Effects of feeding birdsfoot trefoil hay on neutral detergent fiber digestion, nitrogen utilization efficiency, and lactational performance by dairy cows¹

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ABSTRACT

This experiment was conducted to determine effects of feeding birdsfoot trefoil hay-based diets in comparison with an alfalfa hay-based diet on N utilization efficiency, ruminal fermentation, and lactational performance by mid-lactation dairy cows. Nine multiparous lactating Holstein cows (131 ± 22.6 d in milk), 3 of which were rumen fistulated, were fed 3 experimental diets in a replicated 3×3 Latin square design with 3 periods of 14 d of adaptation and 7 d of data and sample collection. Within squares, cows were randomly assigned to diets as follows: alfalfa hay-based diet (AHT), alfalfa and birdsfoot trefoil hay-based diet (ABT), and birdsfoot trefoil hay-based diet (BT). Intakes of dry matter and crude protein were similar across treatments, whereas ABT and BT diets resulted in decreased fiber intake compared with AHT. Feeding BT tended to increase neutral detergent fiber digestibility compared with AHT and ABT. Milk yield tended to increase for cows consuming ABT or BT diets. Milk true protein concentration and yield were greater for cows consuming ABT relative to those fed AHT. Concentration of total volatile fatty acids tended to increase by cows fed BT compared with those fed AHT and ABT. Feeding birdsfoot trefoil hay in a total mixed ration resulted in a tendency to decrease acetate proportion, but it tended to increase propionate proportion, leading to a tendency to decrease acetate-to-propionate ratio. Whereas concentration of ammonia-N was similar across treatments, cows offered BT exhibited greater microbial protein yield relative to those fed AHT and ABT. Cows offered birdsfoot trefoil hay diets secreted more milk N than AHT, resulting in improved N utilization efficiency for milk N. The positive effects due to feeding birdsfoot trefoil hay were attributed to enhanced neutral detergent fiber digestion, and thus

it could replace alfalfa hay in high-forage dairy diets while improving N utilization efficiencies and maintaining lactational performance compared with alfalfa hay. **Key words:** alfalfa hay, birdsfoot trefoil hay, lactating dairy cows, nitrogen utilization efficiency

INTRODUCTION

Forage quality affects feed intake and energy density, lactational performance, cow health, and feed cost. Dairy producers in the western United States use alfalfa (*Medicago sativa* L.) hay for most of the forage portion in dairy diets. Alfalfa is a very palatable legume that is high in CP and low in fiber, and thus feeding alfalfa can maximize intake and production of milk by dairy cattle (Martin and Mertens, 2005). However, the protein in alfalfa is extensively degraded to ammonia in the rumen, resulting in excess N waste excretion. Birdsfoot trefoil (*Lotus corniculatus* L.) is a nonbloating forage legume that is similar to alfalfa in feeding value, and consequently it has a potential to be fed as a main forage to dairy cows with its unique cell wall digestion characteristics as well as condensed tannins (CT; Williams et al., 2011). Within a legume family, evidence exists that lignification has less effect on digestibility in birdsfoot trefoil than alfalfa. At the early bloom stage, Tomlin et al. (1965) demonstrated birdsfoot trefoil cellulose to be digested more rapidly than alfalfa cellulose, despite similar lignin concentrations. McGraw and Marten (1986) reported greater digestibility of birdsfoot trefoil stems than alfalfa stems. Whereas Mowat et al. (1969) found a highly negative correlation ($R^2 = 0.78$) between cell wall lignin concentration and in vitro cell wall digestibility in alfalfa, birdsfoot trefoil cell wall digestibility was not correlated with lignin concentration ($R^2 = 0.13$). Greater cell wall digestibility in birdsfoot trefoil may lead to an increase in DMI by lactating dairy cows (Allen, 2000). In addition, feeding birdsfoot trefoil has been reported to improve N utilization by ruminants due to CT in birdsfoot trefoil (Barry and McNabb, 1999; Min et al., 2003). Moderate concentrations of CT (2.0–4.0% DM) bind protein by hydrogen bonding at near neutral pH (i.e., pH 6.0–7.0) in the

Received January 15, 2015.

Accepted July 12, 2015.

¹Approved as Journal Paper Number 8812 of the Utah Agricultural Experiment Station, Utah State University, Logan.

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rumen to form CT-protein complexes, but dissociate and release bound protein at pH <3.5 in the abomasum (Barry et al., 2001). Using continuous culture, we previously reported that ammonia-N ($\text{NH}_3\text{-N}$) concentration and flow were reduced when replacing alfalfa hay with birdsfoot trefoil hay in dairy TMR (Williams et al., 2011). This in vitro result suggests that incorporating birdsfoot trefoil hay as a CT-containing legume forage into dairy TMR can have a potential to improve environmental performance of dairy operations through a reduction in N excretion. Though feeding birdsfoot trefoil hay has potential benefits over alfalfa hay, establishing stands, the persistence of the stand over time, and the reduction in yield compared with alfalfa pose challenges to incorporating birdsfoot trefoil as a total alfalfa replacement.

Several studies have shown that birdsfoot trefoil fed to dairy cows as a preserved silage (Hymes-Fecht et al., 2013) or fresh forage (Woodward et al., 2000) increased N utilization, reduced N excretion in urine, and shifted N excretion to feces. However, no studies have been conducted to determine if birdsfoot trefoil preserved as a dry hay have the same effects as fresh or wet-preserved forages. Thus, we sought to determine effects of feeding birdsfoot trefoil hay in high-forage dairy diets on nutrient intake and utilization, ruminal fermentation profiles, and lactational performance of dairy cows. We hypothesized that feeding a birdsfoot trefoil hay-based lactation diet would increase intake of DM and digested NDF by dairy cows compared with alfalfa hay-based diet due to increased NDF digestibility, leading to improved lactational performance and N utilization efficiency.

MATERIALS AND METHODS

The dairy cows used in this study were cared for according to the *Guide for the Care and Use of Agricultural*

Animals in Research and Teaching (FASS, 2010) and the Institutional Animal Care and Use Committee at Utah State University. The experiment was conducted at the Caine Dairy Research Center (Wellsville, UT) of Utah State University.

Forage Harvest and Composition

A mature stand of alfalfa hay was the source for the alfalfa hay-containing diets, and it was the second harvest cut the year before the experiment start. 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 to reduce leaf loss, and then baled later that day with a conventional square baler at between 85 and 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 birdsfoot trefoil hay used in this experiment was Norcen variety, planted in fall 2009 on a private ranch (Garland, UT), it was also second cutting harvested the year previous to the experiment by mower-conditioner (model 830) and allowed to sun-cure for 5 d before baling. Chemical composition of the hays and corn silage used to construct experimental diets is reported in Table 1.

Cows, Experimental Design, and Diets

Nine multiparous lactating Holstein cows, 3 of which were surgically fitted with rumen cannula (10-cm i.d., Bar Diamond, Parma, ID), were used for this experiment. Cows began the experiment averaging 131 ± 22.6 DIM. Mean BW was 778 ± 73.6 and 787 ± 65.7 kg at the beginning and the end of the experiment, respectively.

The design of the experiment was a triple 3×3 Latin square, with cannulated cows comprising one square.

Table 1. Chemical composition (means $\pm$ SD) of forages (n = 3)

Item, % of DM (unless otherwise noted)	Forage		
	Corn silage	Alfalfa hay	Birdsfoot trefoil hay
DM, %	30.9 ± 2.26	93.7 ± 0.19	92.7 ± 0.52
OM	94.6 ± 0.21	88.1 ± 0.96	89.5 ± 0.17
CP	5.99 ± 0.003	17.7 ± 0.96	15.8 ± 1.01
NDF	41.7 ± 1.12	34.7 ± 2.28	39.0 ± 3.21
ADF	23.5 ± 0.67	30.0 ± 0.27	33.8 ± 0.63
NFC ¹	47.2 ± 1.45	30.9 ± 1.31	32.8 ± 0.14
IVNDFD, ² % NDF	41.1 ± 1.75	31.2 ± 2.25	35.8 ± 1.12
Lignin	2.72 ± 0.153	6.59 ± 0.674	7.57 ± 0.556
Condensed tannins	ND ³	ND	1.05 ± 0.148

¹NFC = $100 - \text{CP} - \text{NDF} - \text{ether extract} - \text{ash}$.

²IVNDFD = NDF digestibility measured at 30 h of incubation in vitro.

³ND = not determined

Table 2. Ingredients and chemical composition (means $\pm$ SD) of experimental diets fed to dairy cows (n = 3 sample replicates)

Item	Experimental diet ¹		
	AHT	ABT	BT
Ingredient, % of DM			
Alfalfa hay	42.0	20.7	—
Birdsfoot trefoil hay	—	20.7	42.6
Corn silage	15.4	15.4	15.4
Corn grain, flaked	22.5	22.1	21.9
Cottonseed, whole	6.20	6.20	6.20
Soybean meal	1.30	3.50	4.80
Corn DDGS ²	7.10	7.00	6.80
Beet pulp, shreds	1.50	—	—
Fat supplement ³	0.40	1.50	1.20
Calcium carbonate	1.01	1.01	1.01
Salt	0.31	0.31	0.31
Urea	0.70	0.70	0.70
Magnesium oxide	0.18	0.18	0.18
Vitamins and minerals ⁴	0.14	0.14	0.14
Sodium bicarbonate	0.70	0.70	0.70
Nutrient composition, % of DM (unless otherwise noted)			
DM, %	60.3 $\pm$ 4.12	60.0 $\pm$ 3.89	62.1 $\pm$ 1.60
OM	91.0 $\pm$ 1.64	90.4 $\pm$ 0.35	91.8 $\pm$ 0.93
CP	15.9 $\pm$ 0.73	15.5 $\pm$ 0.19	15.2 $\pm$ 0.63
RDP, ⁵ % of CP	53.5	53.4	53.5
RUP, ⁵ % of CP	46.5	46.6	46.5
NDF	41.1 $\pm$ 0.78	39.7 $\pm$ 3.48	38.5 $\pm$ 0.72
ADF	28.8 $\pm$ 0.83	27.6 $\pm$ 2.66	26.9 $\pm$ 0.17
NFC ⁶	31.1	31.9	34.3
NE _L , ⁵ Mcal/kg	1.60	1.60	1.60
Condensed tannins	0.05 $\pm$ 0.016	0.38 $\pm$ 0.062	0.51 $\pm$ 0.087

¹AHT = alfalfa hay-based TMR; ABT = alfalfa hay and birdsfoot trefoil hay-based TMR; BT = birdsfoot trefoil hay-based TMR.

²DDGS = dried distillers grains with solubles.

³Calcium salts of palm oil (EnerGII, Virtus Nutrition LLC, Corcoran, CA).

⁴Formulated to contain (per kg of 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).

⁵Based on tabular value (NRC, 2001).

⁶NFC = 100 – CP – NDF – ether extract – ash.

Within each square, cows were randomly assigned to a sequence of 3 diets during each of the three 21-d periods (14 d of treatment adaptation and 7 d of data and sample collections). The 3 experimental diets included (1) alfalfa hay-based TMR (**AHT**), (2) alfalfa and birdsfoot trefoil hay-based TMR (**ABT**), and birdsfoot trefoil hay-based TMR (**BT**; Table 2), and were composed of 42% alfalfa hay for AHT or 21% alfalfa hay for ABT, whereas birdsfoot trefoil hay comprised 21% of the diet for ABT and 42% of the diet for BT. Corn silage was included in the 3 diets at 15.4% DM. Typical lactating dairy diets in the intermountain west (i.e., Utah, Idaho, Wyoming, Montana, and parts of Arizona and Nevada) contain more alfalfa hay than corn silage. Alfalfa hay is commonly fed to provide 50 to 75% of the dietary forage, with total forage levels averaging

45 to 55% of the dietary DM. The 3 experimental diets maintained forage-to-concentrate ratio at 58:42 on a DM basis.

The diets, excluding corn silage, were processed for approximately 15 min in a TMR wagon (model 455, Roto-Mix, Dodge City, KS), then, before each daily feeding, corn silage was added to each diet, mixed in a feed cart (I. H. Rissler Manufacturing, Curtiss, WI), and weighed out to provide 70% of each individual cows daily feed in the morning and the final 30% in the evening. Cows were fed to allow for 5 to 10% orts, and orts were weighed and collected daily during the week of sampling.

Condensed tannins of alfalfa hay and birdsfoot trefoil hay were isolated from ground samples of the hay (0.5 mm) using the modified HCl-butanol-acetone assay

(Grabber et al., 2013), and a spectrophotometer (Bio-Mate 3, Thermo Fisher Scientific, Madison, WI) was used to quantify the CT. Purified CT from birdsfoot trefoil was used as a standard. Diet concentrations of CT were 0.38 and 0.51% in ABT and BT, respectively (Table 2). Diets were isonitrogenous across treatments, averaging 15.6% CP, and isocaloric (1.60 Mcal/kg). Diets were formulated to meet NRC (2001) recommendations for RDP, RUP, minerals, and vitamins of a mid-lactation dairy cow weighing 780 kg (BCS = 3.0) and producing 36.3 kg of milk/d containing 3.5% fat and 3.0% true protein with 24.9 kg/d of DMI.

Cows were housed in a tiestall barn on a rubber mattress covered with straw and were individually fed. Cows had free access to water. Diets were mixed at 0400 h and fed twice daily. Cows were milked twice daily at 0300 and 1500 h. Milk weights were recorded automatically using Boumatic meters (Madison, WI) for each milking throughout the experiment. Milk samples were collected from 6 consecutive milkings during d 15 through 17 of each experimental period. Individual milk samples were analyzed by the Rocky Mountain DHIA Laboratory (Wellsville, UT) for fat, true protein, lactose, and MUN. Daily milk composition was calculated from the weighted morning and evening observations. Yields of milk fat, true protein, and lactose were calculated by multiplying milk yield from respective day by fat, true protein, and lactose concentrations, respectively, of the milk from each individual cow. To convert milk true protein to milk N, 6.38 was used as the conversion factor (DePeters and Cant, 1992), and total milk N (kg/d) was calculated as milk true protein/6.38 + MUN, where milk true protein and MUN were expressed as kg/d.

Feed Sampling and Analysis

Samples of alfalfa hay, birdsfoot trefoil hay, and corn silage were taken weekly to determine DM concentration, and dietary concentrations of forages and concentrates were adjusted weekly on an as-fed basis to reflect changes in the DM concentrations. Samples of feeds were then composited by period for chemical analysis. Samples of the TMR and orts for each cow were collected daily during the sampling week and stored frozen at -20°C until composited by period for analysis. Then, their composite samples were dried at 60°C for 48 h, ground to pass a 1-mm screen (standard model 4; Arthur H. Thomas Co., Swedesboro, NJ) for chemical analyses. The DM concentrations of 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 in a 550°C muffle furnace oven for 5 h (AOAC International, 2000; method 942.05). Crude protein concentration was determined by combustion methodology (Flash 2000 Automatic Elemental Analyzer, Thermo Fisher Scientific; AOAC International, 2000; method 990.03). 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 manufacturer, 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 from *Bacillus subtilis*; Sigma-Aldrich Corporation, St. Louis, MO).

Apparent total-tract digestibility of CP and NDF were measured during the last week in each period using acid-insoluble ash (AIA) as an internal marker (Van Keulen and Young, 1977). Fecal samples (approximately 100 g, wet weight) were collected for each cow from the rectum twice daily (morning and evening) every 12 h, moving ahead 2 h each day for the 5-d sampling of feces beginning on d 15. This schedule provided 12 representative samples of feces for each cow. Samples were composited across sampling times for each cow, dried at 60°C for 72 h, ground to pass a 1-mm screen (standard model 4), and stored for chemical analysis. Intake of digested NDF was calculated from NDF intake and concentrations of AIA and NDF in diets fed, orts, and feces using the following equation:

$$\begin{aligned} \text{Intake of digested NDF (kg/d)} &= \text{intake} \\ \text{of NDF (kg/d)} &\times \{100 - [100 \times (\text{AIA}_d/\text{AIA}_f) \\ &\times (\text{NDF}_f/\text{NDF}_d)]\}, \end{aligned}$$

where AIA_d = AIA concentration in the diet actually consumed, AIA_f = AIA concentration in the feces, NDF_f = concentration of the NDF in the feces, and NDF_d = concentration of the NDF in the diet actually consumed. Intake of undigested NDF was calculated from the following equation (Eun and Beauchemin, 2005):

$$\begin{aligned} \text{Intake of undigested NDF (kg/d)} &= \text{intake} \\ \text{of NDF (kg/d)} &- \text{intake of digested NDF (kg/d)}. \end{aligned}$$

Ruminal Fermentation Characteristics

Ruminal pH was continuously measured on cannulated cows for 2 consecutive days starting on d 15 using the Lethbridge Research Centre Ruminal pH Measure-

ment System (**LRCpH**; Dascor, Escondido, CA), as described by Penner et al. (2006). Readings in pH buffers 4 and 7 were recorded before placing the LRCpH system in the rumen. Ruminal pH readings were taken every 30 s and stored by the data logger. After about 48 h of continuous pH measurement, the LRCpH was removed from the rumen, washed in 39°C water, and millivolt readings were recorded in pH buffers 4 and 7. The shift in millivolt readings from the electrodes between the start and finish standardization was assumed to be linear and was used to convert the millivolt data to pH units. The daily ruminal pH data were averaged for each minute and summarized as minimum pH, mean pH, and maximum pH. Also, when ruminal pH was less than 5.8, daily episodes, duration (h/d), and area (pH $\times$ min) were calculated. The threshold of 5.8 was chosen because it has been previously described by others (Nocek, 1997; Maekawa et al., 2002; Beauchemin and Yang, 2005) to cause ruminal acidosis.

Samples of whole ruminal contents were collected at 0, 2, 4, 6, 8, 10, and 12 h following the morning feeding on d 20 and 21 of each experimental period from cannulated cows. Rumen samples were collected from different locations in the reticulorumen (ventral sac, reticulum, and feed mat in the dorsal rumen; approximately 250 g each), composited, and filtered through a polyester screen (pore size 355 μ m; B & S H Thompson, Ville Mont-Royal, QC, Canada). Two separate 5-mL aliquots of the rumen samples were conserved and immediately frozen until processed for analyses of $\text{NH}_3\text{-N}$ and VFA. Concentration of $\text{NH}_3\text{-N}$ of rumen contents was determined using methods described by Rhine et al. (1998) after thawing samples and adding 1 mL of 1% sulfuric acid. For VFA determination, 5-mL samples taken at 0 and 6 h post-morning feeding were added to 1 mL of 25% meta-phosphoric acid and quantified using a GLC (model 5890 series II; Hewlett Packard Co., Palo Alto, CA) with a capillary column (30 m $\times$ 0.32 mm i.d., 1- μ m phase thickness, Zebron ZB-FAAP, Phenomenex, Torrance, CA) and flame-ionization detection. The oven temperature was 170°C, held for 4 min, which was then increased by 5°C/min to 185°C, and then by 3°C/min to 220°C, and held at this temperature for 1 min. The injector temperature was 225°C, the detector temperature was 250°C, and the carrier gas was helium (Eun and Beauchemin, 2007).

Urine Sampling and Analysis

Spot urine samples were collected at 0600 and 1800 h on d 18 and 19 in each period from all cows. Urine samples were acidified during collection to a pH <3.8 by addition of 4 M HCl. The acid solution was added to urine in the urine containers during collection, and

then the urine was frozen in separate containers at -20°C . Upon analysis, samples were thawed and composited per cow by period, and aliquots were diluted 1:39 with urine diluent. Diluent was 0.202% sodium L-heptane sulfonic acid and 0.086% ammonium dihydrogen phosphate, acidified to pH 2.1 with 4 M HCl, and directly analyzed for the purine derivatives (**PD**) allantoin, uric acid, xanthine, hypoxanthine, and the pyrimidine pseudouridine, as well as creatinine by using HPLC (Waters Corp., Milford, MA) according to the procedures of Shingfield and Offer (1999). Urinary creatinine was used as a marker to estimate urine volume (Valadares et al., 1999; Leonardi et al., 2003). In calculating urine volume, creatinine output was assumed to average 28 mg/kg of BW as estimated by Whittet (2004). Similar daily creatinine outputs, ranging from 25 to 30 mg/kg of BW, have been reported (McCarthy et al., 1983; Jones et al., 1990). The allantoin and uric acid concentrations, determined from HPLC analysis, were converted to ratios, and purine and pyrimidine production in millimoles per day was determined by the product of creatinine production and the ratio of purine derivatives to creatinine. The ratio of the urinary PD allantoin and uric acid to creatinine was used to estimate the relative differences in microbial CP production (Shingfield and Offer, 1998). Microbial protein production (g/d) was estimated using the equation $\{[\text{PD production, mmol/d} - (0.385 \times \text{BW}^{0.075})] / 0.85\} \times 70 \times 6.25 / (0.13 \times 0.83 \times 1,000)$, as reported by Janicek et al. (2008).

Statistical Analyses

Nutrient data were summarized for each cow by measurement period, providing 9 samples for analysis per period. All data were statistically analyzed using the mixed model procedure in SAS (version 9.3, SAS Institute, 2012). Data for intake, digestibility, milk production, and efficiency were transformed into weekly averages for each cow by measurement period and analyzed with a model that included the effects of dietary treatment and group (square). Cow, period, and cow by period by group were the terms of the random statement. Data for ruminal pH and VFA were analyzed with a model that included the dietary treatment. Cow and period were the terms of the random statement. Data for $\text{NH}_3\text{-N}$ concentration in the rumen were analyzed using the model described above except that the fixed effect of time after feeding was included using the repeated option. For all models used, degrees of freedom were estimated with the Kenward-Roger specification in the models. Means were compared using a protected ($P < 0.05$) least significant difference test. Unless otherwise stated, significance was declared at P

≤ 0.05 , and tendency toward significance at $0.05 < P \leq 0.10$. All results are reported as LSM.

RESULTS AND DISCUSSION

Intake of DM and Nutrients, Digestibility, and Milk Production

Intakes of DM and CP were similar across treatments ($P = 0.33$ and 0.27 , respectively), whereas feeding birdsfoot trefoil hay-containing diets resulted in decreased NDF intake compared with AHT because of the reduced concentration of NDF in ABT and BT (Table 2). Previous studies on the effects of feeding CT-containing forages or CT extracts on feed intake in ruminants have yielded inconsistent results, and focused only on effects of CT on N digestion (Woodward et al., 2001; Benchaar et al., 2008; Dschaak et al., 2011). It is important to note that variations in laboratory procedures and standards used to determine CT concentrations can make comparisons and interpretations difficult between studies. Increased DMI was observed due to feeding birdsfoot trefoil silage-based diets (2.59% CT; Woodward et al., 2001), whereas others found no effect of supplementing CT extract on DMI in either Jersey heifers (Baah et al., 2007; with 0.60% quebracho CT extract) or lactating dairy cows (Benchaar et al., 2008; with 0.45% CT). Conversely, decreases of DMI with CT have been reported either in dairy cows (Dschaak et al., 2011; with 2.25% CT from quebracho CT extract) or in sheep [Barry and McNabb (1999) with 7.5 to 10.0% CT from birdsfoot trefoil pasture; Priolo et al. (2000) with 2.5% CT from carob pulp]. In the current study, our results suggest that the CT in these birdsfoot trefoil hay diets did not negatively affect intake, because the CT concentration of ABT and BT (0.38 and 0.51%, respectively) was less than 5.0% of DM, which is the threshold shown to reduce intake (Patra and Saxena, 2011).

The BT diet had decreased NDF intake, but a tendency for increased NDF digestibility ($P = 0.10$) was noted with the incorporation of birdsfoot trefoil hay in the diets. In vitro NDF digestibility increased with birdsfoot trefoil hay compared with alfalfa hay (Table 1), which may have accelerated NDF digestion in the rumen, leading to a numerical increase in digested NDF intake ($P = 0.20$) and a sizable reduction in undigested NDF intake when cows were fed BT (Table 3). In the current study, 50.5% NDF of the AHT was undigested, but only 44.7% of NDF in the BT was undigested. These differences in digestibility of forages probably altered diet retention time in the rumen, and are related to differences in lignification patterns in stems and leaves of the forage rather than lignin concentration (Hoff-

man et al., 1993; Buxton, 1996). Whereas DMI was similar across dietary treatments in the current study, in a comparative study of commonly cultivated forages, voluntary intake relative to cell wall content substantially increased for birdsfoot trefoil hay compared with alfalfa hay and other forages above the standard deviation of the other forages (Van Soest, 1965). In an early study by Ingalls et al. (1966), an initial rate of disappearance of DM, cell wall constituents, fiber, and lignin were faster for birdsfoot trefoil than for alfalfa. Differences in lignin composition and structure between alfalfa hay and birdsfoot trefoil hay can also influence the digestibility of NDF in the rumen; despite birdsfoot trefoil hay having 15% increased lignin concentration, in vitro NDF digestibility of birdsfoot trefoil hay was numerically greater than alfalfa hay (35.8 vs. 31.2%; Table 1). Recently, Hunt et al. (2014) reported that lignification of stems and leaves of birdsfoot trefoil as plants matured and elongated was different from that of alfalfa, thereby causing potential cell wall differences that could affect digestion rates of NDF in the 2 legume forages. In addition to cell wall chemical differences, MacAdam and Griggs (2013) reported greater NFC concentration in birdsfoot trefoil hay than alfalfa hay when both species were harvested at 6-wk intervals. Because initiation of digestion of fiber by rumen microbes occurs in the cell lumen, a greatly digestible layer in the cells would enhance microbial colonization of fiber and increase digestion of NDF.

Dietary treatment tended to increase ($P = 0.09$) milk and ECM yields, being greater in BT than AHT ($P < 0.04$; Table 3). This is in agreement with Hymes-Fecht et al. (2013), where cows offered birdsfoot trefoil silage increased milk and ECM yields compared with those fed alfalfa silage. Furthermore, those authors reported that milk production was greater for cows consuming low-CT birdsfoot trefoil diets, and milk production increased with CT concentrations within birdsfoot trefoil hay (Hymes-Fecht et al., 2013). Whereas the lowest CT birdsfoot trefoil hay diet was similar to the highest proportion birdsfoot trefoil hay diet in the current study, the results are likely due in part to CT activity in the rumen. Woodward et al. (1999, 2000) reported similar milk yield increases in cows grazing birdsfoot trefoil. The concentrations of CT within the diets of the current study were much less than that in both studies by Woodward et al. (1999, 2000; 2.6% DM and 1.9% DM, respectively), but similar to the lowest CT diet in the study of Hymes-Fecht et al. (2013); this implies that other differences, such as enhanced NDF digestion in birdsfoot trefoil hay used in our study, probably affected milk production through improved NDF digestion and its subsequent effects on ruminal fermentation, but not CT activity in the rumen. Wood-

ward et al. (1999, 2000) and Hymes-Fecht et al. (2013) reported that digestibility of fiber varied in response to CT concentration, suggesting that relationship between CT concentration and effects of digestibility can have varying effects on milk production.

Milk fat and lactose concentration were not different across dietary treatments, whereas milk true protein concentration and yield increased for cows consuming birdsfoot trefoil diets compared with those fed AHT (Table 3). Similar results were found in milk of cows offered diets containing birdsfoot trefoil silage (Hymes-Fecht et al., 2013). However, milk protein concentration and protein yield increased due to feeding birdsfoot trefoil hay, supporting the effect of increased diet digestibility, and contributing to improved diet N use for protein secretion. Dietary treatment led to a tendency in feed efficiency based on milk yield ($P = 0.09$) with its greater value from the BT compared with the ABT ($P = 0.05$), but no effects of treatment on feed efficiency were noted based on ECM yield.

Ruminal Fermentation Characteristics

Dietary treatments in general did not influence ruminal pH profiles with minor effects on minimum and maximum ruminal pH (Table 4). Mean pH aver-

aged 6.47 across treatments, which is typical in cows fed high-forage diets. Similarly, Hymes-Fecht et al. (2013) reported minor effects on ruminal pH by feeding birdsfoot trefoil silage-based diet compared with alfalfa silage-based diet. Dietary NDF concentration for all treatments used in the current study would be adequate to maintain optimal ruminal pH.

Dietary treatment tended to increase ($P = 0.09$) total VFA concentration, with BT being greater than AHT and ABT ($P < 0.04$; Table 4). Dietary treatment resulted in tendencies in proportion of acetate ($P = 0.09$) and propionate ($P = 0.06$); feeding birdsfoot trefoil hay diets increased acetate proportion, whereas the BT increased propionate portion, leading to a tendency in acetate-to-propionate ratio ($P = 0.07$). Effects of feeding CT-containing forages or CT extracts on VFA profiles have been variable. Carulla et al. (2005) reported that VFA concentrations remained unchanged, but molar proportion of propionate increased in sheep fed diets supplemented with CT-containing black wattle (*Acacia mearnsii*). Decreased acetate-to-propionate ratio was reported for cows fed birdsfoot trefoil silage (Hymes-Fecht et al., 2013) and in a continuous culture study (Williams et al., 2011) for TMR diets containing birdsfoot trefoil hay. The tendency for increased total VFA concentration due to feeding the BT in the

Table 3. Intake of DM and nutrients, milk yield and composition, and efficiencies of DM and N used for milk production of lactating dairy cows fed different legume hay-based diets

Item	Dietary treatment ¹			SEM	P-value
	AHT	ABT	BT		
Intake, kg/d					
DM	25.7	26.5	26.7	0.57	0.33
CP	4.16	4.13	4.01	0.10	0.27
NDF	10.6 ^a	10.3 ^{ab}	10.0 ^b	0.30	<0.01
ADF	7.36 ^a	7.10 ^b	6.99 ^b	0.218	0.02
Digestibility					
CP, %	74.2	72.3	75.5	1.18	0.18
NDF, %	50.5	52.3	55.0	1.99	0.10
Digested NDF intake, kg/d	5.25	5.36	5.53	0.283	0.20
Undigested NDF intake, kg/d	5.35 ^a	5.12 ^a	4.47 ^b	0.364	0.01
Yield, kg/d					
Milk	38.3	39.3	40.0	1.94	0.09
ECM	37.4	38.1	39.3	1.55	0.09
Milk composition, %					
Fat	3.37	3.25	3.38	0.145	0.22
True protein	2.84 ^b	2.93 ^a	2.94 ^a	0.080	<0.01
Lactose	4.83	4.82	4.82	0.046	0.83
Milk component yield, kg/d					
Fat	1.28	1.27	1.34	0.055	0.13
True protein	1.08 ^b	1.13 ^a	1.16 ^a	0.047	0.03
Lactose	1.85	1.89	1.92	0.091	0.08
Efficiency					
Milk yield/DMI	1.50	1.47	1.52	0.086	0.09
ECM yield/DMI	1.45	1.45	1.48	0.060	0.57

^{a,b}Means within a row that do not have a common superscript differ at $P < 0.05$.

¹AHT = alfalfa hay-based TMR; ABT = alfalfa hay and birdsfoot trefoil hay-based TMR; BT = birdsfoot trefoil hay-based TMR.

present study suggests enhanced ruminal fermentability by feeding birdsfoot trefoil hay, which can support increased energy supply for lactation. In addition, an increase in propionate and decrease in acetate corresponded to improvements in fiber digestion of forages (Eun and Beauchemin, 2007). It is not uncommon to observe changes in VFA proportions as a direct effect of enhanced fiber digestion in the rumen, implying that feeding birdsfoot trefoil hay may affect microbial growth, shift the metabolic pathways by which specific microbes use substrates, or both. Propionate is quantitatively the most important VFA precursor of glucose synthesis, and therefore has a major effect on hormonal release and tissue distribution of nutrients (Nagaraja et al., 1997). Consequently, increased VFA concentration and propionate proportion as a result of feeding birdsfoot trefoil hay would contribute to improving nutrient supply and utilization, which may have resulted in increases in milk production as well as feed efficiency observed in the current study.

Concentration of $\text{NH}_3\text{-N}$ was similar across all diets, but cows fed BT exhibited greater MCP yield relative to those fed AHT and ABT (Table 4). The most efficient way to improve nutrient utilization in the rumen is to maximize microbial growth by capturing N-containing compounds released from RDP sources and convert that N into rumen microbial cells. Tannin-protein complexes inhibit the fermentation of forage protein to ammonia in the rumen, increasing the amount of protein that reaches the small intestine (Barry et al., 1986; Waghorn et al., 1987). Therefore, CT included in the diet as a supplement or as a component of the forage would be expected to reduce the amount of N

fermented in the rumen. However, no effect of $\text{NH}_3\text{-N}$ concentration in response to dietary treatments in the current study indicates that CT concentration in ABT and BT may have been inadequate to form substantial tannin-protein complexes, one of the most promising effects of CT on ruminal fermentation. Besides adequate N supply, ruminal MCP synthesis also depends on supply of adequate amounts of carbohydrate as an energy and carbon source for the synthesis of peptide bonds (Bach et al., 2005). The Cornell Net Carbohydrate and Protein System suggests a maximum incorporation of 40% of the fermented feed carbohydrate into microbial biomass (Russell et al., 1992). Readily fermentable carbohydrates, such as starch or sugars, are more effective than other sources, such as cellulose, in promoting microbial growth (Stern and Hoover, 1979). However, when cows are fed high-forage diets, such as the ones tested in the current study, the contribution from ruminally fermented NDF to the synthesis of MCP would be greater than high-concentrate diets. Enhanced NDF digestion evidenced by increased VFA concentration by feeding BT may have provided greater energy for MCP yield due to feeding BT. Berthiaume et al. (2010) examined alfalfa cultivars with high- versus low-NDF digestibility in vitro and observed increased total VFA production and increased apparent digestibility of DM and OM, all of which contributed to increased MCP yield.

Utilization of N

Intake of N was not different among treatments, whereas cows consuming both birdsfoot trefoil hay-

Table 4. Ruminal fermentation characteristics of lactating dairy cows fed different legume hay-based diets

Item	Dietary treatment ¹			SEM	P-value
	AHT	ABT	BT		
Minimum pH	5.70 ^a	5.56 ^b	5.70 ^a	0.106	0.05
Maximum pH	7.21 ^a	7.22 ^a	6.91 ^b	0.083	0.05
Mean pH	6.45	6.53	6.37	0.072	0.34
pH <5.8					
Daily episodes, no.	6.00	3.33	3.33	3.707	0.84
Duration, h/d	2.12	0.53	1.16	1.408	0.67
Area, pH × min	16.4	2.75	8.68	10.72	0.60
Total VFA, mM	102	106	119	6.3	0.09
Individual VFA, mol/100 mol					
Acetate (A)	61.1	59.7	59.0	1.93	0.09
Propionate (P)	23.5	24.7	26.1	1.25	0.06
Butyrate	10.8	10.8	10.7	0.56	0.83
A:P	2.62	2.42	2.26	0.201	0.07
Ammonia-N, mg/100 mL	8.33	6.05	6.70	1.244	0.51
Microbial protein production, g/d	1,793 ^b	1,824 ^b	2,115 ^a	93.1	0.01

^{a,b}Means within a row that do not have a common superscript differ at $P < 0.05$.

¹AHT = alfalfa hay-based TMR; ABT = alfalfa hay and birdsfoot trefoil hay-based TMR; BT = birdsfoot trefoil hay-based TMR.

Table 5. Nitrogen utilization of lactating dairy cows fed different legume hay-based diets

Item	Dietary treatment ¹			SEM	<i>P</i> -value
	AHT	ABT	BT		
N intake, g/d	666	662	646	25.1	0.76
Milk N, g/d	184 ^b	194 ^a	197 ^a	7.7	<0.01
Milk N:N intake ²	0.27 ^b	0.29 ^a	0.30 ^a	0.02	0.05
MUN, mg/100 mL	14.1	14.4	14.8	0.68	0.19
Urinary N excretion, ³ g/d	289	299	306	17.0	0.17

^{a,b}Means within a row that do not have a common superscript differ at $P < 0.05$.

¹AHT = alfalfa hay-based TMR; ABT = alfalfa hay and birdsfoot trefoil hay-based TMR; BT = birdsfoot trefoil hay-based TMR.

²Efficiency of use of feed N to milk N.

³Predicted using the following equation: $0.026 \times \text{MUN (mg/100 mL)} \times \text{BW (kg)}$ (Wattiaux and Karg, 2004).

containing diets secreted more milk N than those fed AHT, resulting in improved N utilization efficiency for milk N (Table 5). A similar increase in the N utilization efficiency was reported for birdsfoot trefoil hay silage diets by Hymes-Fecht et al. (2013), where N utilization efficiency increased from 21% for alfalfa hay silage diet to 25% for birdsfoot trefoil hay silage diets. Concentration of MUN, which reflects inefficiency of dietary N utilization and ruminal $\text{NH}_3\text{-N}$, did not differ between treatments. A relatively reduced concentration of CT in ABT and BT may have resulted in no effect on MUN concentration in the present study. Benchaar et al. (2008) and Aguerre et al. (2010) also observed no effect of supplementation of quebracho CT on MUN concentration. Diets also did not affect urinary N excretion.

CONCLUSIONS

Feeding better-quality forage is the cornerstone of any dairy operation, and it considerably affects sustainable dairy production. Our main interest in the current study focused on fiber fermentation and the subsequent milk production performance and changes in N utilization efficiency due to feeding birdsfoot trefoil hay with relatively low CT concentration, which is typical of that available in the western United States. The overall results of the present study support our hypothesis of increased NDF digestion by cows fed birdsfoot trefoil hay-containing TMR, which contributed to increased ruminal fermentation and MCP yield. Furthermore, these increases resulted in improvement in efficiencies of feed and N utilization. However, a lack of effects of feeding birdsfoot trefoil hay on ruminal N degradation was evident by no effect on $\text{NH}_3\text{-N}$ concentration due to a low concentration of CT in birdsfoot trefoil hay fed in the current study. Given the fact that a single source of birdsfoot trefoil hay was used in the dietary treatments tested in the current study, positive results in NDF digestion, ruminal fermentation, and lactational per-

formance reported in this paper should be extrapolated with caution due to potential effects of weather and seasonal effects on forage quality of birdsfoot trefoil hay. Although we reported enhanced digestion of birdsfoot trefoil hay due to its increased NDF digestibility and favorable cell wall structure toward microbial fermentation, we have yet to explore the effects of low-CT concentration diets by feeding birdsfoot trefoil hay on microbial community structure, particularly cellulolytic bacteria and its influence toward microbial growth and energetic efficiency. This direction for future research can offer an opportunity to understand whether ruminal microbial composition and activities could be linked to lactational performance and nutrient utilization efficiency of dairy cows fed birdsfoot trefoil hay as a main forage source.

ACKNOWLEDGMENTS

This study was supported by funds from the USDA-National Institute of Food and Agriculture Organic Agriculture Research and Extension Initiative Grants Program (award number: 2010-51300-21283) and Utah State University Agricultural Experiment Station (Logan, UT). The authors thank K. Neal, J. M. Vera, and C. T. Noviandi at Utah State University for technical assistance and the staff of the Caine Dairy Center (Wellsville, UT) for their conscientious care of the experimental cows.

REFERENCES

- Aguerre, M. J., M. A. Wattiaux, M. C. Capozzolo, P. Lencioni, and C. Cabral. 2010. Effect of quebracho-chestnut tannin extracts at two dietary crude protein levels on performance and rumen fermentation of dairy cows. *J. Dairy Sci.* 93(Suppl. 1):445. (Abstr.)
- Allen, M. S. 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. *J. Dairy Sci.* 83:1598–1624.
- AOAC International. 2000. *Official Methods of Analysis*. Vol. 1 and 2. 17th ed. AOAC International, Gaithersburg, MD.

- Baah, J., M. Ivan, A. N. Hristov, K. M. Koenig, L. M. Rode, and T. A. McAllister. 2007. Effects of potential dietary antiprotozoal supplements on rumen fermentation and digestibility in heifers. *Anim. Feed Sci. Technol.* 137:126–137.
- Bach, A., S. Calsamiglia, and M. D. Stern. 2005. Nitrogen metabolism in the rumen. *J. Dairy Sci.* 88(E-Suppl.):E9–E21.
- Barry, T. N., R. T. Manley, and S. J. Duncan. 1986. The role of condensed tannins in the nutritional value of *Lotus pedunculatus* for sheep. 4. Site of carbohydrate and protein digestion as influenced by dietary reactive tannin concentrations. *Br. J. Nutr.* 55:123–137.
- Barry, T. N., and W. C. McNabb. 1999. The implications of condensed tannins on the nutritive value of temperate forages fed to ruminants. *Br. J. Nutr.* 81:263–272.
- Barry, T. N., D. M. McNeill, and W. C. McNabb. 2001. Plant secondary compounds: Their impact on nutritive value and upon animal production. Pages 445–452 in *Proc. XIX Int. Grass. Conf.*, Sao Paulo, Brazil.
- Beauchemin, K. A., and W. Z. Yang. 2005. Effects of physically effective fiber on intake, chewing activity, and ruminal acidosis for dairy cows fed diets based on corn silage. *J. Dairy Sci.* 88:2117–2129.
- Benchaar, C., T. A. McAllister, and P. Y. Chouinard. 2008. Digestion, ruminal fermentation, ciliate protozoal populations, and milk production from dairy cows fed cinnamaldehyde, quebracho condensed tannin, or *Yucca schidigera* saponin extract. *J. Dairy Sci.* 91:4765–4777.
- Berthiaume, R., C. Benchaar, A. V. Chaves, G. F. Tremblay, Y. Castonguay, A. Bertrand, and A. F. Brito. 2010. Effects of nonstructural carbohydrate concentration in alfalfa on fermentation and microbial protein synthesis in continuous culture. *J. Dairy Sci.* 93:693–700.
- Buxton, D. R. 1996. Quality-related characteristics of forages as influenced by plant environment and agronomic factors. *Anim. Feed Sci. Technol.* 59:37–49.
- Carulla, J. E., M. Kreuzer, A. Machmuller, and H. D. Hess. 2005. Supplementation of *Acacia mearnsii* tannins decrease methanogenesis and urinary nitrogen in forage-fed sheep. *Aust. J. Agric. Res.* 56:961–970.
- DePeters, E. J., and J. P. Cant. 1992. Nutritional factors influencing the nitrogen composition of bovine milk: A review. *J. Dairy Sci.* 75:2043–2070.
- Dschaak, C. M., C. M. Williams, M. S. Holt, J.-S. Eun, A. J. Young, and B. R. Min. 2011. Effects of supplementing condensed tannin extract on intake, digestion, ruminal fermentation, and milk production of lactating dairy cows. *J. Dairy Sci.* 94:2508–2519.
- Eun, J.-S., and K. A. Beauchemin. 2005. Effects of a proteolytic feed enzyme on intake, digestion, ruminal fermentation, and milk production. *J. Dairy Sci.* 88:2140–2153.
- Eun, J.-S., and K. A. Beauchemin. 2007. Assessment of the efficacy of varying experimental exogenous fibrolytic enzymes using in vitro fermentation characteristics. *Anim. Feed Sci. Technol.* 132:298–315.
- FASS. 2010. Guide for the Care and Use of Agricultural Animals in Research and Teaching. 3rd rev. ed. Federation of Animal Science Societies, Champaign, IL.
- Grabber, J. H., W. E. Zeller, and I. Mueller-Harvey. 2013. Acetone enhances the direct analysis of procyanidin- and prodelphinidin-based condensed tannins in *Lotus* species by the butanol-HCl iron assay. *J. Agric. Food Chem.* 61:2669–2678.
- 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.* 76:2632–2643.
- Hunt, S. R., J. W. MacAdam, and T. C. Griggs. 2014. Lignification and tannin localization during the development of birdsfoot trefoil stems. *Crop Sci.* 54:1876–1886.
- Hymes-Fecht, U. C., G. A. Broderick, R. E. Muck, and J. H. Graber. 2013. Replacing alfalfa or red cover silage with birdsfoot trefoil silage in total mixed rations increases production of lactating dairy cows. *J. Dairy Sci.* 96:460–469.
- Ingalls, J. R., J. W. Thomas, M. B. Tesar, and D. L. Carpenter. 1966. Relations between ad libitum intake of several forage species and gut fill. *J. Anim. Sci.* 25:283–289.
- Janicek, B. N., P. J. Kononoff, A. M. Gehman, and P. H. Doane. 2008. The effect of feeding dried distillers grains plus solubles on milk production and excretion of urinary purine derivatives. *J. Dairy Sci.* 91:3544–3553.
- Jones, S. J., D. L. Starkey, C. R. Calkins, and J. D. Crouse. 1990. Myofibrillar protein turnover in feed-restricted and realimented beef cattle. *J. Anim. Sci.* 68:2707–2715.
- Leonardi, C., M. Stevenson, and L. E. Armento. 2003. Effect of two levels of crude protein and methionine supplementation on performance of dairy cows. *J. Dairy Sci.* 86:4033–4042.
- MacAdam, J. W., and T. C. Griggs. 2013. Irrigated birdsfoot trefoil variety trial: Forage nutritive value. Accessed Nov. 15, 2014. http://extension.usu.edu/files/publications/publication/AG_Forages_2013-02pr.pdf.
- Maekawa, M., K. A. Beauchemin, and D. A. Christensen. 2002. Effect of concentrate level and feeding management on chewing activities, saliva production, and ruminal pH of lactating dairy cows. *J. Dairy Sci.* 85:1165–1175.
- Martin, N. P., and D. R. Mertens. 2005. Reinventing alfalfa for dairy cattle and novel uses. Pages 299–311 in *Proc. 35th California Alfalfa and Forage Symposium*, Visalia, CA. University of California Cooperative Extension, Davis.
- McCarthy, F. D., W. G. Bergen, and D. R. Hawkins. 1983. Muscle protein turnover in cattle of differing genetic backgrounds as measured by urinary N-methylhistidine excretion. *J. Nutr.* 113:2455–2463.
- McGraw, R. L., and G. C. Marten. 1986. Analysis of primary spring growth of four pasture legume species. *Agron. J.* 78:704–710.
- Min, B. R., T. N. Barry, G. T. Attwood, and W. C. McNabb. 2003. The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: A review. *Anim. Feed Sci. Technol.* 106:3–19.
- Mowat, D. N., M. L. Kwain, and J. E. Winch. 1969. Lignification and in vitro cell wall digestibility of plant parts. *Can. J. Plant Sci.* 49:499–504.
- Nagaraja, T. G., C. J. Newbold, C. J. Van Nevel, and D. I. Demeyer. 1997. Manipulation of ruminal fermentation. Pages 523–632 in *The Rumen Microbial Ecosystem*, 2nd ed. P. N. Hobson and C. S. Stewart, ed. Blackie Academic and Professional, New York, NY.
- NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.
- Nocek, J. E. 1997. Bovine acidosis: Implications on laminitis. *J. Dairy Sci.* 80:1005–1028.
- Patra, A. K., and J. Saxena. 2011. Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. *J. Sci. Food Agric.* 91:24–37.
- Penner, G. B., K. A. Beauchemin, and T. Mutsvangwa. 2006. An evaluation of the accuracy and precision of a stand-alone submersible continuous ruminal pH measurement system. *J. Dairy Sci.* 89:2132–2140.
- Priolo, A., G. C. Waghorn, M. Lanza, L. Biondi, and P. Pennisi. 2000. Polyethylene glycol as a means for reducing the impact of condensed tannins in carob pulp: effects on lamb growth performance and meat quality. *J. Anim. Sci.* 78:810–816.
- Rhine, E. D., G. K. Sims, R. L. Mulvaney, and E. J. Pratt. 1998. Improving the Bertholot reaction for determining ammonium in soil extracts and water. *Soil Sci. Soc. Am. J.* 62:473–480.
- Russell, J. B., J. D. O'Connor, D. G. Fox, P. J. Van Soest, and C. J. Sniffen. 1992. A net carbohydrate and protein system for evaluating cattle diets: I. Ruminal fermentation. *J. Anim. Sci.* 70:3551–3561.
- SAS Institute. 2012. SAS/STAT User's Guide. Release 9.3. SAS Inst. Inc., Cary, NC.
- Shingfield, K. J., and N. W. Offer. 1998. Evaluation of milk allantoin excretion as an index of microbial protein supply in lactating dairy cows. *Anim. Sci.* 67:371–385.
- Shingfield, K. J., and N. W. Offer. 1999. Simultaneous determination of purine metabolites, creatinine, and pseudouridine in ruminant urine by reversed-phase high-performance liquid chromatography. *J. Chromatogr. B Biomed. Sci. Appl.* 723:81–94.

- Stern, M. D., and W. H. Hoover. 1979. Methods for determining and factors affecting rumen microbial protein synthesis: A review. *J. Anim. Sci.* 49:1590–1603.
- Tomlin, D. C., R. R. Johnson, and B. A. Dehority. 1965. Relationship of lignification to in vitro cellulose digestibility of grasses and legumes. *J. Anim. Sci.* 24:161–165.
- Valadares, R. F., G. A. Broderick, S. C. Valadares Filho, and M. K. Clayton. 1999. Effect of replacing alfalfa silage with high moisture corn on ruminal protein synthesis estimated from excretion of total purine derivatives. *J. Dairy Sci.* 82:2686–2696.
- Van Keulen, J., and B. A. Young. 1977. Evaluation of acid-insoluble ash as a natural marker in ruminant digestibility studies. *J. Anim. Sci.* 44:282–287.
- Van Soest, P. J. 1965. Symposium on factors influencing the voluntary intake of herbage by ruminants: Voluntary intake in relation to chemical composition and digestibility. *J. Anim. Sci.* 24:834–843.
- Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74:3583–3597.
- Waghorn, G. C., M. J. Ulyatt, A. John, and M. T. Fisher. 1987. The effect of condensed tannins on the site of digestion of amino acids and other nutrients in sheep fed on *Lotus corniculatus* L. *Br. J. Nutr.* 57:115–126.
- Wattiaux, M. A., and K. L. Karg. 2004. Protein level for alfalfa and corn silage-based diets: II. Nitrogen balance and manure characteristics. *J. Dairy Sci.* 87:3492–3502.
- Whittet, K. M. 2004. Factors affecting variability in urinary creatinine and purine derivative excretion in beef cattle. MS Thesis. Univ. of Nebraska, Lincoln.
- Williams, C. M., J.-S. Eun, J. W. MacAdam, A. J. Young, V. Fellner, and B. R. Min. 2011. Effects of forage legumes containing condensed tannins on methane and ammonia production in continuous cultures of mixed ruminal microorganisms. *Anim. Feed Sci. Technol.* 166:364–372.
- Woodward, S. L., M. J. Auldist, P. J. Laboyrie, and E. B. L. Jansen. 1999. Effect of *Lotus corniculatus* and condensed tannins on milk yield and milk composition of dairy cows. *Proc. N.Z. Soc. Anim. Prod.* 59:152–155.
- Woodward, S. L., P. J. Laboyrie, and E. B. L. Jansen. 2000. *Lotus corniculatus* and condensed tannins—Effects on milk production by dairy cows. *Asian-australas. J. Anim. Sci.* 13(Suppl.):521–552.
- Woodward, S. L., G. C. Waghorn, M. J. Ulyatt, and K. R. Lassey. 2001. Early indications that feeding *Lotus* will reduce methane emissions from ruminants. *Proc. N.Z. Soc. Anim. Prod.* 61:23–26.