Effects of nonstructural carbohydrate concentration in alfalfa on fermentation and microbial protein synthesis in continuous culture¹

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ABSTRACT

Insufficient readily fermentable energy combined with extensive degradation of proteins in alfalfa (*Medicago sativa* L.) may result in poor forage N utilization by ruminants. Using the inherent genetic variability and differences between harvests, our objective was to compare the effect of contrasting concentrations of nonstructural carbohydrates (NSC) in alfalfa on rumen fermentation and microbial protein synthesis. Individual genotypes of the alfalfa cultivar AC Caribou grown near Québec City, Québec, Canada, were harvested at the vegetative and early flowering stages, dried at 55°C, ground, and analyzed for soluble carbohydrates (fructose + sucrose + glucose + pinitol) and starch. Approximately 20 genotypes having, respectively, the highest and lowest NSC concentrations were pooled to constitute 2 contrasted 1-kg forage samples. Samples of high- (17.9% DM) and low- (7.4% DM) NSC alfalfa were respectively allocated to separate dual-flow fermenters in a completely randomized design with 3 replications. Rumen inoculum was obtained from 4 ruminally fistulated cows in early lactation that were fed a TMR with a 50:50 forage to concentrate ratio. A 10-d incubation period was used, with the first 6 d serving as an adaptation period followed by 4 d of sampling with solid and liquid dilution rates in the fermenters set at approximately 2.0 and 4.3%/h, respectively. High versus low NSC concentration in alfalfa significantly enhanced the apparent digestibility of OM (59.1% for high-NSC alfalfa vs. 54.4% for low-NSC alfalfa) and DM (60.0 vs. 54.3%) and the true digestibility of DM (74.1 vs. 64.7%). Increasing NSC concentration in alfalfa (high vs. low) significantly decreased ruminal pH (6.85

vs. 7.08) and $\text{NH}_3\text{-N}$ concentration (26.0 vs. 33.6 mg/dL) and increased total VFA concentration (94.9 vs. 83.0 mM). Molar proportions of acetate, isobutyrate, and isovalerate significantly decreased, whereas molar proportions of propionate and butyrate significantly increased with high-NSC alfalfa, resulting in a more glucogenic fermentation. More importantly, microbial N flow (263 vs. 230 mg/d) and bacterial N efficiency (41.1 vs. 29.6% of available N), measured using ^{15}N as a microbial marker, both significantly increased with the high-NSC alfalfa. These results indicate that increasing the concentration of NSC in alfalfa promotes a glucogenic fermentation and enhances microbial N synthesis in the rumen.

Key words: dairy cow, nonstructural carbohydrate, microbial N, rumen fermentation

INTRODUCTION

Alfalfa (*Medicago sativa* L.) is the predominant forage species fed to dairy cows in North America. The concentration of soluble proteins in alfalfa is high and generally exceeds the capacity of rumen microbes to utilize N efficiently. Excess NPN is excreted via the urine, which may contribute to water and air pollution (NRC, 2001) and adversely affect the health and reproduction of the cow (McCormick et al., 1999). Van Soest (1994) suggested increasing water-soluble carbohydrates (**WSC**) and starch as an approach to improving alfalfa protein utilization by ruminants. Others (Johnson, 1976) proposed that the synchronized release of N and NSC (soluble carbohydrates + starch) in the rumen could maximize rumen capture of soluble N. However, recent research suggested that the ratio between soluble N and rumen-fermentable energy, coming mostly from NSC, is a much more important determinant of ruminal N losses than synchrony in the rates of N and energy release (Dewhurst et al., 2000; Huhtanen and Shingfield, 2005).

It is generally recognized that there is genetic variation in the type and concentrations of NSC in grasses

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(McDonald et al., 1991). In the UK, grass breeders have produced ryegrass cultivars (Humphreys, 1989) with greater WSC concentrations (high-sugar grasses). These cultivars have increased the efficiency of N use by zero-grazed dairy cows (Miller et al., 2001), the BW gain of grazing lambs (Lee et al., 2001), and the DMI (Lee et al., 2002b), microbial N flows to the duodenum (Lee et al., 2002b), and efficiency of microbial protein synthesis in beef cattle (Merry et al., 2006). In vitro studies (Lee et al., 2002a; 2003; Merry et al., 2006) have also helped to determine the effects of increasing the supply of WSC to the rumen microbial population under controlled conditions. Generally, an increase in WSC concentration in perennial ryegrass available to rumen microbes has been shown to increase the ruminal glucogenic:lipogenic VFA ratio and to reduce ruminal ammonia concentrations (Lee et al., 2002a; 2003; Merry et al., 2006). These findings indicate the potential for improved animal productivity of high-WSC forages. This improvement is likely mediated through an increase in the efficiency of N and energy utilization as a result of reduced ruminal ammonia concentrations and changes in the type of energy-yielding substrates (e.g., VFA) absorbed from the rumen associated with high-WSC forages.

However, to our knowledge, little research has been conducted to improve the NSC concentration in alfalfa through breeding and management practices or to document the effect of increasing NSC concentration on the efficiency of microbial synthesis in the rumen. In this study, we used the inherent genetic variability and differences between harvests to evaluate the effect of alfalfa forage with highly contrasting NSC concentration on in vitro ruminal function parameters and efficiency of microbial protein synthesis measured in a continuous culture system.

MATERIALS AND METHODS

Plant Material

More than 500 genotypes of the alfalfa cultivar AC Caribou grown as spaced plants in a field site near Québec City, Québec, Canada (46°47'N, 71°07'W; elevation = 65 m) were individually harvested on September 13, 2004 at the late vegetative stage of development (Fick and Mueller, 1989). Within 15 min after cutting, forage samples were transferred to a large draft oven, dried at 55°C, and subsequently ground using a Wiley mill (standard model 3; Arthur H. Thomas Co., Philadelphia, PA) fitted with a 1-mm screen. Each sample was scanned using a monochromator NIRS instrument (Foss NIRSystems 6500, Silver Spring, MD). A subset of 115 samples was chemically analyzed for soluble car-

bohydrates (fructose + sucrose + glucose + pinitol) by HPLC (Brito et al., 2008) and for starch using a colorimetric method as described previously (Bertrand et al., 2003). Results from this subset were used to calibrate and validate an equation allowing the prediction of NSC concentration (soluble carbohydrates + starch) for all samples; the validation statistics were all acceptable ($n = 20$; $SD = 4.4\%$ DM; standard error of prediction corrected for the bias = 0.45% DM; $R^2 = 0.99$). The 50 genotypes with superior NSC and the 50 genotypes with inferior NSC were identified and harvested again on July 28, 2005 at the flowering stage of development, dried, and chemically analyzed in duplicate as described previously. The average NSC concentration was 13.5% DM in 2004 for the 50 genotypes with superior NSC and 8.4% DM in 2005 for the 50 genotypes with inferior NSC; we chose these 2 groups of genotypes to maximize the difference in NSC concentration. Ground material of genotypes having, respectively, the highest NSC concentrations in the 2004 harvest and the lowest NSC concentrations in the 2005 harvest was pooled to constitute 2 highly contrasting 1-kg samples. Among the 20 low-NSC genotypes that were pooled to constitute 1 of the 2 pooled samples, soluble carbohydrate concentration varied between 5.4 and 7.0% DM, starch concentration varied between 1.3 and 2.3% DM, and NSC concentration varied between 6.7 and 8.7% DM. Among the 23 high-NSC genotypes, soluble carbohydrate concentration varied between 9.5 and 12.8% DM, starch concentration varied between 5.1 and 10.4% DM, and NSC concentration varied between 15.5 and 23.0% DM. Subsequent chemical determination of carbohydrates in the 2 pooled samples confirmed their contrasted concentrations (17.9 vs. 7.4% DM for high- and low-NSC alfalfa, respectively; Table 1).

Experimental Design and Treatments

The experiment was conducted as a completely randomized design with 2 dietary treatments and 3 replications per treatment. The experimental period lasted 10 d, with the first 6 d serving as an adaptation period allowing equilibration of microbial populations followed by 4 d of sampling. The 2 experimental treatments were high- versus low-NSC alfalfa. The source of inoculum, feeding rate, and solid and liquid dilution rates were as described in Fraser et al. (2007).

Source of Inoculum

Mixed rumen contents were collected 2 h after the morning feeding from 4 ruminally fistulated, lactating Holstein dairy cows that were fed an early-lactation diet (16.7% CP, 34.4% NDF) consisting of 50% forage

Table 1. Chemical composition of alfalfa with high and low concentrations of NSC administered to the continuous culture fermenters¹

Item % of DM unless stated	High NSC	Low NSC
DM, %	94.1 ± 0.03 ¹	93.9 ± 0.02
OM	90.6 ± 0.07	90.2 ± 0.05
CP	22.4 ± 0.31	28.1 ± 0.18
NDF	25.8 ± 0.15	28.7 ± 0.34
ADF	22.0 ± 0.61	24.0 ± 0.42
Acid detergent lignin	7.0 ± 0.79	6.3 ± 0.45
Fructose	0.41 ± 0.019	0.30 ± 0.023
Sucrose	8.48 ± 0.095	3.74 ± 0.062
Glucose	0.71 ± 0.009	0.51 ± 0.098
Pinitol	2.13 ± 0.187	1.75 ± 0.103
Starch	6.15 ± 0.011	1.07 ± 0.008
NSC	17.87 ± 0.298	7.37 ± 0.158

¹Values are means ± SD.

and 50% concentrate on a DM basis (Table 2). Mixed rumen contents for use in the fermenters were squeezed through 4 layers of cheesecloth into an insulated thermos. Prior to their utilization for the incubation, the pooled rumen contents were analyzed to determine the natural abundance of ¹⁵N (i.e., background concentration). All procedures with the cows were performed in accordance with the guidelines of the Canadian Council on Animal Care (CCAC, 1993).

Continuous Culture System

A 6-unit, dual effluent system similar to that described by Hoover et al. (1976) was used in this study. Each fermenter had a 1.3-L nominal capacity. At the beginning, 350 mL of warmed McDougall's buffer (pH 8.2; McDougall, 1948) modified to contain 0.5 g/L of (NH₄)₂SO₄, 950 mL of strained rumen fluid, and 12 g of alfalfa (DM basis) were added to each fermenter. Thereafter, the fermenters were manually fed with 24 g (DM basis) of alfalfa per day, divided into 2 equal feedings at 0600 and 1800 h. Fermenters were maintained at a constant temperature of 39°C and were infused with a constant stream of CO₂ at a rate of 20 mL/min to maintain anaerobic conditions. Infusion of artificial saliva and flow of filtered liquid were set to maintain solid and liquid dilution rates of approximately 2.0 and 4.3%/h, respectively. Daily effluent was collected in a 4.0-L container immersed in an ice water bath (−4°C) to inhibit microbial growth.

Measurements

Alfalfa Digestibility. From d 7 to 10, daily effluent was homogenized and a 400-mL subsample was taken and centrifuged (20,000 × g, 30 min, 4°C). The resulting supernatant was discarded and the solid fraction (indigestible portion + microbial fraction) was harvested

and dried in a forced-air oven at 55°C for 48 h for the determination of DM, OM, NDF, ADF, and total N.

Fermentation End-Products. Fermenter pH was recorded at 2-h intervals daily using a pH meter (model 4505, Chemtrix Inc., Hillsboro, OR). On d 6 to 10, just before morning feeding, subsamples (4.0 mL) were taken from the fermenter liquid, preserved as described previously (Fraser et al., 2007), and stored at −20°C until analyzed for NH₃ and VFA concentrations.

Microbial Protein Synthesis. Microbial protein synthesis was estimated using ¹⁵N as a microbial marker. On d 1, McDougall's buffer solution was further modified by replacing the (NH₄)₂SO₄ with labeled (¹⁵NH₄)₂SO₄ (0.5 g/L; Sigma Chemical Co., St. Louis, MO; minimum ¹⁵N enrichment = 10.01 atom %). From d 7 to 10, the total effluents collected were weighed, homogenized, and subsampled. The first subsample was centrifuged (20,000 × g, 30 min, 4°C) to isolate residual (microbial + undigested feed) N (RN). A second subsample was centrifuged at low speed (500 × g, 10 min, 4°C) to remove feed particles. A 60-mL sample of the supernatant was centrifuged (20,000 × g, 30 min, 4°C) to isolate microbial cells (MN). The resulting pellets (RN and MN) were washed using deionized water and centrifuged 3 times (20,000 × g, 30 min, 4°C), resuspended in distilled water, frozen at −20°C until they were lyophilized, weighed for DM determination, and ground with a ball mill (Mixer Mill MM2000, Retsch, Haan, Germany) to a fine powder. Samples of RN and

Table 2. Ingredients, DM, and chemical composition of the early-lactation diet fed to ruminal contents donor cows

Item, % of DM unless stated	Value
Ingredient	
Barley silage	30.6
Alfalfa hay	9.8
Barley grain, steam-rolled	47.5
Corn gluten meal	3.36
Canola meal	2.91
Soybean meal	2.91
Beet molasses	0.49
Canola oil	0.90
Vitamin–mineral mix	0.72
Calcium carbonate	0.67
Dicalcium phosphorus	0.36
Monosodium phosphate	0.04
Binding agent ¹	0.15
DM and chemical composition	
DM	39.0
OM	92.7
CP	16.3
NDF	33.5
ADF	18.6
Acid detergent lignin, % of NDF	12.9
Starch	45.5
NE _L , Mcal/kg	1.60

¹Aka, Bear River Zeolite of Canada Corp., Lethbridge, Alberta, Canada.

MN pellets were pooled across sampling days and analyzed for total N and ^{15}N concentrations.

Chemical Analyses

Analytical DM concentration of the diets and effluent feed residue from the fermenters were determined by drying at 135°C for 2 h (AOAC, 1990). Ash concentration was determined by combustion at 550°C overnight, and OM was subsequently calculated as 100 minus the percentage of ash (AOAC, 1990). The NDF and ADF concentrations were determined according to Van Soest et al. (1991). Heat-stable α -amylase and sodium sulfite were used in the NDF procedure. Acid detergent lignin was determined as outlined in AOAC (1990; method 973.18). Total concentration in alfalfa samples, effluent feed residues, and undigested feed residues were determined by dry combustion (Model NA 1500, Carlo Erba Instruments, Rodano, MI, Italy). Crude protein was calculated as $\text{N} \times 6.25$.

Concentration of VFA was determined by GLC (Hewlett Packard Model 5890, Agilent Technologies, Santa Clara, CA) equipped with a 30-m (0.32-mm i.d.) Zebron FFAP column (Phenomenex, Florence, CA). The concentration of $\text{NH}_3\text{-N}$ was determined using the phenol-hypochlorite procedure (Weatherburn, 1967) adapted for a Technicon Autoanalyzer II. Total N and ^{15}N concentrations of RN and MN were determined by flash combustion using a Model NA 1500 nitrogen analyzer (Carlo Erba Instruments, Rodano, Milan, Italy) and isotope ratio mass spectrometry (VG Isotech, Middlewich, UK), respectively.

Calculations

Yield of MN (milligrams/day) was estimated using the equation

$$\text{MN} = (\text{APE in RN} / \text{APE in MN}) \times \text{RN},$$

where APE (atom percent excess) in RN is the percentage excess of ^{15}N in the microbial plus undigested feed residue, APE in MN is the percentage excess of ^{15}N in the microbial fraction of the effluent, and RN is the total N in the microbial plus undigested feed residue (milligrams; Wang et al., 2000).

Statistical Analyses

Data were analyzed as repeated measures using the MIXED procedure of SAS (SAS Institute, 2006). Means were compared using the least squares means linear hypothesis test with treatments (high- vs. low-NSC alfalfa), days of sampling, and their interaction included

as fixed effects, and fermenter nested within (treatment $\times$ day of sampling) as a random effect. Significance was declared at $P < 0.05$.

RESULTS AND DISCUSSION

Chemical Composition of Alfalfa

The high- and low-NSC alfalfa had NSC concentrations of 17.9 and 7.4%, NDF concentrations of 40.1 and 41.6%, and CP concentrations of 22.4 and 28.1% of DM, respectively (Table 1). Concentrations of all measured NSC (fructose, sucrose, glucose, pinitol, and starch) were higher in high- than in low-NSC alfalfa. Concentrations of starch (6.2 vs. 1.1% of DM) and sucrose (8.5 vs. 3.7% of DM) contributed the most to the differences in NSC concentration between high- and low-NSC alfalfa, respectively. Such changes could be nutritionally beneficial to ruminants; it was previously shown that supplementation of forage grasses with starch (Bach et al., 1999) or an 80:20 inulin:sucrose solution (Lee et al., 2003) reduced $\text{NH}_3\text{-N}$ concentration in the rumen. Increased concentrations of soluble carbohydrates and starch in high- versus low-NSC alfalfa were associated with lower concentrations of NDF, ADF, and CP. Burns et al. (2007) reported that the accumulation of NSC in alfalfa cut at sundown was generally associated with a reduction in the concentrations of NDF and its constituent fiber fractions, whereas Humphreys (1989) reported a negative genetic correlation between WSC and CP in perennial ryegrass. Our study highlights the extent of the variability of NSC concentration in alfalfa because of its inherent genetic variability and differences between harvests; the latter can be partly related to an effect of the stage of development. The innovative approach of pooling genotypes from both ends, in terms of NSC concentration, of an alfalfa population allowed us to show the potential benefits that could result from breeding or management practices used to increase NSC concentration in forage legumes.

Nutrient Digestion

Apparent digestibility of DM and OM and true digestibility of DM were greater ($P < 0.05$) in the high- than in the low-NSC alfalfa, whereas apparent digestibility of NDF and ADF was numerically higher but not significantly different (Table 3). Values for ADF and NDF apparent digestibility observed in the present study are similar to those reported by Orozco-Hernandez et al. (1997) in alfalfa harvested at the same stage of development. We have recently published the results of an in vivo study showing that, in baleage made from the same alfalfa cultivar harvested at sundown compared

Table 3. Effects of high and low concentrations of NSC in alfalfa (treatment) and sampling day on digestibility of DM, OM, and fiber in the dual-flow continuous culture system

Item	Treatment		SED ¹	P-value		
	High NSC	Low NSC		Treatment	Sampling day	Treatment × day
True digestibility, %						
DM	74.1	64.7	2.09	0.002	0.89	0.82
Apparent digestibility, %						
DM	60.0	54.3	2.08	0.02	0.79	0.99
OM	59.1	54.4	1.99	0.04	0.77	0.99
NDF	58.5	56.3	2.58	0.42	0.91	0.99
ADF	54.8	52.1	3.40	0.47	0.83	0.98

¹SED = standard error of the differences of means.

with sunup, the apparent digestibility of OM in the sundown-cut alfalfa increased concomitantly to an increase of 2.3 percentage units in NSC concentration (Brito et al., 2008). Our results, both in vitro and in vivo, confirm that an increase in NSC concentration of at least 2.3 percentage units improves alfalfa DM digestibility, mostly through a reduction in NDF concentration. Similar to our previous in vivo findings (Brito et al., 2008), ruminal digestion of NSC was deemed almost complete in the conditions of the present study.

Ruminal Environment

As a consequence of the increased DM digestibility (Table 3), ruminal total VFA concentration was higher ($P = 0.04$) and ruminal pH was lower ($P = 0.02$) for the high- than for the low-NSC alfalfa (Table 4). Molar proportions of acetate, isobutyrate, and isovalerate were lower ($P \leq 0.007$) for the high- compared with the low-NSC alfalfa, whereas the proportions of propionate and butyrate were higher ($P \leq 0.006$). Consequently, the acetate:propionate ratio was lower for the high-NSC alfalfa. Bach et al. (1999) reported that supplementing a lush pasture with cracked corn, a source of starch, resulted in higher molar proportions of propionate, butyrate, and branched-chain VFA, lower molar proportions of acetate, and a lower acetate:propionate ratio. The increased proportion of butyrate is consistent with the results presented by Lee et al. (2002b) for steers offered perennial ryegrass selected for higher WSC concentration. Water-soluble carbohydrates have been shown to increase either acetate or butyrate (Huhtanen, 1998). Although the reasons for different fermentation patterns of WSC are not clear, it may be related to methane production because methanogenesis is a prerequisite to acetate but not to butyrate (Demeyer and Van Nevel, 1975). Branched-chain VFA (isobutyrate and isovalerate) are derived primarily from

dietary proteins (Gorosito et al., 1985). Therefore, the lower CP concentration of the high-NSC alfalfa might explain why isobutyrate and isovalerate molar proportions were reduced. Ribeiro et al. (2005) also showed that branched-chain VFA molar proportions linearly decreased with the addition of sucrose to alfalfa hay and concluded that changes in branched-chain VFA were related to the decrease in N digestibility associated with sucrose addition. We reported the same phenomenon (Berthiaume et al., 2006) in an in vitro study comparing alfalfa harvested after 0 versus 12 h of daylight. However, we observed an increase in N truly digested in the rumen when cows were fed alfalfa mowed at sundown compared with alfalfa mowed at sunup (Brito et al., 2009). This apparent discrepancy may have to do with the longer wilting time associated with sundown cutting and harvesting. Papadopoulos and McKersie (1983) showed that wilted alfalfa suffered less proteolysis.

N Metabolism

Concentration of $\text{NH}_3\text{-N}$ ($P = 0.02$) in the daily effluent was lower with high- compared with low-NSC alfalfa (Table 4). Considering the greater N intake with the low-NSC alfalfa (987 mg/d) than the high-NSC alfalfa (795 mg/d) as a result of the unexpectedly large difference in CP concentration between the 2 treatments (28.1 and 22.4% DM for low- and high-NSC alfalfa, respectively), all the N flow data were reported relative to N ingested. True N flow, as a proportion of N input (dietary + infused), increased ($P = 0.007$) with the high- compared with the low-NSC alfalfa (Table 5). More importantly, the MN flow increased ($P < 0.001$) with the high-NSC alfalfa, supporting our hypothesis that high NSC concentration in alfalfa would increase microbial protein synthesis. Brito et al. (2009) also observed an increase in microbial NAN flow at

Table 4. Effect of high and low concentrations of NSC in alfalfa (treatment) and sampling day on ruminal pH and concentrations of VFA and ammonia measured in the dual-flow continuous culture system

Item	Treatment			P-value		
	High NSC	Low NSC	SED ¹	Treatment	Sampling day	Treatment × day
Ruminal pH	6.85	7.08	0.057	0.02	0.26	0.90
Total VFA, mmol/L	94.9	83.0	5.13	0.04	0.04	0.72
VFA, mol/100 mol						
Acetate	61.4	65.2	1.24	0.007	0.27	0.91
Propionate	20.3	18.4	0.57	0.006	0.52	0.98
Butyrate	11.6	9.0	0.55	0.002	0.58	0.88
Isobutyrate	1.6	1.9	0.06	<0.001	0.81	0.59
Valerate	2.7	2.4	0.17	0.23	0.30	0.92
Isovalerate	2.0	2.6	0.14	<0.001	0.25	0.86
Caproate	0.5	0.5	0.06	0.60	0.37	0.99
Acetate:propionate	3.03	3.59	0.167	0.006	0.41	0.97
NH ₃ -N, mg/L	260	336	25.0	0.02	0.76	0.94

¹SED = standard error of the differences of means.

the omasum in alfalfa cut at sundown compared with that cut at sunup. Interestingly, supplementation of lush pasture with starch (Bach et al., 1999) increased bacterial N flow, as observed in the present study (Table 5), whereas infusion with incremental doses of an inulin:sucrose mixture (Lee et al., 2003) decreased bacterial N flow measured in vitro, suggesting that the type of carbohydrate provided is important. The current treatments had no effect on the flow of N from dietary origin expressed as a percentage of N input (27.8 and 28.0% for high- and low-NSC alfalfa, respectively; Table 5). This suggests that the reported increases in DM and OM digestibilities are the result of an increase in the concentration of the NSC fraction that is deemed completely digested.

Microbial Efficiency

The efficiency of microbial protein synthesis (expressed as grams of bacterial-N per kilogram of OM apparently digested in the rumen) was not affected by treatments (Table 5). Higher bacterial efficiencies have sometimes been associated with both higher (Hussein et al., 1991) and lower (Bach et al., 1999) bacterial yields. The ultimate goal of ruminant nutrition should be to maximize microbial growth and the amount of RDP that is captured into rumen microbial cells, not only to improve the supply of AA to the small intestine but also to decrease N losses. Therefore, Bach et al. (2005) suggested that efficiency of microbial protein synthesis is not a good estimator of the efficiency of available N

Table 5. Effects of high and low concentrations of NSC in alfalfa (treatment) and sampling day on N degradation and efficiency of microbial protein synthesis in the dual-flow continuous culture system

Item	Treatment			P-value		
	High NSC	Low NSC	SED ¹	Treatment	Sampling day	Treatment × day
Dietary N, mg/d	795	987				
N infused, mg/d	91	95				
N flow, mg/d						
Total	737	831	30.8	0.02	0.56	0.95
Ammonia	226	299	20.4	0.01	0.68	0.90
True	510	532	24.0	0.38	0.73	0.99
Microbial	263	230	9.5	0.005	0.49	0.97
Dietary	247	302	15.4	0.005	0.89	0.98
N flow, % of N input						
True	57.5	49.2	2.42	0.007	0.73	0.99
Microbial	29.7	21.3	0.95	<0.001	0.45	0.93
Dietary	27.8	28.0	1.56	0.94	0.91	0.99
EMPS ²	22.6	21.6	1.65	0.57	0.63	0.99
Bacterial-N/feed N, %	33.1	23.3	1.10	<0.001	0.50	0.94
Bacterial-N/available N, ³ %	41.1	29.6	2.19	0.007	0.68	0.98

¹SED = standard error of the differences of means.

²EMPS = efficiency of microbial protein synthesis (grams of bacterial-N per kilogram of OM apparently digested in the rumen).

³N intake – undegraded-N (Bach et al., 1999).

capture by the rumen microbes. They proposed calculating the efficiency of N use by ruminal microbes as grams of bacterial N per gram of available N, where available N is calculated as N input (dietary + artificial saliva) minus undegraded N. Using this expression of efficiency, high-NSC alfalfa resulted in a greater ($P = 0.007$) efficiency of N utilization by bacteria. This is consistent with the lower accumulation of $\text{NH}_3\text{-N}$ in the rumen with high- compared with low-NSC alfalfa. When expressed as grams of bacterial N per 100 grams of feed N, the N in high-NSC alfalfa was utilized more efficiently by rumen bacteria ($P < 0.001$) than the N in the low-NSC alfalfa. However, even with the high-NSC alfalfa, N use efficiency reported in the present study (33.1%) was similar to that reported by Bach et al. (1999) with a grass-legume pasture diet (32.8%) but lower than that reported by others (Merry et al., 2006) with red clover (54%).

CONCLUSIONS

Using the inherent genetic variability and differences between harvests, we observed that a group of alfalfa genotypes with a high NSC concentration (17.9% DM for high-NSC alfalfa vs. 7.4% DM for low-NSC alfalfa) was more digestible and resulted in a decrease in ruminal pH, an increase in total ruminal VFA concentration, and a shift toward a more glucogenic fermentation in an in vitro continuous culture system. The observed reduction in rumen ammonia and improvements in MN flow (263 vs. 230 mg/d) and bacterial utilization of available N (41.1 vs. 29.6%) with high versus low NSC alfalfa confirm the possibility of improving the supply of microbial N to the small intestine and of decreasing N losses in forage-fed ruminants by feeding alfalfa with a high NSC concentration. Our in vitro study provides evidence that increased NSC concentration in alfalfa enhances MN synthesis in the rumen, but in vivo studies are needed to confirm these results with dairy cows fed at greater feeding and passage rates.

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