Performance, digestion, nitrogen balance, and emission of manure ammonia, enteric methane, and carbon dioxide in lactating cows fed diets with varying alfalfa silage-to-corn silage ratios

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ABSTRACT

Two trials were conducted simultaneously to study the effects of varying alfalfa silage (AS) to corn silage (CS) ratio in diets formulated to avoid excess protein or starch on lactating dairy cow performance, digestibility, ruminal parameters, N balance, manure production and composition, and gaseous emissions [carbon dioxide (CO₂), methane (CH₄), and ammonia-N (NH₃-N)]. In trial 1 all measurements, except gas emissions, were conducted on 8 rumen-cannulated cows in replicated 4 × 4 Latin squares. In trial 2, performance and emissions were measured on 16 cows randomly assigned to 1 of 4 air-flow controlled chambers in a 4 × 4 Latin square. Dietary treatments were fed as total mixed rations with forage-to-concentrate ratio of 55:45 [dietary dry matter (DM) basis] and AS:CS ratios of 20:80, 40:60, 60:40, and 80:20 (forage DM basis). Measurements were conducted the last 3 d of each 21-d period. Treatments did not affect DM intake, DM digestibility, and milk/DM intake. However, responses were quadratic for fat-and-protein-corrected milk, fat, and protein production, which reached predicted maxima for AS:CS ratio of 50:50, 49:51, and 34:66, respectively. Nitrogen use efficiency (milk N/N intake) decreased from 31 to 24 g/100 g as AS:CS ratio increased from 20:80 to 80:20. Treatments did not alter NH₃-N/milk-N but tended to have a quadratic effect on daily NH₃-N emission. Treatments had a quadratic effect on daily CH₄ emission, which was high compared with current literature; they influenced CH₄ emission per unit of neutral detergent fiber (NDF) intake and tended to influence CO₂/NDF intake. Ruminal acetate-to-propionate ratio and total-tract NDF digestibility increased linearly with increasing AS:CS ratio. In addition, as AS:CS ratio increased from 20:80 to 80:20, NDF digested increased linearly from 2.16 to 3.24 kg/d, but CH₄/digested NDF decreased linearly from 270 to 190 g/kg. These 2 counterbalancing effects

likely contributed to the observed quadratic response in daily CH₄ emission, which may have been influenced also by increasing starch with increasing CS in the diet as reflected by the increased ruminal propionate molar proportion. Overall, production performances were greatest for the intermediate AS:CS ratios (40:60 and 60:40), but daily excretion of urine, manure, fecal N, urinary urea N, and urinary N decreased with increasing proportion of CS in the diet, whereas daily CH₄ emission was reduced for the 2 extreme AS:CS ratios (20:80 and 80:20). However, the proportion of AS and CS in the diet did not affect CH₄/fat-and-protein corrected milk.

Key words: greenhouse gas, rumen fermentation, nitrogen utilization, forage, soybean

INTRODUCTION

The 2 main forages fed to dairy cows in the Midwest of the United States are alfalfa silage (AS) and corn silage (CS). Compared with CS, AS has greater CP and NPN (NRC, 2001). Thus, feeding increasing AS:CS ratios in the forage portion of a diet often leads to increased dietary CP and NPN, which has been associated with lower N utilization efficiency (NUE; g of milk N/100 g of N intake) and greater manure N excretion (Wattiaux and Karg, 2004; Brito and Broderick, 2006), and consequently greater manure NH₃-N emission (Powell et al., 2008; Aguerre et al., 2012) and indirect nitrous oxide (a potent greenhouse gas) emission from the manure (Dijkstra et al., 2013). However, reducing dietary CP (James et al., 1999) and RDP (Monteny et al., 2002) may mitigate NH₃-N emission. In this study we hypothesized that formulating diets with increasing proportion of AS would not alter NH₃-N emission, if CP, RDP, and RUP remained within the general guidelines of the NRC (2001).

Increasing the proportion of AS relative to CS in the diet alters forage NDF composition substantially because at similar NDF content, AS NDF has greater lignin and lower hemicellulose content than CS NDF (NRC, 2001). Similarly, fractional rate of NDF diges-

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tion is likely to be greater because fractional rate of NDF digestion is greater for alfalfa compared with CS (Herrick et al., 2012). Recent findings indicated that the amount of NDF digested in the digestive tract of lactating dairy cows increased with increasing AS:CS ratio (Brito and Broderick, 2006; Spanghero and Zanfi, 2009). More NDF digestion may result in more methane (CH_4) and carbon dioxide (CO_2) emission because these greenhouse gases are produced during enteric fermentation of fiber. Although, Doreau et al. (2014) reported lower daily CH_4 emission with CS compared with grass silage in the diet, the difference waned when expressed per kilogram of OM digested. Changes in NDF composition associated with plant maturity (Boadi and Wittenberg, 2002) and plant species (Chaves et al., 2006) have been shown to alter enteric CH_4 emission under grazing conditions. However, the hypothesis that the difference in NDF composition between AS and CS affects CH_4 emission remain to be fully explored. Hassanat et al. (2013) reported no change in daily CH_4 emission and CH_4 /FCM when replacing AS with CS; however, dietary ether extract (EE), which is known to have an inhibitory effect on CH_4 emission (Mores et al., 2013), decreased substantially as the proportion of CS increased in the diet. Thus our main objectives were to study the effects of AS:CS ratio in the forage portion of diets balanced for RDP and RUP and a constant level of EE on lactating dairy cow performance; apparent total-tract digestibility; ruminal fermentation characteristics; N balance; manure production and composition; and emission of CH_4 , CO_2 , and $\text{NH}_3\text{-N}$.

MATERIALS AND METHODS

Cows and Experimental Design

The experiments were conducted with cows from the herd of the US Dairy Forage Research Center, at Prairie du Sac, Wisconsin. Cows were housed in tie stalls bedded with rubber mats and wheat straw (except in trial 2). Care and handling of the animals were conducted as outlined in the guidelines of the Research Animal and Resource Committee at the University of Wisconsin–Madison. Two trials were conducted simultaneously with the same experimental diets and basic experimental design: 4×4 Latin squares with 21-d periods and treatment sequences balanced for carryover effects. Trial 1 measured performance, rumen parameters, apparent digestibility, and N balance of 8 rumen-cannulated lactating Holstein cows. One Latin square included 4 primiparous cows of (means $\pm$ SD) 536 ± 41 kg of BW and 136 ± 4 DIM, whereas the other Latin square included 4 multiparous cows (683 ± 27 kg of BW and 37 ± 17 DIM) that were randomly assigned

to dietary treatments. Trial 2 measured performance and gaseous emission from 16 multiparous cows (653 ± 83 kg of BW and 85 ± 34 DIM). Cows were randomly assigned to 1 of 4 air-flow controlled chambers, and chambers were randomly assigned to 1 of 4 dietary treatment sequences.

Trials 1 and 2: Diets and Performance Measurements

Each of the 4 experimental dietary treatment had a forage-to-concentrate ratio of 55:45 (DM basis) but a different AS:CS ratio in the forage portion of the diet: 20:80, 40:60, 60:40, and 80:20 (DM basis; Table 1). Aside from AS and CS, few feed ingredients were used in constructing the treatments in an attempt to minimize confounding effects. Ingredient composition of the concentrate portion of the diet was adjusted to maintain NRC (2001) predicted RDP and RUP above 10 and 6% of dietary DM, respectively, and to avoid excess CP or starch in the diet. Thus, as AS increased and CS decreased, solvent soybean meal (SBM) was replaced with expeller SBM, and the proportion of ground corn grain increased at the expense of the soybean by-products (Table 1). Diets were offered to each cow individually as TMR once daily at 0800 h allowing for 10%orts (as-fed basis). Also, the as-fed proportions of concentrate mix, mineral and vitamin premix, and forages in the TMR were adjusted weekly based on forage DM analysis.

During the last 4 d of each period, daily samples of approximately 0.5 kg of AS, CS, corn grain, solvent SBM, expeller SBM, soy hulls, and feed refusals were collected and stored at -20°C . Individual feed ingredients were composited by period on an equal-weight basis (as fed), dried at 60°C (forced-air oven) for 48 h, and ground to pass a 1-mm Wiley mill screen (Arthur H. Thomas, Philadelphia, PA). Feed-refusal samples were composited by period according to daily as-fed refusal weights for each individual cow (trial 1) or chamber (trial 2). The composite samples were dried at 60°C for 48 h and ground to pass a 1-mm screen of a Wiley mill (Arthur H. Thomas). Ground feed samples and refusal samples were analyzed for total N (Leco FP-2000 Nitrogen Analyzer, Leco Instruments Inc., St. Joseph, MI), analytical DM at 100°C for 24 h, ash (AOAC International, 1996; method 942.05), and NDF using α -amylase (Sigma no. A3306; Sigma Chemical Co., St. Louis, MO) with sodium sulfite and corrected for ash according to Van Soest et al. (1991) and adapted for Ankom²⁰⁰ Fiber Analyzer (Ankom Technology, Fairport, NY), and ADF was analyzed using AOAC method 973.18 (AOAC International, 1996). Fatty acid content was determined with the procedure of Sukhija and Palmquist (1988), and starch was determined with

Table 1. Ingredients and chemical composition of dietary treatments

Item	AS:CS ratio, ¹ g/g in 100 g of forage DM			
	20:80	40:60	60:40	80:20
Ingredient, % of diet DM				
Alfalfa silage	11.0	22.0	33.0	44.0
Corn silage	44.0	33.0	22.0	11.0
Ground corn grain	20.9	24.2	27.6	30.9
Solvent soybean meal	16.6	11.1	5.5	—
Expeller soybean meal	—	2.2	4.4	6.6
Soy hulls	5.0	5.0	5.0	5.0
Mineral and vitamins ²	2.5	2.5	2.5	2.5
Laboratory measurements				
DM, % as fed	52.4	53.6	55.0	56.4
Ash, % of DM	4.5	5.2	5.8	6.4
CP, % of DM	16.6	17.0	17.5	18.0
NDF, % of DM	27.3	27.6	27.9	28.1
ADF, % of DM	18.8	19.8	20.9	21.9
Starch, % of DM	29.6	28.0	26.5	24.0
FA, % of DM	3.3	3.4	3.4	3.5
Gross energy, Mcal/kg of DM	4.36	4.36	4.36	4.37
Calculated composition				
Forage NDF, % of NDF	76.5	75.7	74.9	74.1
Forage NDF, % of DM	20.9	20.9	20.9	20.8
Alfalfa-silage NDF, % of DM	4.1	8.3	12.4	16.6
Corn-silage NDF, % of DM	16.8	12.6	8.4	4.2
ADL, ³ % of DM	2.3	2.9	3.4	4.0
Hemicellulose, ⁴ % of DM	8.5	7.7	7.0	6.2
Cellulose, ⁵ % of DM	16.5	16.9	17.5	17.9
Ether extract, ⁶ % of DM	4.3	4.4	4.4	4.5
NFC, ⁷ % of DM	47.4	45.9	44.4	42.9
RDP, ⁸ % of DM	10.0	10.6	11.2	11.9
RUP, ⁸ % of DM	6.5	6.4	6.3	6.1

¹Ratio of alfalfa silage (AS) to corn silage (CS) in the forage portion of a TMR containing 55% forage (DM basis).

²Premix composition: guaranteed minimum concentration (as-fed basis): Ca 14.7%, salt 10.3%, Na 13.9%, Mg 4.5%, K 0.4%, S 0.9%, Se 14 mg/kg, vitamin A 282,192 IU/kg, vitamin D 55,116 IU/kg, vitamin E 1,334 IU/kg, and monensin 440 g/t.

³Calculated based using NRC (2001) feed composition tables.

⁴Hemicellulose = NDF – ADF.

⁵Cellulose = ADF – ADL.

⁶Ether extract = FA + 1.

⁷NFC = 100 – (CP + NDF + ether extract + ash).

⁸Estimated using NRC (2001) with BW, DMI, and milk yield observed during the trial.

the procedure of Knudsen (1997). Nonfiber carbohydrates were calculated according to NRC (2001) using CP, NDF, ash, and FA + 1 to estimate EE. Dietary ingredients were pooled across periods into a single sample and analyzed for gross energy using Parr 6400 adiabatic bomb calorimeter (Parr Instrument Company, Moline, IL). The chemical composition of the diets was computed from laboratory measurements and proportion of feed ingredients in the TMR. In addition, NRC (2001) was used to predict selected dietary characteristics (EE, RUP, RDP, ADL, and cellulose).

Average DMI per cow (trial 1) and per chamber (trial 2) was computed for the last 4 d of each period based on daily records of TMR offered and refused for each cow and the 100°C DM contents of the computed TMR and analyzed feed refusals. Individual cow milk

production was recorded at each of 2 daily milking (0600 and 1700 h) during both trials and summarized per cow (trial 1) or per chamber (trial 2) for the last 4 d of each period. Milk samples from morning and evening milking were collected on d 19 and 20 of each period and analyzed for fat, true protein, lactose, and MUN by infrared analysis (AgSource Milk Analysis Laboratory, Menomonie, WI) with a Foss FT6000 (Foss North America Inc., Eden Prairie, MN), and SCC was determined with a Fossomatic 500 FC (Foss Food Technology Corp., Hillerød, Denmark). Average daily concentration and production of milk components were computed using morning and evening milk production as weighing factor. Milk production and concentrations of fat, true protein, and lactose were used to compute NE_L (NRC, 2001) and fat-and-protein-corrected milk

(IDF, 2010). Cow BW was recorded during 2 consecutive days at the end of each period, and the mean was used as BW for each period.

Trial 1: Collection of Feces and Urine and Rumen Sampling

Feces were collected daily for 3 consecutive days starting after the evening milking of d 18. Stainless steel pans ($1.23 \times 0.38 \times 0.076$ m) were placed in the gutter behind each cow, and once a day fecal materials were transferred into a large container and total weight was recorded before thorough mixing and sampling. Fecal samples (500 g) were stored at -20°C . Upon thawing, samples were dried at 60°C (forced-air oven) for 72 h; ground to pass a 1-mm Wiley mill screen (Arthur H. Thomas Co.); composited by daily excretion weight (on 60°C DM basis); and analyzed for 100°C DM, ash, total N, NDF, and ADF with the same methods as described above. Urines were collected using indwelling Foley catheters (24 French, 75-mL balloons) on the same 3 d as the fecal collection. Catheters emptied into large carboys with 500 to 700 mL of 50% H_2SO_4 (final pH <4). Each day urines were weighed, and 2-mL aliquots were diluted immediately with 10 mL of 0.072 N H_2SO_4 and stored at -20°C for later analysis of total N. Apparent digestibility of DM, OM, CP, NDF, ADF, and hemicellulose were calculated from daily intakes and fecal excretions. In the calculation, however, a factor of 1.099 was used to adjust the mass of feces and urines to an estimated 24-h excretion because the actual collection period lasted for approximately 22 h because of milking interruptions. On d 19 of each period at 0 (prefeeding), 6, 12, and 18 h after feeding, samples were collected from 5 locations in the rumen of each cow using a metal filter probe fitted with a plunger for manual suction. Samples were pooled immediately for pH determination using a calibrated portable pH meter (Twin pH-meter model B-213, Spectrum Technologies Inc., Plainfield, IL). Then, 2 samples of pooled rumen fluid were collected. The first sample was acidified with 50% H_2SO_4 and frozen until analysis for VFA by GLC (Perkin Elmer Autosystem, Norwalk, CT) as outlined in Bal et al. (2000), whereas the second sample was mixed with 50% trichloroacetic acid and frozen until analysis for $\text{NH}_3\text{-N}$ (Chaney and Marbach, 1962).

Trial 2: Gaseous Emissions

Emissions were measured from 4 airflow-controlled chambers, 2 of which were located on each side of the central alley of a modified tie-stall barn. Each chamber ($9.1 \times 6.0 \times 2.9$ m; 161 m^3 of air space) contained 4 tie-stalls and a floor-to-ceiling retractable curtain wall

behind the gutter. A retractable curtain separated also the adjacent chambers. One inlet supplied air to all 4 chambers. The inlet included an air duct with a fan, an airflow station, and a gas sampler. Each chamber had a separate outlet including an air duct with a fan, an airflow station, and a gas sampler. A 10% higher airflow from the outlet compared with the inlet was used to maintain negative pressure in the chambers to minimize risk of gas leakage before measurements. For more information on technical aspects of the chambers design, calibrations, and function, see Powell et al. (2007) and Aguerre et al. (2011). Gas-analyzer calibration was conducted routinely, air-flow measurements were checked regularly, and previous results indicated all chambers functioned similarly in regard to CH_4 emission (Arndt et al., 2011). Emissions were recorded during the last 3 d of each period. Recordings were initiated after lowering and sealing the curtains on d 18 at approximately 1800 h (after evening milking), discontinued at 0500 h the next morning for milking and TMR delivery, and resumed from approximately 0900 until 1600 h (before the next evening milking). This protocol was repeated until opening of the curtains for morning milking of d 21. Air samples were sequentially drawn from each of the 5 gas samplers (1 inlet and 4 outlets) using a computer-controlled sequencer (Mark 3 Intelligent Sampler; California Analytical Instruments, Orange, CA) and analyzed for NH_3 , CH_4 , CO_2 , and H_2O concentration (parts per million volume) with a photo-acoustic multigas monitor (Innova Model 1412; AirTech Instruments, Ballerup, Denmark). During periods 1, 3, and 4, air of each chamber and the inlet was sampled every 14 min and 11 s, and during period 2 each chamber and inlet was sampled every 5 min and 38 s. Four airflow stations in the 4 outlet ducts and one in the inlet duct measured airflows every minute. Further technical description can be found in Aguerre et al. (2011). Grams of gases emitted during each sampling interval was calculated as the product of concentration in the outlet air (g/m^3) corrected for concentration in the inlet air (g/m^3) and the volumetric air flow (m^3). The first 30 min of data collected after curtains were lowered and sealed were omitted, leading to an average of 1,122 min (18 h and 42 min), which were multiplied by a factor of 1.283 to derive daily emission (g/d).

Statistical Analysis

For both trials, data were analyzed using the MIXED procedure of SAS (SAS Institute, 2004). Performance data including DMI, $\text{DMI}/\text{BW}^{0.75}$, NDF intake/BW, concentration of milk components, and production of milk, milk components, and NE_L , as well as milk/DMI for trial 1 and trial 2 were combined because they were

obtained from individual cows fed the same experimental diets simultaneously in both trials, and preliminary analysis indicated no square effects and no treatment-by-square effects. For each period, a single average value expressed on a per-cow basis was obtained for each experimental unit (cow in trial 1 and chamber in trial 2). Thus a weighing factor of 4 was applied to data of trial 2 because of the presence of 4 cows in each chamber. These data were analyzed with the following model:

$$Y_{ijkl} = \mu + S_i + C_{j(i)} + P_k + T_l + S_i \times T_l + e_{ijkl}$$

where Y_{ijkl} is the dependent variable, μ the overall mean, S_i is the fixed effect of square ($i = 1$ to 3), $C_{j(i)}$ the random effect of experimental unit j (cow in trial 1 and chamber in trial 2) within square i ($j = 1$ to 4), P_k is the fixed effect of period ($k = 1$ to 4), T_l is the fixed effect of treatment ($l = 1$ to 4), $S_i \times T_l$ is the interaction between square i and treatment l , which was retained for $P \leq 0.25$ only, and e_{ijkl} the residual error assumed to be independent and normally distributed. Milk/DMI, milk fat and lactose concentrations, SCC, and MUN were not normally distributed. The SCC data were analyzed using natural logarithm, whereas the other not normally distributed data were analyzed using ranks. The square-by-treatment interaction term was retained for milk fat concentration ($P = 0.25$) and milk fat production ($P = 0.23$).

Digestibility and rumen parameters were averaged in a single value per cow and period and analyzed with the same model as described above, except that the square effect (S_i) applied only to the rumen-cannulated cows of trial 1 (with $i = 1$ to 2, primiparous vs. multiparous cows). In this case, the interaction term square by treatment was retained for percentage digestibility of hemicellulose ($P = 0.07$), intake of digestible hemicellulose ($P = 0.22$), and fecal N excretion ($P = 0.22$).

Gas emission data of trial 2 were analyzed with chambers as experimental unit, but for convenience of data interpretation, all results were reported on a per-cow basis after dividing chamber-level data by 4 (the number of cows in each chamber). For each response variable the measurements obtained between d 18 and 21 were averaged in a single value per period and per chamber. Data were analyzed with the following model:

$$Y_{ikl} = \mu + C_i + P_k + T_l + e_{ikl}$$

where Y_{ikl} is the dependent variable, μ is the overall mean, C_i is random effect of chamber effect ($i = 1$ to 4), and P_k , T_l , and e_{ikl} are as described above.

Tendency was declared for $0.05 < P \leq 0.10$. When significance was declared ($P \leq 0.05$), treatment means

were separated using protected least significant difference. In addition, preplanned orthogonal contrasts were used to test for linear and quadratic effects. When a quadratic effect was declared, the response variable was regressed on AS:CS ratio to obtain regression-equation coefficients, which were used to predict maximal response and corresponding dietary AS:CS ratio.

RESULTS AND DISCUSSION

Diet Composition and Cow Performance

Compared with CS, AS had greater CP (24.4 vs. 6.1%; DM basis), almost identical NDF (37.7 vs. 38.2%), more ADF (34.4 vs. 24.3%), lower hemicellulose (3.3 vs. 13.9%), and much lower starch (0.81 vs. 35.0%) and NFC (23.7 vs. 49.6%). Dietary treatments were formulated to keep dietary CP, RDP, and RUP within the general guidelines of the NRC (2001) as the proportion of AS increased in the diet, and to keep dietary starch from becoming excessive as the proportion of CS increased in the diet. As indicated in Table 1, dietary treatments varied in narrow ranges for CP, RDP, RUP, EE, NDF, and gross energy. However, with every 20% stepwise change in AS:CS ratio, 4.2% of CS NDF was substituted for 4.1% of AS NDF, leading to essentially no change in forage NDF content but a 1%-unit change in ADF content. Dietary starch changed with the AS:CS ratio in the diet as in other research focused on the effects of dietary proportions of AS and CS (Krause and Combs, 2003; Brito and Broderick, 2006; Hassanat et al., 2013). In this trial, there was a 1.6%-unit change in dietary starch for every 20% stepwise change in AS:CS ratio.

Dry matter intake (24.6 ± 3.4 kg/d) and DMI per unit of metabolic BW did not differ among treatments. However, NDF intake as a percentage of BW increased linearly from 1.03 to 1.09 as dietary AS increased and CS decreased in the diet (Table 2). These values were slightly lower than the maximum NDF intake capacity of 1.1 to 1.2% of BW suggested by Mertens (1987). No treatment effects were detected for milk yield (37.8 ± 4.4 kg/d), but there was a quadratic response for milk NE_L production and fat-and-protein-corrected milk production with predicted maximum of 26.8 Mcal/d and 36.5 kg/d when AS:CS ratio 49:51 and 50:50, respectively. Treatments did not influence milk fat content ($3.60 \pm 0.36\%$) but milk fat production was lower in the 2 extreme treatments (20:80 and 80:20 AS:CS) than in the 2 intermediate treatments (40:60 and 60:40 AS:CS). Predicted maximum fat production was 1.40 kg/d when AS:CS ratio was 49:51. Brito and Broderick (2006) and Hassanat et al. (2013) found linear decreases in both fat content and fat yield with

increasing proportion of CS at the expense of AS in the diet. These authors interpreted these results as an effect associated with increasing starch leading to low ruminal pH, incomplete ruminal biohydrogenation of FA with negative consequence on de novo synthesis of milk fatty acids in the udder (Bauman and Griinari, 2001). However, in the present study treatment had no effects on ruminal pH (see below), and milk fat yield only numerically declined with the highest level of CS in the diet (AS:CS 20:80, Table 2). Interestingly, the reduction in milk fat production observed here for the other extreme treatment (AS:CS 80:20) was not observed by Brito and Broderick (2006) and Hassanat et al. (2013). Change in milk protein content was linear but change in protein production was both linear and quadratic (with a predicted maximum at 1.15 kg/d when AS:CS was 34:66). Increasing dietary starch by altering the proportion of corn grain (Ferraretto et al., 2011), the proportions of corn grain and CS (Oba and Allen, 2003a), or the proportion of CS alone (Hassanat et al., 2013) has been associated with greater milk protein content and production. Starch had a stimulating effect on microbial protein synthesis and flow from the rumen (Oba and Allen, 2003b; Jenkins and McGuire, 2006). Also, postruminal supply of starch increased arterial glucose concentration and blood flow to the mammary gland and influenced the metabolic pathways involved

in casein synthesis (Rius et al., 2010). Data of Table 2 suggested that these possible mechanisms may have exerted their effects starting when starch level was above 25% of dietary DM (i.e., above AS:CS of 80:20) but tapered off when approaching 30% of dietary DM (i.e., when AS:CS was 20:80). In our study, MUN increased linearly with increasing AS and decreasing CS in the diet, but neither Brito and Broderick (2006) nor Hassanat et al. (2013) reported such effect. Other authors have shown that MUN reflected excess N intake (Broderick and Clayton, 1997; Nousiainen et al., 2004) or an inefficient capture of N by rumen microbes for lack of energy (Hristov et al., 2005).

Apparent Digestibility (Trial 1)

For cows in trial 1, DMI (23.7 ± 3.73 kg/d) and OM intake (22.4 ± 3.50 kg/d) did not differ among treatments (Table 3). However, as AS increased and CS decreased in the forage portion of the diet, intake of CP, NDF, and ADF increased linearly, whereas the intake of hemicellulose decreased linearly. No effect of treatment was detected for apparent digestibility of DM, OM, and CP, which averaged 70.2 ± 3.27 , $72.6 \pm 3.06\%$, and $73.0 \pm 3.30\%$, respectively. These results agreed with Krause and Combs (2003) and Ruppert et al. (2003) but contrasted with those of Hassanat et al.

Table 2. Cow performance¹

Item	AS:CS ratio ² (g/g in 100 g of forage DM)				SEM ³	P-value	
	20:80	40:60	60:40	80:20		Linear	Quadratic
BW, kg	637	640	639	639	12.0	0.67	0.72
DMI, kg/d	24.0	24.7	25.0	24.8	0.61	0.21	0.34
DMI/BW ^{0.75} , kg/kg	0.190	0.194	0.197	0.195	0.004	0.20	0.36
NDF intake, % of BW	1.03	1.07	1.09	1.09	0.04	0.01	0.35
Milk production							
Milk, kg/d	37.7	38.3	37.9	37.3	0.97	0.46	0.14
Milk NE _L ⁴ , Mcal/d	25.9	26.8	26.6	25.8	0.78	0.69	0.01
FPCM ⁵ , kg/d	34.3	35.5	35.3	34.2	1.01	0.76	0.01
Milk/DMI, kg/kg	1.60	1.56	1.53	1.52	—	0.20	0.36
Milk composition							
Fat, %	3.49	3.61	3.67	3.62	—	0.26	0.14
Protein, %	3.04 ^a	3.00 ^{ab}	2.98 ^b	2.90 ^c	0.05	<0.01	0.16
Lactose, %	4.92	4.94	4.92	4.89	0.05	0.22	0.27
Milk NE _L , Mcal/kg	0.69	0.70	0.70	0.69	—	0.67	0.05
SCC, $\times 10^3$ cells/mL	94.1	78.2	100.3	122.9	0.01	0.22	0.31
MUN, mg/dL	14.3	14.9	15.2	15.4	—	0.02	0.44
Milk component yield							
Fat, kg/d	1.33	1.40	1.40	1.35	0.05	0.68	0.05
Protein, kg/d	1.15 ^a	1.16 ^a	1.13 ^a	1.08 ^b	0.03	<0.01	0.04
Lactose, kg/d	1.88	1.91	1.87	1.83	—	0.50	0.24

^{a-c}Least squares means within a row not sharing a common superscript differ ($P < 0.05$) based on protected LSD.

¹Cow performance for cows in trial 1 ($n = 8$) and trial 2 ($n = 4$).

²Ratio of alfalfa silage (AS) to corn silage (CS) in the forage portion of a TMR containing 55% forage (DM basis).

³When no SEM was reported, data were analyzed using ranks.

⁴Diet effect was declared a tendency ($0.05 < P \leq 0.10$).

⁵FPCM (fat-and-protein-corrected milk) = milk (kg/d) $\times$ $[0.1226 \times \text{fat} (\%) + 0.0776 \times \text{true protein} (\%) + 0.2534]$ (IDF, 2010).

Table 3. Intake and digestibility¹

Item	AS:CS ratio, ² g/g in 100 g of forage DM				SEM	P-value	
	20:80	40:60	60:40	80:20		Linear	Quadratic
Intake, kg/d							
DM	22.3	23.8	24.6	24.0	0.99	0.12	0.21
OM	21.3	22.7	23.2	22.5	0.93	0.25	0.21
CP	3.69 ^b	4.07 ^{ab}	4.30 ^a	4.32 ^a	0.17	<0.01	0.21
NDF ³	6.07	6.57	6.85	6.76	0.26	0.02	0.18
ADF	4.18 ^c	4.73 ^b	5.13 ^{ab}	5.27 ^a	0.20	<0.01	0.23
Hemicellulose	1.89 ^a	1.85 ^{ab}	1.71 ^b	1.48 ^c	0.07	<0.01	0.08
Apparent total-tract digestibility, %							
DM	68.7	69.9	71.2	70.9	1.23	0.11	0.53
OM	71.4	72.2	73.7	73.2	1.15	0.16	0.57
CP	73.4	72.7	73.2	72.8	1.23	0.74	0.92
NDF	35.5 ^b	43.2 ^a	47.3 ^a	48.1 ^a	2.31	<0.01	0.15
ADF	39.1 ^b	43.9 ^{ab}	48.1 ^a	47.5 ^a	1.87	<0.01	0.16
Hemicellulose	27.4 ^b	41.2 ^a	44.5 ^a	49.9 ^a	4.23	<0.01	0.32
Apparently digested, kg/d							
DM	15.4	16.7	17.5	17.1	0.8	0.11	0.29
OM	15.3	16.4	17.1	16.5	0.8	0.21	0.30
CP ³	2.71	2.96	3.15	3.15	0.14	0.02	0.35
NDF	2.16 ^b	2.83 ^a	3.24 ^a	3.27 ^a	0.22	<0.01	0.17
ADF	1.64 ^b	2.06 ^{ab}	2.47 ^a	2.50 ^a	0.16	<0.01	0.23
Hemicellulose	0.52	0.76	0.77	0.76	0.08	0.07	0.16

^{a-c}Least squares means within a row not sharing a common superscript differ ($P < 0.05$) based on protected LSD.

¹Intake and digestibility measured in trial 1 ($n = 8$).

²Ratio of alfalfa silage (AS) to corn silage (CS) in the forage portion of a TMR containing 55% forage (DM basis).

³Diet effect was declared a tendency ($0.05 < P \leq 0.10$).

(2013), who found that increasing AS at the expense of CS in the diet decreased digestibility of DM and OM but increased CP digestibility. As the AS:CS ratio in the diet increased from 20:80 to 80:20, the digestibility of NDF, ADF, and hemicellulose increased linearly from 35.5 to 48.1%, from 39.1 to 47.5%, and from 27.4 to 49.9%, respectively, which agreed with other reports (Ruppert et al., 2003; Brito and Broderick, 2006; Hassanat et al., 2013). These results indicated that NDF and ADF digestibility of AS was greater than for CS. Nevertheless, as indicated in Table 3, this effect was due to a lower digestibility of NDF and hemicellulose for the 20:80 AS:CS treatment compared with the other treatments. However, this drop was not likely associated with unfavorable rumen pH (see below). Extrapolations of data to AS:CS ratio of 100:0 and 0:100 led to estimates of NDF digestibility of 54 and 33% for diets that would contain AS or CS as a single forage source, respectively. Similar extrapolation for ADF yielded digestibility estimates of 52.0 and 37.3% for diets that would contain AS and CS as a single forage source, respectively. These estimates of AS NDF and CS NDF digestibility when included in a diet of 55:45 forage-to-concentrate ratio should be reliable because forage NDF and ADF made up more than 75% of the respective dietary totals.

Dietary treatments did not affect the amount of apparently digested DM and OM, which averaged 16.6

± 2.94 and 16.3 ± 2.80 kg/d, respectively. However as AS:CS ratio in the diet increased from 20:80 to 80:20, the amount of CP, NDF, and ADF apparently digested increased linearly from 2.71 to 3.15 kg/d, from 2.16 to 3.24 kg/d, and from 1.64 to 2.50 kg/d, respectively.

Rumen Parameters (Trial 1)

No effects of treatments were detected on rumen pH, which averaged 6.48 ± 0.13 . Unless a large amount of dietary buffers was included in the diet, as in the study by Brito and Broderick (2006), rumen pH generally dropped with more CS and less AS in the diet (Krause and Combs, 2003; Hassanat et al., 2013). Rumen stoichiometry associated with the change in acetate-to-propionate ratio shown in Table 4 should have resulted in a change in rumen pH. However more saliva production and buffering capacity may have resulted from replacing part of AS with CS because such substitution has increased hours of rumination time (Grant et al., 1995; Krause and Combs, 2003) and time spent chewing per kilogram of DMI (Krause and Combs, 2003). Dietary treatments did not affect total VFA concentration (102 ± 10.7 mM), but the molar proportion of acetate increased linearly and the molar proportion of propionate decreased linearly, resulting in a linear increase in acetate-to-propionate ratio as AS increased and CS decreased in the diet (Table 4). These

results agreed with Hassanat et al. (2013) and may be explained in part from the increase in the amount of digested NDF (Table 3) and the reduction in dietary starch supply (Table 1) with increasing AS:CS ratio in the diet. The molar proportion of butyrate (12.6 ± 1.94 mol/100 mol of VFA) and valerate (1.93 ± 0.69 mol/100 mol of VFA) was not affected by dietary treatments, but molar proportion of branched-chain VFA decreased linearly with increasing AS and decreasing CS in the diet. This net effect was associated with a linear decrease in 2-methyl-butyrate, a linear increase in isobutyrate, and a reduction in isovalerate for the 2 highest levels of AS compared with the 2 highest levels of CS in the diet (Table 4). These shifts may have reflected decreased rumen “true” protein degradation associated with increasing AS in the diet or the replacement of solvent SBM with expeller SBM (Table 1). Rumen $\text{NH}_3\text{-N}$ concentration increased linearly with increasing AS:CS ratio in the diet (Table 4) as reported by Brito and Broderick (2006) and Hassanat et al. (2013). This effect may have resulted from the combined effect of increased RDP and decreased NFC supply (Table 1), because Hristov et al. (2005) found a positive correlation between NFC supply and $\text{NH}_3\text{-N}$ utilization by rumen microbes.

Manure Excretion and Nitrogen Partitioning (Trial 1)

Both wet and dry fecal excretions were similar among treatments (50.0 ± 9.03 and 7.03 ± 1.15 kg/d, respectively), but daily manure excretion (wet feces plus urine) and urinary output increased linearly, whereas urinary N concentration and urinary urea-N concentration decreased linearly with increasing AS in the diet (Table 5). Fecal N concentration increased at an

increasing rate with every stepwise increase in AS and decrease in CS in the diet; both linear and concave quadratic effects were significant ($P < 0.01$ and $P = 0.04$, respectively; Table 5). Wattiaux and Karg (2004) also reported increased N concentration in feces and lower N concentration in urine when AS was the main forage source compared with CS. As in this trial, Brito and Broderick (2006) reported increasing urine output with increasing AS:CS ratio in the diet, which may have been the result of the higher potassium content of AS compared with CS. This increase in urine output was responsible for the 21% increase in manure output per unit of milk (Table 5).

Increasing AS and decreasing CS in the diet led to a linear increase in N intake, fecal N, and urinary N but a linear decrease in milk N (Table 6). The urinary N-to-fecal N ratio (1.30 ± 0.17 g/g) did not differ among dietary treatments. The same pattern of change in fecal N and urinary N was in the studies by Brito and Broderick (2006) and Hassanat et al. (2013); however, in the former study N intake increased and milk N remained unaffected, but in the latter study N intake remained unaffected and milk N decreased. Nitrogen use efficiency declined linearly with an increase in AS and a decrease in CS in the diet (Table 6), which may have been due in part to reduced CP, lower RDP combined with higher RUP, and higher dietary starch (see above and Table 1). For this study, linear regression showed the following relationship: $\text{NUE} = 21.3 + 0.200 \times \text{CS}$ ($R^2 = 0.96$), where CS is the percentage of CS in dietary DM, and therefore, in the range of 11 to 44% of dietary DM (Table 1), every 10-percentage-unit increase in CS at the expense of AS increased NUE by 2 percentage units. The data of Brito and Broderick (2006) for which CS ranged from 0 to 51% of dietary

Table 4. Rumen parameters¹

Item	AS:CS ratio, ² g/g in 100 g of forage DM				SEM	P-value	
	20:80	40:60	60:40	80:20		Linear	Quadratic
pH	6.50	6.46	6.47	6.48	0.05	0.74	0.36
$\text{NH}_3\text{-N}$, mg/dL	6.04 ^b	8.16 ^a	8.02 ^a	9.23 ^a	0.77	<0.01	0.49
Total VFA, mM	96.8	104.8	100.9	105.4	3.02	0.12	0.56
VFA, mol/100 mol							
Acetate (A)	58.4 ^c	60.2 ^{bc}	61.8 ^{ab}	62.5 ^a	0.33	<0.01	0.51
Propionate (P)	23.6 ^a	22.7 ^{ab}	21.5 ^{ab}	20.3 ^b	0.82	0.01	0.91
Butyrate	12.9	12.5	12.2	12.8	0.01	0.70	0.15
Valerate	2.18	1.83	1.78	1.91	0.98	0.30	0.20
Branched-chain VFA	2.90	2.86	2.73	2.66	0.10	0.05	0.86
2-Methylbutyrate	1.28 ^a	1.14 ^{ab}	1.08 ^b	0.93 ^c	0.29	<0.01	0.86
Isobutyrate	0.92 ^b	0.95 ^b	0.97 ^b	1.06 ^a	0.07	<0.01	0.40
Isovalerate	0.70 ^{ab}	0.76 ^a	0.67 ^b	0.67 ^b	0.21	0.11	0.20
A:P	2.55 ^c	2.71 ^{bc}	2.89 ^{ab}	3.11 ^a	0.17	<0.01	0.79

^{a-c}Least squares means within a row not sharing a common superscript differ ($P < 0.05$) based on protected LSD.

¹Rumen parameters measured in trial 1 ($n = 8$).

²Ratio of alfalfa silage (AS) to corn silage (CS) in the forage portion of a TMR containing 55% forage (DM basis).

Table 5. Manure excretion and composition¹

Item	AS:CS ratio, ² g/g in 100 g of forage DM				SEM	P-value	
	20:80	40:60	60:40	80:20		Linear	Quadratic
Manure as is, kg/d	68.9 ^b	77.5 ^a	78.8 ^a	81.6 ^a	3.9	<0.01	0.10
Feces as is, kg/d	48.6	51.7	50.2	49.5	2.8	0.83	0.12
Feces DM, % as is	14.3	14.0	14.1	14.1	0.24	0.58	0.10
Feces DM, kg/d	6.92	7.19	7.06	6.95	0.34	0.95	0.26
Feces N, % of DM	2.26 ^b	2.46 ^{ab}	2.59 ^{ab}	2.70 ^a	0.04	<0.01	0.04
Urine, kg/d	20.2 ^d	25.8 ^c	28.6 ^b	32.0 ^a	1.4	<0.01	0.11
Urine N, g/100 mL	0.98 ^b	0.89 ^{ab}	0.85 ^a	0.77 ^a	0.04	<0.01	0.75
Urine urea-N, ³ g/100 mL	0.71	0.64	0.64	0.58	0.03	0.01	0.78
Manure/milk, kg/kg	1.89 ^c	2.08 ^b	2.17 ^b	2.29 ^a	0.07	<0.01	0.29
Manure/FPCM, ⁴ kg/kg	2.73 ^c	2.97 ^b	3.03 ^{ab}	3.22 ^a	0.09	<0.01	0.79

^{a-d}Least squares means within a row not sharing a common superscript differ ($P < 0.05$) based on protected LSD.

¹Manure excretion and composition measured in trial 1 ($n = 8$).

²Ratio of alfalfa silage (AS) to corn silage (CS) in the forage portion of a TMR containing 55% forage (DM basis).

³Diet effect was declared a tendency ($0.05 < P \leq 0.10$).

⁴FPCM (fat-and-protein-corrected milk) = milk (kg/d) $\times$ [0.1226 $\times$ fat (%) + 0.0776 $\times$ true protein (%) + 0.2534] (IDF, 2010).

DM yielded the following regression: $\text{NUE} = 28.6 + 0.134 \times \text{CS}$ ($R^2 = 0.99$), whereas the data of Hassanat et al. (2013) yielded the following relationship: $\text{NUE} = 26.5 + 0.076 \times \text{CS}$ ($R^2 = 0.95$) when CS ranged from 0 to 56% of dietary DM. The varying intercepts among these regressions indicated that NUE when AS is the sole forage may vary substantially across studies. Although in all 3 studies confounding effects existed such as changes in levels of dietary CP and starch, replacing AS with CS in the diet consistently increased NUE.

Gaseous Emissions (Trial 2)

Data presented in Table 7 indicated that cows in trial 2 during which gas emissions were measured performed

substantially better than cows in trial 1. In trial 2, the lowest DMI and milk production averaged 26.3 and 40.3 kg/d, respectively.

Dietary treatments tended to have a quadratic effect on daily $\text{NH}_3\text{-N}$ emission (Table 7). The predicted maximum was 20.4 g/d at 52:48 AS:CS ratio. Average daily $\text{NH}_3\text{-N}$ emission of this study (15.7 g/d per cow) was similar to values reported by Powell et al. (2008) and other studies conducted in tie-stall barns (Jungbluth et al., 2001, reported values ranging from 6 to 21 g/d per cow; Bluteau et al., 2009, reported values ranging from 3.8 to 18.2 g/d per cow). These emission values were considerably lower than values reported for free-stall barn, which averaged 109 g/d per cow when alleys were scraped (Aguerre et al., 2010)

Table 6. Nitrogen partitioning¹

Item	AS:CS ratio, ² g/g in 100 g of forage DM				SEM	P-value	
	20:80	40:60	60:40	80:20		Linear	Quadratic
Daily values, g of N/d							
Intake	590 ^b	651 ^{ab}	689 ^a	692 ^a	26.9	<0.01	0.21
Fecal	156 ^b	177 ^a	184 ^a	188 ^a	11.0	<0.01	0.07
Urinary	197 ^b	229 ^a	241 ^a	248 ^a	12.5	<0.01	0.06
Milk	180 ^a	179 ^a	172 ^{ab}	166 ^b	6.3	<0.01	0.32
Unaccounted	57	65	81	89	23	0.20	0.82
Urinary urea	142 ^c	166 ^b	184 ^{ab}	189 ^a	11.6	<0.01	0.18
Manure ²	353 ^c	407 ^b	425 ^{ab}	437 ^a	23.4	<0.01	0.06
Ratios, g/100 g							
Urinary N/fecal N	1.27	1.29	1.32	1.32	0.05	0.44	0.85
NUE ³	30.7 ^c	27.6 ^b	25.1 ^{ab}	24.1 ^a	1.04	<0.01	0.27
Manure N/milk N	60.0	62.4	61.7	63.2	2.50	0.33	0.82

^{a-c}Least squares means within a row not sharing a common superscript differ ($P < 0.05$) based on protected LSD.

¹Nitrogen partitioning measured in trial 1 ($n = 8$).

²Ratio of alfalfa silage (AS) to corn silage (CS) in the forage portion of a TMR containing 55% forage (DM basis).

³Manure N = fecal N + urinary N.

⁴N use efficiency = milk N/N intake.

Table 7. Cow performances and gaseous emissions¹

Item	AS:CS ratio, ² g/g in 100 g of forage DM				SEM	P-value	
	20:80	40:60	60:40	80:20		Linear	Quadratic
Performance							
DMI, kg/d	26.3	26.5	26.6	26.5	0.69	0.68	0.80
N intake, g/d	698 ^c	723 ^{bc}	745 ^{ab}	764 ^a	18.4	<0.01	0.78
NDF intake, kg/d	7.17	7.30	7.40	7.47	0.18	0.05	0.76
GE intake, ³ Mcal/d	115	115	116	116	3.02	0.61	0.80
Milk, kg/d	40.3	41.0	41.0	40.4	1.52	0.92	0.25
Fat, %	3.72 ^b	3.84 ^a	3.87 ^a	3.80 ^{ab}	0.07	0.05	0.01
Protein, %	3.03 ^a	2.99 ^{ab}	2.96 ^b	2.87 ^c	0.02	<0.01	0.23
FPCM, ⁴ kg/d	37.8	39.0	39.1	37.9	1.42	0.96	0.04
Emissions ⁵							
NH ₃ -N, g/d	14.82	16.38	16.52	15.12	1.17	0.73	0.06
NH ₃ -N/N intake, g/100 g	2.16 ^{ab}	2.30 ^a	2.26 ^a	1.98 ^b	0.20	0.09	0.02
NH ₃ -N/milk N, g/kg	0.078	0.086	0.088	0.084	0.008	0.36	0.24
CH ₄ , g/d	697	743	729	683	23	0.53	0.05
CH ₄ /DMI, ⁶ g/kg	26.6	28.1	27.5	25.7	0.55	0.27	0.03
CH ₄ /NDF intake, g/kg	97.2 ^a	101.8 ^{ab}	98.6 ^{ab}	91.5 ^b	1.86	0.05	0.02
CH ₄ /GE intake, ⁶ Mcal/100 Mcal	8.04	8.50	8.31	7.78	0.17	0.24	0.03
CH ₄ /FPCM, g/kg	18.5	19.1	18.7	18.0	0.56	0.43	0.26
CO ₂ , g/d	21,934	22,279	22,014	21,204	724	0.33	0.31
CO ₂ /DMI, g/kg	835	841	831	799	18	0.19	0.34
CO ₂ /NDF intake, g/kg	3,061	3,051	2,981	2,838	71	0.06	0.39
CO ₂ /GE intake, g/Mcal	7.31	7.30	7.24	6.89	0.29	0.33	0.57
CO ₂ /FPCM, g/kg	584	573	566	560	21	0.23	0.87

^{a-c}Least squares means within a row not sharing a common superscript differ ($P < 0.05$) based on protected LSD.

¹Cow performances and gaseous emissions measured in trial 2 ($n = 4$).

²Ratio of alfalfa silage (AS) to corn silage (CS) in the forage portion of a TMR containing 55% forage (DM basis).

³GE intake = gross energy intake.

⁴FPCM (fat-and-protein-corrected milk) = milk (kg/d) $\times$ [0.1226 $\times$ fat (%) + 0.0776 $\times$ true protein (%) + 0.2534] (IDF, 2010).

⁵Emission data reported on a per-cow basis after dividing chamber-level data by 4 (the number of cows in each chamber).

⁶Diet effect was declared a tendency ($0.05 < P \leq 0.10$).

or 182 g/d per cow when alleys were flushed (Hollman et al., 2008). Swensson and Gustafsson (2002) found higher release of NH₃-N from free-stall barn with liquid manure than from tie-stall barn with solid manure. Factors explaining NH₃-N emission from diverse housing systems have been reviewed extensively (Rotz, 2004; Sommer et al., 2006). Although daily NH₃-N emission varied in a narrow range, the quadratic effect occurred in spite of a linear increase in urinary urea-N excretion across dietary treatments (Table 6). Dietary treatments tended ($P = 0.09$) to have a linear effect and did have a quadratic effect on NH₃-N/N intake (Table 7) with a predicted maximum of 2.31 g/100 g at 45:55 AS:CS ratio. The change in NH₃-N/N intake was more related to large differences in N intake (i.e., the numerator) than NH₃-N emission (i.e., the denominator).

Methane emission data (Table 7) were for the most part similar to values previously reported by our laboratory with cows at similar production levels (Aguerre et al., 2011). Daily CH₄ emission, however, was unexpectedly high and was more than 3 standard deviations above the mean reported in the meta-analysis of Ramin and Huhtanen (2013), but DMI was also unusu-

ally high: 2.9 standard deviations above the mean. Although undetected instrument error could not be ruled out to explain high daily CH₄ emission observed here, high level of DMI was likely a contributing factor. In contrast, CH₄/DMI and CH₄-energy/GE intake in this trial were each within 1.5 standard deviations of their respective mean in the aforementioned meta-analysis. Daily CH₄ emission, CH₄/DMI, CH₄/NDF intake, and CH₄-energy/GE intake showed quadratic responses to dietary treatments (Table 7) with predicted maximum of 742 g/d, 28.0 g/kg of DMI, 101 g/kg of NDF intake, and 8.48 Mcal/100 Mcal at 48:52, 46:54, 43:57, and 46:54 AS:CS ratio, respectively. Similar to our results, Hassanat et al. (2013) observed a quadratic response for CH₄ emissions, CH₄/DMI, and CH₄-energy/GE intake with increasing AS and decreasing CS in the forage portion of the diet. These authors suggested that dietary starch, which was higher with more CS in the diet, led to decreased rumen pH, protozoa, cellulolytic bacteria, and methanogens. However, in the present study rumen pH did not decrease with increasing dietary starch content as CS increased in the diet. In contrast, CH₄/NDF intake was influenced by dietary treatment (Table

Figure 1. Grams of methane per kilogram of NDF with increasing percentage of alfalfa silage at the expense of corn silage in a TMR containing 55% forage (DM basis); $Y = 297 - 1.39X$ ($R^2 = 0.97$).

7). To explore this data further, NDF digestibility data (trial 1) were combined with NDF intake (trial 2) to calculate CH_4 emission per kilogram of digested NDF. These calculations resulted in 2.54, 3.15, 3.50, and 3.59 kg/d of digested NDF and 274, 236, 208, and 190 g of CH_4 per kilogram of digested NDF for AS:CS ratios: 20:80, 40:60, 60:40, and 80:20, respectively. These emissions followed a linear relationship when regressed against the percentage of AS in dietary forage (Figure 1). The negative slope indicated a decrease of 1.39 g of CH_4 per kilogram of digested NDF for every percentage unit increase in AS and decrease in CS in the forage portion of the diet. Therefore, our data suggested type of carbohydrate in forage NDF may have affected CH_4 emission. The same analysis with the data of Hassanat et al. (2013) yielded also a high R^2 (0.92) and a negative slope (-0.23). This comparatively lower slope may have been related to a CH_4 -inhibitory effect of EE (Mores et al., 2013), which increased from 3.4 to 7.0% as AS increased from 0 to 100% of the forage in the diet. Nevertheless, both studies indicated that emission per kilogram of digested NDF increased as CS was included in the diet at the expense of AS, and furthermore they provided similar estimates of CH_4 emission for AS as the sole forage in the diet (122 and 158 g/kg of NDF fermented in Hassanat et al., 2013, and this study, respectively). Apart from the cellulose and hemicellulose in forage NDF, other dietary carbohydrates influenced CH_4 emission (Moe and Tyrrell, 1979; Hindrichsen et al. 2005), and further research is particularly needed on the effect of starch on CH_4 emission (Haque et al., 2014).

No treatment effect was found for any CO_2 measurements, except that CO_2 /NDF intake tended to decrease linearly with increasing AS:CS ratio. Most of the CO_2 emitted by cows results from their metabolism rather than rumen fermentation. Although CO_2 emission may

be more related to metabolic activity (i.e., level of production) than changes in dietary components, the change in CO_2 /NDF intake observed here was consistent with the conjecture that differential fermentation pattern may be associated with differential gas production pattern (CO_2 vs. CH_4).

CONCLUSIONS

Increasing AS and decreasing CS in the diet and adjusting ground corn grain, solvent SBM, and expeller SBM to maintain adequate supply of RDP and RUP did not affect DMI or milk yield but had a quadratic effect on yields of fat-and-protein-corrected milk, fat, and protein. In addition, increasing AS and decreasing CS in the diet reduced NUE and increased excretion of manure, manure N, and urinary urea-N. The low NH_3 -N emissions and minimal effect of dietary treatments on NH_3 -N emissions were likely associated with housing condition used in this study (tie-stall barn). Increasing AS and decreasing CS increased NDF intake, total-tract NDF digestibility, amount of NDF fermented, and the molar proportion of acetate in ruminal VFA but decreased the molar proportion of propionate, which most likely reflected the effects of changes in both fiber and starch fermentation across dietary treatments. In this trial daily CH_4 emission were unusually high, but the pattern of change in daily CH_4 emission was quadratic, whereas the change in acetate-to-propionate ratio was linear. Data suggested that fermentation of CS NDF yielded substantially more CH_4 than the fermentation of AS NDF. Thus, as AS increased and CS decreased in the diet, the greater CH_4 emission expected from greater amount of NDF fermented was counterbalanced by a decreasing emission per gram of NDF fermented. Although emission of CH_4 per kilogram of milk did not vary among treatments, CH_4 production was the net effect of interacting factors, namely, the amount of fiber fermented, the source of fiber, and most likely the amount of starch fermented.

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