×5 Latin square design using the PROC MIXED of SAS (SAS Institute, 2002–2003). A single mean observation was computed for each cow over the last week of each period ($n = 20$). Model sums of squares from the data were separated into overall mean, cow (within square), square, period, treatment (effect of diet), square $\times$ treatment interaction, and overall error. All variables were considered fixed, except cow (within square) and overall error, which were considered random. Model sums of squares for ruminal data collected at different times after feeding (pH and concentrations of ammonia, total free AA, VFA, and other metabolites) were separated into overall mean, cow, period, treatment (effect of diet), whole plot error, hours postfeeding (repeated measures), hours post feeding $\times$ treatment interaction, and subplot error. The repeated measures analyses were performed using the SP(POW) structure of SAS. For all statistical analyses, significance was declared at $P \leq 0.05$ and trends at $0.05 \leq P \leq 0.10$. The PDIFF option of SAS (SAS Institute, 2002–2003) was used to test for treatment differences among least squares means; differences are reported only if the F-test for dietary treatment was significant ($P \leq 0.05$).

RESULTS AND DISCUSSION

Silage Composition

A large number of differences in chemical composition were observed among the silages fed in this trial, almost all of which reached significance at $P \leq 0.01$

(Table 1). As determined using a butanol-HCl assay, total CT concentrations ranged from about 8 g/kg in LBFT to 16 g/kg in HBFT populations compared with about 2 g/kg in RC; concentrations were comparable to levels previously reported for these forages (Grabber, 2008). Although a small degree of variation in DM content existed among the AL and BFT silages, all 4 fell in the range of 34 to 41%, which was similar to the DM target during wilting. However, RC at 60% DM was much dryer than the other forages. Dry matter content affects the rate and quality of the fermentation during ensiling (Muck and Dickerson, 1988) and may account for some of the variation in fermentation products between RC and the other forages. As expected, RC silage was lowest in CP content. Previously, we observed RC to be about 2 percentage units lower in CP than AL, but the RC was also lower in NDF and ADF than AL in those trials (Broderick et al., 2001). In the present study, RC silage had greater content of NDF, ADF, and ash than the other forages; this indicated that the RC was much more mature than the other forages and dissimilar from RC fed in earlier trials. Greater maturity of RC impaired the comparison of its nutritional value with that of the other forages. Our initial analysis indicated that AL and the 3 BFT silages were similar in CP content. However, over the course of the trial, mean CP content of NBFT and HBFT were both lower than mean CP content in AL and LBFT (Table 1); this resulted in the diets containing those silages being numerically lower in CP concentration (Table 2). As in a previous ensiling study with these forages (Grabber and Coblenz, 2009), RC had substantially greater proportions of NDIN, ADIN, and N fraction B3 (Sniffen et al., 1992) than other silages, reflecting the reaction of PPO with *o*-diphenols in that forage (Jones et al., 1995). Significant differences were detected among the other 4 forages in these N fractions, but overall, these were numerically small and likely had little effect on N utilization. Diets containing the AL and BFT silages contained similar NDIN, ADIN, and N fraction B3 (Table 2).

Although AL had the greatest proportion of total N present as ammonia, AL and the BFT silages were similar in total AA-N and AL was lower than LBFT and NBFT in the proportion of NPN (Table 1). Silage made from alfalfa commonly has in excess of 50% total N present as NPN (Broderick, 1995); 52% NPN is a typical value for AL. Condensed tannins have generally been reported to suppress proteolysis of ensiled forages and to reduce NPN formation (Albrecht and Muck, 1991; Lorenz et al., 2010), but that effect was not observed in the present study. Grabber and Coblenz (2009) reported that CT in BFT caused modest shifts in N from NPN mainly to fraction B2. Although signifi-

cant differences in ammonia-N were detected, no values were in excess of 10%, a value indicative of extensive clostridial fermentation (McDonald et al., 1991). As expected, RC contained the lowest proportions of total N present as ammonia, total AA, and NPN (Table 1). As noted previously, these results indicated that the PPO enzyme system was more effective in limiting NPN formation than CT; *o*-quinones in RC were found to dramatically shift N from NPN to fractions B2 and B3 (Grabber and Coblenz, 2009).

Rate of pH decline, which represents a balance between production of fermentation acids and the buffering capacity of the ensiled material, is another factor influencing NPN formation: if pH decreases slowly, greater amounts of forage protein usually are hydrolyzed by plant proteases in the silo (Muck et al., 2003). Differences in final silage pH were observed, with AL and LBFT having higher values than the other 3 forages (Table 1). Although final pH may not reflect the rate of pH decline, that LBFT had higher pH and greater N in total AA and NPN than HBFT suggested that pH may have been a factor in the extent of protein breakdown in BFT silages. Final silage pH is strongly influenced by lactic acid concentrations because of lactic acid has a lower acid dissociation constant (pKa) relative to acetic acid and other VFA (Muck et al., 2003), and higher lactate levels in RC, NBFT, and HBFT were related to lower pH in those forages. Greater concentrations of acetate are related to improved aerobic stability (Muck, 2004); AL silage had more acetate than the other 4 forages. Other small, but significant differences were

observed in concentrations of fermentation products (Table 1) but these probably had little effect on silage quality. Moreover, it is noteworthy that ethanol and butyrate, 2 important anti-quality factors (McDonald et al., 1991), were either present in similar low levels (ethanol) or not detected (butyrate) in the legume silages fed in this trial.

Animal Responses

Although diets were formulated to 17% CP and 28% NDF, shifts in forage composition over the course of the trial resulted in greater CP content in the AL and RC diets and 2 to 3 percentage units lower NDF concentration in the diets based on BFT silages (Table 2). No differences were noted in N intake, despite differences in CP concentration among diets (Table 3). The RC diet also had higher starch content, due to its greater content of high-moisture corn, and greater NDIN and N fraction B3, reflecting the greater contribution of N from RC forage (Table 1). Note that the dietary composition data derived from direct chemical analysis of TMR samples collected during the trial and, thus, reflect sampling as well as analytical variation.

No effect was observed of legume silage source on DMI in this trial (Table 3). In the case of BFT silages, CT concentrations were far below levels thought to depress intake (Patra and Saxena, 2011). Previous lactation trials with cattle indicated that intake of diets containing RC silage was similar or somewhat lower than diets containing AL silage (Broderick et al., 2001).

Table 3. Effect of dietary forage source on production

Item	Silage source ¹					SEM	P-value
	AL	RC	LBFT	NBFT	HBFT		
N intake, g/d	713	730	685	637	684	25.8	0.07
DMI, kg/d	24.7	25.6	24.7	24.0	25.2	0.93	0.69
BW change, kg/d	-0.01	0.03	0.53	0.15	0.43	0.349	0.17
Milk, kg/d	30.9 ^c	30.8 ^c	33.5 ^b	34.6 ^{ab}	35.4 ^a	1.07	<0.01
Milk/DMI	1.27 ^{bc}	1.24 ^c	1.38 ^{abc}	1.49 ^a	1.42 ^{ab}	0.065	0.01
ECM, kg/d	30.0 ^{bc}	29.3 ^c	31.8 ^{ab}	33.4 ^a	33.8 ^a	1.43	<0.01
ECM/DMI	1.23 ^{bc}	1.18 ^c	1.30 ^{abc}	1.43 ^a	1.37 ^{ab}	0.067	0.02
Fat, %	3.74	3.72	3.57	3.86	3.74	0.158	0.74
Fat, kg/d	1.18 ^{bc}	1.16 ^c	1.23 ^{abc}	1.35 ^a	1.32 ^{ab}	0.077	0.03
True protein, %	3.19 ^a	3.06 ^b	3.19 ^a	3.14 ^a	3.21 ^a	0.049	<0.01
Protein, kg/d	1.00 ^c	0.95 ^c	1.07 ^b	1.08 ^{ab}	1.14 ^a	0.038	<0.01
Lactose, %	4.69	4.73	4.65	4.71	4.71	0.054	0.54
Lactose, kg/d	1.48 ^b	1.48 ^b	1.60 ^a	1.62 ^a	1.69 ^a	0.057	<0.01
SNF, %	8.78	8.68	8.75	8.74	8.82	0.086	0.13
SNF, kg/d	2.77 ^c	2.71 ^c	2.98 ^b	3.01 ^{ab}	3.15 ^a	0.104	<0.01
Milk-N/N-intake, %	22.3 ^{bc}	21.0 ^c	24.9 ^{ab}	27.5 ^a	26.6 ^a	1.24	<0.01
MUN, mg/dL	11.4 ^a	11.0 ^{ab}	11.4 ^a	9.7 ^c	10.1 ^{bc}	0.63	0.04
Plasma urea-N, mg/dL	17.0 ^a	14.2 ^b	13.8 ^b	12.7 ^b	14.6 ^b	0.89	0.02

^{a-c}Least squares means within the same row without a common superscript differ ($P < 0.05$).

¹AL = alfalfa; RC = red clover; LBFT = low condensed tannin (CT) birdsfoot trefoil; NBFT = normal CT birdsfoot trefoil; HBFT = high CT birdsfoot trefoil.

Body weight change and milk content of fat, lactose, and SNF were not influenced by diet. However, switching from AL silage to LBFT silage containing 8 g/kg of CT increased milk yield by 8%, whereas feeding HBFT with 2-fold greater CT levels increased milk yield by an additional 6% (Table 3). Feeding BFT silage in place of AL silage increased milk/DMI and ECM/DMI as well as yields of ECM, fat, and lactose by 10 to 12%, although no differences among BFT silages were detected. It was noted that milk/DMI and ECM/DMI ranged from 1.2 to 1.5 and, thus, were low to normal in this study. Differences among the BFT were found for milk protein content and yield of protein and SNF; these traits were higher on HBFT, intermediate on NBFT, and lower on LBFT. Similar responses in milk yields were attributed to CT when fresh BFT was fed, with and without polyethylene glycol, as the sole feed source for dairy cows (Woodward et al., 1999; Woodward et al., 2000). Woodward et al. (2006) found that supplementation of *L. corniculatus* silage to grazing dairy cows increased yield of milk and milk solids compared with supplementing equal DM from ryegrass silage. Yields of milk and milk components from feeding RC silage were lower than the BFT treatments and similar to AL silage. Although previous lactation studies also indicated that milk yields on diets containing RC silage were similar to that on diets containing AL silage (Broderick et al., 2001), greater maturity, and DM content at harvest of the RC fed in this trial may have impaired production.

Mean N efficiency improved from about 21 to 22% for AL and RC silage to an average 26.3% for the BFT silages. Milk urea-N, which reflects inefficiency of N utilization (Noussainen et al., 2004), was lower for NBFT and HBFT, the 2 higher CT silages, than on AL and LBFT. The concentration of MUN was lowest for NBFT, the diet with the numerically lowest N intake. Urea nitrogen in blood plasma usually is paralleled by MUN (Broderick and Clayton, 1997), but plasma urea was elevated only for AL relative to the other 4 diets. Improved N utilization on CT-containing diets likely was mediated partly through greater RUP supply. Waghorn et al. (1987) observed 50% greater abomasal flow of essential AA and a 67% increase in intestinal digestion of essential AA in sheep fed fresh-cut BFT with active CT compared with fresh-cut BFT treated with polyethylene glycol to inactivate the CT. A subsequent study with sheep utilizing ³⁵S-labeled fresh BFT and polyethylene glycol indicated that CT delayed and somewhat depressed absorption of essential AA from the intestine, but total amounts absorbed were increased due to greater flux into the abomasum (Wang et al., 1996b). Thus, CT in BFT appears to increase the supply of essential AA for ruminants. In other studies, levels of RUP in RC silage greatly exceeded those

in AL and BFT silages (Grabber and Coblenz, 2009; Grabber et al., 2011). The absence of a milk yield or N efficiency response with RC silage is consistent with the suggestion of Vanhatalo et al. (2009) that utilization of RUP from RC may be limited by low concentrations of absorbable Met.

Urinary excretion of urea-N was affected by dietary legume silage and was lowest for NBFT, highest for AL, and intermediate for the other silages (Table 4). However, no other significant differences for urinary excretion were detected in this study. A companion study indicated that CT in BFT silage diverted N excretion from urine to feces, which resulted in reduced ammonia emissions from manure (Misselbrook et al., 2005). Fraser et al. (2000) observed proportionately lower urinary-N excretion and increased N retention in sheep when RC or BFT replaced dietary AL, with greater N retention on BFT than RC.

Apparent digestibilities of DM, OM, NDF, ADF, and hemicellulose (HC; NDF – ADF) were highest for the RC diet (Table 4). Greater DM digestibility of the RC diet probably was due partly to its higher content of high-moisture corn (Table 2). However, fiber digestibility also was greater for RC compared with AL and BFT, despite RC being more mature and excessively dry at harvest. Higher fiber digestibility for RC compared with AL has been reported previously (Fraser et al., 2000; Broderick et al., 2001). Compared with AL, RC has been reported to have a somewhat greater HC in NDF (as was also observed in the present study), lower lignin, and a similar rate but 36% higher extent of in situ NDF digestion (Coblenz et al., 1998). Hoffman et al. (1997) compared RC and AL harvested at 2 maturities over 2 yr: the in situ NDF digestion rate was greater for AL but potentially digestible NDF was greater for RC in yr 1, with no difference found in yr 2. Milk yield was similar in both years but RC diets resulted in lower milk protein yield in yr 1 and lower milk protein concentration in yr 2. In the current study, digestibility of DM, OM, and fiber components was higher, or tended to be higher, for HBFT, intermediate for AL, and lower for NBFT and LBFT. The HBFT diet was always greater than LBFT and NBFT for digestibility of DM, OM, NDF, and HC, and the NBFT diet was greater than LBFT for ADF digestibility. This was surprising because of literature suggesting that CT in BFT have little or no effect on OM and fiber digestion (Waghorn et al., 1987; Chiquette et al. 1989; Waghorn and Shelton, 1997). Findings on apparent CP digestibility in the current trial were more consistent with those reported in the literature: AL was highest, RC and LBFT were intermediate, and NBFT and HBFT were lowest (Table 4). The most common previous observation is that increasing CT somewhat

Table 4. Effect of dietary forage source on urinary excretion and apparent digestibility

Item	Silage source ¹					SEM	P-value
	AL	RC	LBFT	NBFT	HBFT		
Urinary excretion							
Volume, L/d	28.5	27.0	26.8	27.5	28.5	0.94	0.57
Urea-N, g/d	150 ^a	137 ^b	142 ^{ab}	124 ^c	133 ^{bc}	4.1	<0.01
Total-N, g/d	205	202	213	183	189	10.4	0.25
Urea-N/total-N, %	77.0	75.9	70.5	72.0	75.0	3.24	0.60
Apparent digestibility, %							
DM	71.0 ^b	74.5 ^a	70.2 ^b	71.4 ^b	73.5 ^a	0.47	<0.01
OM	72.3 ^c	76.9 ^a	71.4 ^c	72.3 ^c	74.5 ^b	0.47	<0.01
NDF	47.3 ^{bcd}	59.9 ^a	43.1 ^e	45.6 ^{de}	49.0 ^b	0.86	<0.01
ADF	48.4 ^b	57.2 ^a	45.4 ^c	48.0 ^b	50.1 ^b	0.81	<0.01
Hemicellulose ²	45.0 ^b	64.4 ^a	37.5 ^c	39.9 ^c	46.3 ^b	1.21	<0.01
N	68.5 ^a	67.5 ^{ab}	66.1 ^{abc}	65.0 ^c	65.7 ^{bc}	0.92	0.05

^{a-e}Least squares means within the same row without a common superscript differ ($P < 0.05$).

¹AL = alfalfa; RC = red clover; LBFT = low condensed tannin (CT) birdsfoot trefoil; NBFT = normal CT birdsfoot trefoil; HBFT = high CT birdsfoot trefoil.

²Hemicellulose = NDF – ADF.

reduced total-tract N digestibility (Waghorn et al., 1987; Chiquette et al., 1989; Waghorn and Shelton, 1997).

Ruminal ammonia and total free AA concentrations (Table 5), which are indicative of dietary protein degradation (Broderick, 1995), were lowest for the RC diet and were either not different among the other 4 diets (ammonia) or were similar except for a lower value for HBFT (total AA). Ruminal concentrations of isovalerate and total branched-chain VFA (**BCVFA**), which derive from microbial catabolism of branched-chain AA (Van Soest, 1994), were highest on AL, lowest on RC, and intermediate on the BFT-based diets; this pattern was similar to that observed for ruminal total free AA.

Others have reported reduced ruminal ammonia and BCVFA due to presence of dietary CT (Wang et al., 1994). That the ruminal total free AA and BCVFA followed a pattern of lowest concentration for the BFT with the highest CT is consistent with reduced AA catabolism (due to suppressed NPN formation in the silo) and protein degradation (due to inhibition of ruminal proteolysis) with the HBFT diet. Numerous reports exist of reduced ruminal protein degradation in the presence of active dietary CT (Chiquette et al., 1989; Wang et al., 1996a; McMahon et al., 2000) and increased supply of metabolizable essential AA due to CT action (Waghorn et al., 1987; Wang et al., 1996b). The trend for lower ruminal pH with the LBFT diet

Table 5. Effects of dietary forage source on ruminal metabolism

Item ²	Silage source ¹					SEM	P-value
	AL	RC	LBFT	NBFT	HBFT		
pH	6.39	6.58	6.28	6.42	6.46	0.063	0.08
Ruminal metabolite concentration, mM							
Ammonia	8.7 ^a	5.3 ^b	8.9 ^a	7.8 ^a	7.5 ^a	0.53	<0.01
Free AA	11.6 ^{ab}	6.4 ^c	14.1 ^a	11.5 ^{ab}	10.2 ^b	1.17	<0.01
Acetate	59.9	62.1	65.4	48.6	45.0	9.04	0.37
Propionate	18.1	16.5	18.1	18.8	17.4	1.25	0.71
Acetate:propionate ratio	3.47	3.66	3.67	2.54	2.65	0.520	0.33
Butyrate	9.65	9.09	9.91	9.97	9.14	0.856	0.91
Valerate	1.58	1.18	1.58	1.47	1.42	0.169	0.52
Isobutyrate	1.15	0.80	1.04	0.99	0.90	0.076	0.09
Isovalerate	1.69 ^a	0.99 ^c	1.56 ^{ab}	1.61 ^a	1.28 ^{bc}	0.102	<0.01
BCVFA ²	2.85 ^a	1.80 ^c	2.60 ^a	2.59 ^{ab}	2.19 ^{bc}	0.159	<0.01
Total VFA	92.1	91.5	97.6	81.5	75.1	10.09	0.46
Lactate	0.38	0.66	2.03	0.78	0.94	0.403	0.07
Ethanol	0.11	0.28	0.69	0.52	0.15	0.188	0.18

^{a-c}Least squares means within the same row without a common superscript differ ($P < 0.05$).

¹AL = alfalfa; RC = red clover; LBFT = low condensed tannin (CT) birdsfoot trefoil; NBFT = normal CT birdsfoot trefoil; HBFT = high CT birdsfoot trefoil.

²Branched-chain VFA (isobutyrate + isovalerate).

reflected the trend for higher ruminal lactate for that diet. Otherwise, no other effects ($P \geq 0.18$) were observed of source of dietary legume silage on ruminal metabolite concentrations.

Overall, we conclude that presence of CT in BTF improved N utilization by the lactating dairy cow. Condensed tannins in AL are limited to the seed coat, but genetic engineering efforts are underway to expand CT expression to herbage (Verdier et al., 2012). Diets based on AL are often supplemented with or replaced by feeds containing more RUP to improve dairy cow performance. This can, however, exacerbate N excretion in manure, increase feed costs, lead to suboptimal dietary fiber levels, or increase reliance on high-input row crops. Because AL is a primary component of many dairy diets, CT expression in AL herbage could improve the performance and sustainability of dairy farms. Replacing AL silage with RC silage has been reported to improve N efficiency in lactating cows (Broderick et al., 2001). Consequently, the expression of PPO with suitable *o*-diphenol substrates is also being pursued as a means for improving alfalfa protein utilization by dairy cows (Sullivan and Hatfield, 2006; Sullivan and Zarnowski, 2011).

CONCLUSIONS

The most important observation in this experiment was that replacing dietary silage made from AL with silage made from the CT-containing forage BFT increased yields of milk and milk components by improving efficiency of utilization of dietary DM and N. Generally, only small differences in production were observed among the 3 BFT studied; however, milk protein content and yield of milk, protein and SNF were greater for the BFT silage with the highest CT (16 g/kg) compared with the lowest CT (8 g/kg of DM). Small differences in ruminal total free AA and BCVFA concentrations suggested that greater CT content in BFT resulted in somewhat lower ruminal catabolism of dietary protein. Lower NPN and higher NDIN in RC silage were related to reduced ruminal N degradation. Organic matter and fiber digestibility also were greater for RC than on the other legume silage diets; these differences did not translate into improved lactational performance, possibly because RC was too mature and too dry at harvest in the present trial. Results of this study indicate that BFT is a valuable forage and that replacing dietary AL with CT-containing forage would increase production and nutrient efficiency in lactating dairy cows.

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