Urine volume and nitrogen excretion are altered by feeding birdsfoot trefoil compared with alfalfa in lactating dairy cows[1]

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ABSTRACT: Legumes that contain condensed tannins may have lower ruminal protein degradation than alfalfa. The present study investigated the effects of feeding birdsfoot trefoil (*Lotus corniculatus* L.) hay on lactational performance and N utilization and excretion. Eight multiparous Holstein cows in midlactation (150 ± 22.3 d-in-milk) were randomly assigned to 2 treatments [alfalfa hay-based total mixed ration (**AHT**) or birdsfoot trefoil hay-based total mixed ration (**BHT**)] in a crossover design with 2 experimental periods. Each experimental period lasted 17 d (14 d of adaptation and 3 d of sampling and total collection). Hays comprised approximately 50% of DM in experimental diets. There were no treatment effects on dry matter intake (DMI; 21.4 vs. 20.7 kg/d), milk yield (29.4 vs. 28.1 kg/d), milk fat concentration (3.20% vs. 3.21%), and milk protein concentration (3.20% vs. 3.16%) for AHT and BHT, respectively. In addition, dietary treatments did not affect milk yield/DMI or energy-corrected milk yield/DMI. In contrast, apparent crude protein digestion decreased in cows fed BHT compared with those fed AHT (60.7% vs. 69.1%). Concentration of milk urea-N decreased by feeding BHT compared with AHT (11.9 vs. 13.3 mg/100 mL), whereas total N excretion did not differ between AHT and BHT diets. However, cows fed BHT excreted more N in feces (194 vs. 168 g/d), whereas urinary N excretion was lower compared with cows fed AHT. The shift of N to feces resulted in a decrease in urinary N:fecal N ratio in cows fed BHT relative to those fed AHT. Overall results in the current study suggest that feeding birdsfoot trefoil in dairy diets shifts routes of N from urine to feces compared with feeding alfalfa hay, with little effect on lactational performance. Reduction in urinary N and any impact on environment may be attributed to functional effect of condensed tannins in birdsfoot trefoil hay.

Key words: alfalfa hay, birdsfoot trefoil hay, condensed tannins, total collection

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INTRODUCTION

By definition, sustainability is "meeting society's present needs without compromising the ability of future generations to meet their own needs." The 3 contributing pillars of sustainability are environmental responsibility, economic viability, and social acceptability (USDA-EPA, 1999), which have increased public awareness and investigations of sustainability within animal production agriculture. Among all nutrients in animal agricultural production systems, N and

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P are the two which have caused concerns because of their effects on water, air quality, and eutrophication (USDA-EPA, 1999). Although all dietary N sources should eventually be excreted or incorporated into a protein product, the efficiency of feed N conversion to milk N rarely exceeds 30%, which means 70% of feed N is generally wasted via excretion. Of the excreted portion, 30% is through feces and the remaining portion via urine in the form of urea (VandeHaar and St-Pierre, 2006). Excreted N amount and pathway are of the most critical environmental concerns; urinary N is more volatile than fecal N and can quickly convert into ammonia, which adds to particulate pollution. Feeding diets reduced in CP can substantially reduce urinary N excretion and increase N utilization efficiency. However, a decrease in dietary protein concentration can have negative effects on productivity if the diet is not well-balanced (Lee et al., 2011, 2012).

Alfalfa (*Medicago sativa*) is legume forage that is commonly fed to livestock. However, the protein available in alfalfa is quickly degradable in the rumen, which causes inefficient use of N by ruminants (Brito and Broderick, 2006). Ahlgren (1956) stated that in the early 1900's birdsfoot trefoil (**BFT**) hay was an important pasture crop in the northern part of the United States due to high adaptability and yield. The use of BFT hay as a harvested feedstuff became less common as alfalfa cultivars became more available. Birdsfoot trefoil is a forage that contains condensed tannins (**CT**s) and has been shown to increase BW gain in cattle and sheep, wool growth, milk yields, and reproductive performance in many studies compared with nontannin-containing forage sources (Patra and Saxena, 2011). Condensed tannins available in BFT can attach to soluble proteins in the rumen and form insoluble CT-protein complexes. Subsequently, when the complexes reach the abomasum, these complexes are released and may be absorbed in the small intestine (Waghorn, 2008), which results in a reduction in ruminal protein degradation to ammonia-N (NH_3-N; Weiss et al., 2009). Grabber (2009) showed that rations containing moderate levels of CT, around 2% to 4% of DM, decreased proteolysis in the rumen up to 50%. The formation of CT-protein complexes provides a natural route for the reduction of N waste by dairy cows. Also, studies have shown that BFT fed to dairy cows as a fresh forage (Woodward et al. 2000) or preserved silage (Hymes-Fecht et al., 2013) shifted excretion of N from urine to feces.

The objectives of the current study were to evaluate the effects of BFT or alfalfa hay on milk yield and composition, nutrient digestibility, and outputs of nitrogenous metabolites in urine and feces of dairy cows offered 2 different legume forages as the major forage source. The overall hypothesis in this study was that feeding BFT hay compared with alfalfa hay to lactating dairy cows would alter the N excretion pathway from urine toward feces and that CT in BFT hay-containing diet would reduce milk urea nitrogen (**MUN**) concentration and urinary N excretion due to the reduction in ruminal protein degradation.

MATERIALS AND METHODS

The dairy cows used in this study were cared for according to the Live Animal Use in Research Guidelines of the Institutional Animal Care and Use Committee at Utah State University, Logan, UT. The study was conducted at the Caine Dairy Research Center (Wellsville, UT) of the Utah State University from September through November 2014.

Hays, Cows, and Experimental Diets

The alfalfa hay used in the current study was the third cutting of a mature stand (Gunner variety) raised on clay-silt soil (Cache Junction, UT). Forage was cut at prebloom stage with a conventional mower conditioner (Model 830, John Deere, Moline, IL). The cut forage was allowed to sun cure for 2 d in the field, turned once with a rake, and then baled (model 575, New Holland Inc., New Holland, PA) at 85 to 87% DM. The hay was stored in metal hay barns until the time of the trial. Hay was bright green, fine stemmed, and mold free. The BFT hay tested in this experiment was Norcen variety, planted on a subirrigated field consisting primarily of calcareous silty loam soil (Logan, UT). It was then allowed to sun cure for 5 d then baled in small, 45-kg bales (model 440, International Harvester, Racine, WI) at 83 to 85% DM. Samples of each forage (approx. 2 kg) were oven-dried to a constant dry weight, ground to pass a 2-mm screen, and analyzed using NIRS (Utah State University Analytical Lab, Logan UT). Chemical composition of the hays and corn silage used to construct experimental diets is reported in Table 1.

Eight multiparous Holstein dairy cows in midlactation (DIM = 150 ± 22.3; average BW = 721 ± 56.3 kg at the beginning of trial) were paired by DIM, current milk production, and BW and were randomly assigned to 1 of 2 dietary treatments in a crossover design. Dietary treatments were comprised of two 17-d periods. The first 14

Table 1. Chemical composition (means ± SD) of fed forages (n = 3)

	Forage		
Item, % of DM	Alfalfa hay	Birdsfoot trefoil hay	Corn silage
DM	93.1 ± 0.25	89.4 ± 3.47	40.9 ± 0.87
OM	89.3 ± 0.76	88.1 ± 0.37	92.4 ± 0.33
CP	20.4 ± 0.93	15.9 ± 0.25	6.70 ± 0.16
NDF	37.8 ± 1.60	39.4 ± 0.38	43.4 ± 1.12
ADF	31.4 ± 0.79	32.7 ± 0.93	34.2 ± 0.67
Ether extract	2.35 ± 0.08	2.29 ± 0.08	3.21 ± 0.10
NFC[1]	30.9 ± 1.14	34.7 ± 0.19	38.1 ± 2.15
Condensed tannins	ND[2]	2.80 ± 0.21	—

[1]NFC = 100 − CP − NDF − ether extract − ash.

[2]Not detected.

d of each period were used for diet adaptation, and the last 3 d were used for sampling and data collection, with a 5-d washout period between experiment periods. Cows were housed individually in tie-stalls fitted with rubber mattresses covered with straw and allowed free access to water. Cows were milked twice daily at 0400 and 1600 h, and milk production was recorded during adaptation (Perfection 3000 Boumatic Weigh Meter System, Boumatic, Madison, WI). During total collection period, cows were milked in the stall and milk yield was measured at each milking using portable milk meters (Waikato Milking Systems NZ Ltd, Hamilton, New Zealand) calibrated for accuracy by Rocky Mountain DHI (Nibley, UT). Milk samples were taken by proportional sampler during the morning and afternoon milkings on days 15, 16, and 17 of each experimental period. Milk samples were stored at 4 °C and preserved with Broad Spectrum Microtabs II (D & F Control Systems Inc., San Ramon, CA). Individual milk samples were analyzed by Rocky Mountain DHIA Laboratory (Nibley, UT). Fat, true protein, and lactose concentration were obtained using mid-infrared wave-band procedures (Bentley 2000, Bentley Instruments, Caska, MN). Milk urea N was analyzed using the Berthelot enzymatic procedure on a ChemSpec 150 Analyzer (Bentley Instruments) (Herrick et al., 2018). Milk component yields were calculated on the basis of weighted milk yield of morning and evening milk samples.

Dietary treatments consisted of alfalfa hay-based total mixed ration (**TMR**) (**AHT**; 46.0% alfalfa hay, 3.59% oat hay, 18.5% corn silage, and 31.9% concentrate on a DM basis) and BFT hay-based TMR (**BHT**; 49.4% BFT hay, 18.0% corn silage, and 32.8% concentrate on a DM basis; Table 3). The forage-to-concentrate ratio of the diets offered to cows was 68:32. To keep this ratio between treatments, oat hay was added to the AHT, as alfalfa hay had less NDF and more CP. To supply enough CP, soybean and canola meal mixture was used in both diets but was included at a slightly greater concentration in the BHT (Table 2). Cows were individually fed twice daily for 110% of expected daily intake, with 50% of allotted feed fed at 0600 h and 50% fed at 1500 h. Weights of feed offered and refused were recorded daily, and samples were taken during the sampling week to determine DM. Every 3 d, required forage would be shredded and used for TMR preparation. The forage and other ingredients were mixed for 15 min in a TMR wagon (Model 455, Roto-Mix, Dodge City, KS) before feeding. Diets were designed to be isonitrogenous and isocaloric across treatments averaging 16.4% CP and 1.62 Mcal/kg, respectively (Table 2) but due to unexpected changes in the quality of the BFT, the BHT diet ended up slightly lower in CP and NDF. Diets were formulated to meet NRC (2001) recommendations for RDP, RUP, NDF, ADF, NE_L, minerals, and vitamins sufficient for the production of 30 kg of milk using Cornell-Penn-Miner software, version 3.0.8.1.

Data Collection and Sampling

Samples of alfalfa hay, BFT hay, and corn silage were obtained once a week to determine DM concentration, and dietary concentrations of forages and concentrates were adjusted every week on an as-fed basis to reflect changes in the concentrations of nutrients due to change in DM. Sampled feeds were composited by period for chemical analysis. Samples of TMR and orts for each cow were taken on a daily basis during the sampling week, composited by period, and stored frozen at −20 °C until analyzed.

Total urine collections were obtained from all cows on days 15 to 17 using indwelling Foley catheters (26 French, 75-cc balloon; C. R. Bard, Inc.,

Table 2. Ingredients and chemical composition (means ± SD) of experimental diets fed to lactating dairy cows

	Experimental diet[1]	
Item	AHT	BHT
Ingredient, % of DM		
Alfalfa hay	47.0	—
Birdsfoot trefoil hay	—	49.4
Oat hay	4.6	—
Corn silage	18.5	18.0
Corn grain, flaked	16.2	16.1
Soybean meal	5.33	7.13
Canola meal	5.33	7.13
Calcium carbonate	1.01	1.01
Salt	0.31	0.31
Urea	0.70	0.70
Magnesium oxide	0.18	0.18
Sodium bicarbonate	0.70	0.70
Vitamins and minerals[2]	0.14	0.14
Chemical composition, % of DM		
DM, %	57.5 ± 2.19	56.3 ± 0.89
OM	90.9 ± 0.41	91.5 ± 1.08
CP	16.4 ± 0.17	15.6 ± 1.01
RDP, % of CP[3]	64.5	62.7
RUP, % of CP[3]	35.5	37.3
NDF	34.6 ± 1.14	35.7 ± 0.68
ADF	25.2 ± 1.49	26.6 ± 0.78
NFC[4]	39.6	38.8
NE_L,[3] Mcal/kg	1.58	1.60
Condensed tannins	0.06 ± 0.030	1.36 ± 0.154

[1]AHT = alfalfa hay-based TMR; BHT = birdsfoot trefoil hay-based TMR.

[2]Formulated to contain (per kg DM): 13.4 mg of Se (from sodium selenate), 550 mg of Cu (from copper-AA complex), 2,412 mg of Zn (from zinc-AA complex and zinc sulfate), 2,290 mg of Mn (from manganese-AA complex), 33 mg of Co (from cobalt carbonate), 185,045 IU of vitamin A, 22,909 IU of vitamin D, 616 IU of vitamin E, and 285 mg of Rumensin (Elanco Animal Health, Greenfield, IN).

[3]Based on tabular value (NRC, 2001).

[4]NFC = 100 − CP − NDF − ether extract − ash.

Covington, GA). Catheters were inserted in the bladder of each cow using local anesthesia under direction of the farm veterinarian on day 14 of each experimental period and drained into clean 25-L plastic containers using polypropylene tubing. In each container, 480 mL of 4 N H_2SO_4 was included to acidify the urine, as it entered the containers. Cows were fitted to containers at 0200, and these were emptied at 0800, 1500, and 2100 h to obtain daily composite volume and samples (final pH < 3). After the weight of the acidified urine was recorded, 2 sets of 100-mL aliquots were taken at each collection time point, combined for each day, the pH confirmed to be <3, and then stored at −20 °C until the analysis of urinary N concentration.

Total fecal collections were taken from all cows on days 15 to 17 using special portable wooden boxes (made in the research lab, 150 × 60 cm). The boxes were located at the end of each stall covering the rear area accessible by each cow, so feces could only be evacuated in the boxes. Immediately after a cow defecated, the feces were weighed and placed in a sealed 38-L plastic container. Following mixing of feces, a 200-g sample was obtained each 5 to 7 h during total collection days, and then, the plastic containers were emptied, excess feces discarded, and samples stored −20 °C. All daily subsamples were mixed, and a 400-g sample was retained. Samples were dried in a forced air oven at 55 °C, ground to pass a 1-mm screen (Standard Model 4; Arthur H. Thomas Co., Swedesboro, NJ), and stored in airtight containers at −20 °C pending further chemical analysis.

Ruminal fluid samples were obtained and measured separately using an esophageal tube 4 h after the morning feeding (Geishauser, 1993) on days 15, 16, and 17 of each experimental period for each cow (totaling 12 measurements per treatment per period). The first 200 mL of ruminal fluid was discarded to avoid contamination from saliva, and then, 200 mL was collected for analysis. The pH of the ruminal fluid was measured within 5 min of collecting the samples from each cow using a portable pH meter (Oakton pH 6; Oakton Instruments, Vernon Hills, IL). Five milliliters of the ruminal fluid was mixed with 1 mL of 1% sulfuric acid and stored frozen (−40 °C) for analysis of NH_3-N. Another 5 mL of the ruminal fluid was retained and mixed with 1 mL of 25% metaphosphoric acid and then stored at −40 °C until determination of VFA profiles.

Chemical Analyses

Concentration of DM in pooled hays, diets, and orts per period was determined by drying at 55 °C for 48 h in a forced air oven. Dried samples were ground to pass a 1-mm screen (Standard Model 4) and stored at −20 °C for chemical analyses. The DM concentrations of the samples were used to calculate intakes of DM and nutrients. Analytical DM concentration of samples was determined by oven drying overnight at 105 °C, and OM was determined by ashing at 550 °C for 5 h (AOAC, 2000; method 942.05). Concentrations of CP were determined using an automated N combustion analyzer (Elementar, Analysensysteme GmbH, Hanau, Germany; AOAC, 2000; method 968.06). Concentrations of NDF and ADF were determined

sequentially using a fiber analyzer (200/220, Ankom Technology Corp., Macedon, NY) according to the methodology supplied by the company, which is based on the methods described by Van Soest et al. (1991). Sodium sulfite was used in the procedure for NDF determination, and samples were pretreated with heat-stable amylase (Type XI-A form Bacillus subtilis; Sigma–Aldrich Corporation, St. Louis, MO). Nutrient compositions of fecal samples were determined using the same procedures used for feed sample analyses.

Condensed tannins of alfalfa and BFT hay were isolated from ground samples of the hay (0.5 mm) using the modified HCl–butanol–acetone assay (Grabber et al., 2013). A spectrophotometer (Bio-Mate 3, Thermo Fisher Scientific, Madison, WI) was used to quantify the CT as described by Grabber et al. (2013) and Broderick et al. (2017).

Urine samples were thawed and composited per cow by period. Urinary urea-N was analyzed using a commercial kit (Stanbio Urea-N Kit 580, Stanbio Laboratory Inc., San Antonio, TX). Urinary N was analyzed on freeze-dried urine samples using a Costech ECS 4010 C/N/S elemental analyzer (Costech Analytical Technologies Inc., Valencia, CA).

Apparent total-tract digestibility of DM and nutrients was measured using total fecal collection. Fecal samples were collected for each cow from the fecal collection. Samples were composited across sampling times for each cow and processed as mentioned earlier, and the percentage of digestibilities was calculated based on the following equation:

$$\%\ \text{of Digestibility} = [\text{Intake (kg/d)} - \text{Excreted (kg/d)} / \text{Intake (kg/d)}] \times 100$$

Concentration of NH_3-N in the ruminal contents was determined as described by Rhine et al. (1998), using a plate reader (MRXe; Dynex Technologies Inc., Chantilly, VA). Ruminal VFA were separated and quantified using a gas chromatography (Model 6890 Series II; Hewlett-Packard Co., Palo Alto, CA) with a capillary column (30 m × 0.32 mm i.d., 1-µm phase thickness, Zebron ZB-FAAP; Phenomenex Inc., Torrance, CA) and flame-ionization detection. The oven temperature was held at 170 °C for 4 min, increased to 185 °C at a rate of 5 °C/min, then increased by 3 °C/min to 220 °C, and held at this temperature for 1 min (Eun and Beauchemin, 2007). The injector and the detector temperatures were 225 and 250 °C, respectively, and the carrier gas was helium.

Statistical Analysis

Data were analyzed using PROC MIXED of SAS Individual cow was the experimental unit for all variables. The model for all diet parameters reported in the current study included the fixed effects of group, dietary treatment, with cow within group and period within group designated as random effects. The following model was used:

$$Y_{ijkl} = \mu + \delta_l + \beta_{i(l)} + \alpha_j + \gamma_k + \alpha\gamma_{jk} + \varepsilon_{ijkl}$$

where Y_{ijkl} is the response due to subject i, treatment j, period k, and sequence l; μ is overall mean; δ_l is fixed effect due to subject i nested within sequence l; $\beta_{i(l)}$ is random effect due to subject i nested within sequence l; α_j is fixed effect due to treatment j; γ_k is fixed effect due to period k; and ε_{ijkl} is fixed interaction effect due to treatment j and period k.

Rumen fermentation parameters were analyzed using the same model as above, but included a repeated measures statement. Degrees of freedom were adjusted using the Kenward–Roger option. Differences were considered significant at $P \le 0.05$, and trends were discussed at $0.05 < P \le 0.10$. Least squares means are reported throughout.

RESULTS AND DISCUSSION

Characteristics of Experimental Diets

Concentrations of CP were 20.4% and 15.9% for alfalfa and BFT hay, respectively (Table 1). Concentrations of NDF and ADF were greater in BFT hay compared with alfalfa hay. Birdsfoot trefoil hay contained 2.8% CT, whereas alfalfa hay did not contain CT. Diet concentrations of CT were 0.06% and 1.36% in AHT and BHT, respectively (Table 2). Nutritive value of *Lotus* species including BFT is often considered similar or superior to each one of alfalfa, ryegrass, and white clover (Barry and McNabb, 1999). Concentrations of CP in *Lotus* species ranges from 16% to 24%, whereas NDF concentration varies between 28% and 36% and DM digestibility is 72% to 78% (Blumenthal and McGraw, 1999). According to Holt et al. (2013), alfalfa containing 20.7% CP is considered to be of high quality. In addition, OM digestibility of BFT was lower than AH.

Intake and Digestibility of DM and Nutrients

In the present study, intakes of DM, NDF, and ADF did not differ between treatments (Table 3).

Our findings for DMI are in agreement with Christensen et al. (2015) who reported no difference in DMI between cows fed with alfalfa hay and those with BFT hay. Likewise, these results are in agreement with Hymes-Fecht et al. (2013) who also reported no differences in DMI when dairy cows were fed with BFT silage of similar CT concentration compared with alfalfa silage. Previous studies on the effects of CT extracts or feeding CT-containing forages on feed intake in ruminants have resulted in contradictory results. Although increased DMI was reported in dairy cows fed BFT silage-based diets (2.59% CT) compared with perennial ryegrass, (Woodward et al., 2001), Baah et al. (2007), and Benchaar et al. (2008) did not observe any effect of supplementing CT extract on DMI in Jersey heifers (0.60% CT from quebracho extract) or lactating dairy cows (0.45% CT). In contrast, decreased DMI was reported in a study by Priolo et al. (2000) where sheep were fed with carob pulp with 2.5% CT. Similarly, Dschaak et al. (2011) showed decreased DMI when dairy cows were fed a quebracho CT extract-containing diet (2.3% CT).

Apparent digestibilities of DM, NDF, and ADF did not differ between AHT and BHT diets, but OM (71.1% vs. 75.6%) and CP (60.7% vs. 69.1%) digestibilities were decreased by feeding BHT ($P < 0.01$; Table 3). In contrast, Grosse Brinkhaus et al. (2016) reported no changes in OM digestibility when cows were fed diets containing 3 different concentrations of CT provided by alfalfa, sainfoin, or BFT in pellets (0, 35.5, and 5.0 g of CT/kg of DM, respectively). These authors also reported greater N digestibility for BFT compared with alfalfa (Grosse Brinkhaus et al., 2016), which is inconsistent with present results. Decreased OM digestion observed in our study may have been caused by differences in maturity between BFT and alfalfa as reflected by increased numerically NDF and ADF concentrations in the former compared with the latter hay. In addition, reduced total-tract N digestibility probably originated from CT-protein complex in the rumen.

Table 3. Least squares means of intake and apparent digestibility of DM and nutrients in lactating dairy cows fed different legume hay-based diets

	Dietary treatment[1]			
Item	AHT	BHT	SEM	*P*
Intake, kg/d				
DM	21.4	20.7	0.83	0.46
CP	3.41	3.16	0.143	0.09
NDF	7.22	7.70	0.321	0.18
ADF	5.87	5.91	0.283	0.79
Total-tract digestibility, %				
DM	68.0	64.8	1.46	0.20
OM	75.6	71.1	1.02	<0.01
CP	69.1	60.7	1.71	<0.01
NDF	55.6	51.7	1.90	0.18
ADF	52.4	46.1	2.89	0.21
Digested NDF intake, kg/d	4.00	4.30	0.312	0.75
Undigested NDF intake, kg/d	3.24	3.37	0.214	0.87
BW, kg	721	721	29.5	0.86

[1]AHT = alfalfa hay-based TMR; BHT = birdsfoot trefoil hay-based TMR.

Milk Production and Efficiency

Milk yield averaged 28.8 kg/d and was not different between dietary treatments (Table 4). In addition, energy corrected milk (**ECM**) and fat corrected milk (**FCM**) yields were similar between AHT and BHT. Dietary treatments did not affect milk components and their yields. Also, feeding either AHT or BHT resulted in similar feed efficiencies. No difference in milk yield in the current study is in agreement with those of Christensen et al. (2015) and Grosse Brinkhaus et al. (2016). In contrast, Woodward et al. (2000) and Hymes-Fecht et al. (2013) reported increased milk yield in cows fed with BFT compared with those fed with

Table 4. Least square means of milk yield and composition of lactating dairy cows fed different legume hay-based diets

	Dietary treatment[1]			
Item	AHT	BHT	SEM	*P*
Yield, kg/d				
Milk	29.4	28.1	2.64	0.47
ECM	28.9	27.2	2.47	0.31
3.5% FCM	27.8	26.6	2.34	0.38
Milk composition, %				
Fat	3.20	3.21	0.162	0.67
True protein	3.20	3.16	0.100	0.35
Lactose	4.56	4.54	0.118	0.80
MUN, mg/100 mL	13.3	11.9	0.37	<0.01
Milk component yield, kg/d				
Fat	0.93	0.89	0.082	0.40
True protein	0.95	0.86	0.080	0.13
Lactose	1.36	1.29	0.145	0.39
Solids-not-fat	2.57	2.40	0.250	0.30
Feed efficiency				
Milk yield/DMI	1.39	1.35	0.106	0.59
3.5% FCM/DMI	1.35	1.26	0.134	0.31
ECM yield/DMI	1.35	1.30	0.106	0.49

[1]AHT = alfalfa hay-based TMR; BHT = birdsfoot trefoil hay-based TMR.

perennial ryegrass, alfalfa, or red clover silage. The concentration of CT in the BFT used in current study was similar to that reported by Woodward et al. (2000) and Hymes-Fecht et al. (2013), which averaged 1.9% and 1.6%, respectively. However, factors other than CT may have influenced the lack of response in milk production when feeding BHT vs. AHT. In the current study, the CP concentration of alfalfa hay was 4.5 percentage units greater than that in BFT hay. We observed a noticeable reduction in OM digestibility in cows fed BHT vs. AHT, thus suggesting that reduced BFT hay quality (i.e., lower CP concentration, higher NDF and ADF) may have impaired a potential improvement in milk yield.

The concentration of MUN, which reflects inefficiency of N utilization (Nousiainen et al., 2004), was decreased in cows fed BHT compared with those fed AHT (11.9 vs. 13.3 mg/dL; Table 4). Similarly, Hymes-Fecht et al. (2013) reported the lowest MUN concentration in cows fed BFT diet containing the medium concentration of CT (1.2% DM), which is comparable to the CT concentration of our study. Urea nitrogen in blood plasma usually is paralleled by MUN (Broderick and Clayton, 1997). We did not measure PUN, but as ruminal ammonia concentration decreased significantly in BHT diet compared with AHT, the PUN concentration should have been decreased, which led to lower MUN concentration in BHT diet compared with AHT.

Ruminal Fermentation Characteristics

Mean ruminal pH was not different between treatments (Table 5) and was typical for cows fed high-forage diets as reported by Brito et al. (2008). Dietary NDF concentration for both treatments fed herein seemed to be sufficient to maintain optimal ruminal pH. Similarly, Hymes-Fecht et al. (2013) and Broderick et al. (2017) reported minor effects on ruminal pH by feeding a BFT silage-based diet compared with an alfalfa silage-based diet. Feeding BHT tended to decrease total VFA concentration, whereas the concentration of NH_3-N declined in response to feeding BHT compared with AHT (5.50 vs. 9.90 mg/100 mL, respectively). Condensed tannin-protein complexes inhibit the degradation of forage protein to NH_3-N in the rumen, thereby increasing the amount of dietary protein that reaches the small intestine (Waghorn et al., 1987). Despite similar RDP supply calculated using the NRC (2001) model, the concentration of ruminal NH_3-N differed between treatments, probably due to CT effects on ruminal proteolysis. Lowered ruminal NH_3-N concentration has been frequently reported when feeding CT-containing forages such as sainfoin (Azuhnwi et al., 2013) or replacing alfalfa silage with different dietary concentrations of BFT silage (Broderick et al., 2017).

Table 5. Least square means of ruminal fermentation characteristics of lactating dairy cows fed different legume hay-based diets (n = 3 sample replicates)

	Dietary treatment[1]			
Item	AHT	BHT	SEM	*P*
Mean pH	6.27	6.37	0.09	0.45
Total VFA, mM	112.7	110.3	5.76	0.07
Individual VFA, mol/100 mol				
Acetate (A)	66.7	66.3	0.61	0.48
Propionate (P)	20.2	20.4	0.60	0.75
Butyrate	9.42	10.2	0.26	0.15
Isobutyrate	0.79	0.63	0.032	<0.01
Isovaleric	1.24	1.01	0.109	<0.01
Valeric	1.32	1.31	0.050	0.74
Caproic	0.295	0.345	0.0382	0.38
A:P	3.37	3.32	0.11	0.73
NH_3-N, mg/100 mL	9.90	5.50	0.579	<0.01

[1]AHT = alfalfa hay-based TMR; BHT = birdsfoot trefoil hay-based TMR.

Feeding BHT decreased molar proportions of isobutyrate and isovalerate compared with AHT. As these branched-chain VFA derived from AA degradation, the decreased proportions of the branched-chain VFA may support decreased ruminal CP degradation because of CT-soluble protein complex formation.

Utilization of N

There was a trend for lower N intake in BHT compared with AHT (Table 6), the same trend was observed for milk protein yield. Urinary N excretion was greater for AHT compared with BHT (272 vs. 227 g/d, respectively), whereas fecal N excretion was lower in AHT in relative to BHT (168 vs. 194 g/d, respectively). Hence, the total amounts of urinary plus fecal N excretion (manure) did not differ between the treatments (437 vs. 422 g/d, respectively, for AHT and BHT), but the route of excretion was altered. Several studies have shown that dairy cows fed with BFT as a fresh forage (Woodward et al. 2000), preserved silage (Hymes-Fecht et al., 2013; Broderick et al., 2017), or hay (Grosse Brinkhaus et al., 2016) shifted excretion of N from urine to feces (Hymes-Fecht et al., 2013). One possible explanation for more fecal N excretion

Table 6. Least square means of N partitioning of lactating dairy cows fed different legume hay-based diets determined from total collection of feces and urine (n = 3 sample replicates)

	Dietary treatment[1]			
Item	AHT	BHT	SEM	*P*
N intake, g/d	548	505	23.1	0.09
Milk protein output, g/d	947	864	80.4	0.13
Milk N output, g/d	158	145	13.4	0.13
Milk NPN output, g/d	5.95	5.67	0.548	0.41
Total urine output, kg/d	26.0	20.4	1.24	<0.01
Urinary N output, g/d	272	227	14.1	<0.01
Urinary urea-N, mg/100 mL	680	754	56.2	0.31
Urinary urea-N output, g/d	169	156	11.9	0.39
Fecal output, wet, kg/d	47.3	50.1	2.01	0.13
Fecal output, dry, kg/d	6.89	7.17	0.27	0.41
Fecal N, %	2.47	2.66	0.04	<0.01
Fecal N output, g/d	168	194	6.18	<0.01
N output of feces and urine, g/d	437	422	18.0	0.24
Total N output (milk + urine + feces), g/d	588	561	22.6	0.82
Milk N:N intake[2]	0.28	0.29	0.032	0.39
UN:FN[3]	1.62	1.17	0.061	0.03
MkN:MaN[4]	0.36	0.34	0.044	0.82

[1]AHT = alfalfa hay-based TMR; BHT = birdsfoot trefoil hay-based TMR.

[2]Efficiency of use of feed N to milk N.

[3]UN:FN = ratio of urinary N to fecal N, where urinary N and fecal N are expressed in g/d.

[4]MkN:MaN = ratio of milk N to manure N, where milk N and manure N are expressed in g/d.

in BHT compared with AHT in the present study is the formation of CT-protein complex, which did not get digested in the small intestine. Cows fed BHT showed reduced total urine output and urinary N output, perhaps as a result of decreased N intake and ruminal NH_3-N concentration. Grosse Brinkhaus et al. (2016) observed that urinary excretion of N and its percentage of N intake were lower in sainfoin than in alfalfa, but not in BFT fed cows. Moreover, Broderick et al. (2017) reported the lowest urinary N excretion in cows fed the diet containing BFT silage with the greatest concentration of CT when they fed 3 different levels of BFT silage.

Urinary N-to-fecal N ratio decreased in the cows fed BHT compared with those fed AHT (Table 6) due to a sizable decrease in urinary N excretion. Urinary N is known to be the most environmentally volatile N (Varel et al., 1999) because in the environment, microbial ureases react with urinary N (Muck, 1982), and urea is rapidly hydrolyzed to ammonia and volatilized into the environment (James et al., 1999). Therefore, the decreased urinary N-to-fecal N ratio in response to feeding BFT hay in the current study suggests additional environmental benefits, which may eventually impact N management on farms. However, this environmental benefit would probably be less noticed in the shadow of lower productive benefits of BFT feeding herein.

CONCLUSIONS

The most important finding of this experiment was the reduced urinary N, MUN, ruminal NH_3-N concentrations, and urine volume exhibited in cows fed BHT compared with those fed AHT. Another great finding of the study was feeding BFT hay resulted in changing the N excretion pathway from urine to feces, which can benefit the environment substantially by reducing volatile N compounds into it. Feeding BFT hay in a high-forage lactation diet did not influence digestibilities for DM, NDF, and ADF compared with alfalfa hay; however, it decreased CP and OM digestibilities compared with alfalfa. These results imply that CT from the BFT hay exhibited protein-binding activity in the rumen that reduced protein degradation. We did not determine how the shift in N excretion by feeding BFT hay influenced farm N management or urine volume effect on water footprint in the present study, which could guide comprehensive benefits of feeding BFT hay toward sustainable dairy production. Moreover, a potential following study would be studying the physiological path of urine formation and identifying how it is influenced from CT-containing diets.

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