Protein Level for Alfalfa and Corn Silage-Based Diets: I. Lactational Response and Milk Urea Nitrogen

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ABSTRACT

This study was designed to evaluate lactational responses of cows fed corn silage (CS) or alfalfa silage (AS) as primary forage source when the diet was balanced for recommended (RP) or excessive (HP) amounts of rumen degradable protein (RDP) and undegradable protein (RUP) according to the recommendations of the National Research Council (NRC). A second objective was to evaluate different sources of variations in milk urea N (MUN). The total mixed rations included 55% forage on a dry matter (DM) basis as either 14% CS and 41% AS or 14% AS and 41% CS. Diets were offered to 48 multiparous Holstein cows (body weight = 652 kg) that were assigned randomly to treatments arranged as a 2×2 factorial in 12 complete blocks based on calving date. Data collected during wk 4 to 12 of lactation were adjusted to those obtained from a pretreatment diet fed during wk 1 to 3. Crude protein (CP) averaged 16.5, 18.0, 16.2, and 17.1% of DM in the AS-RP; AS-HP; CS-RP; and CS-HP diets, respectively. Overall DM intake (DMI) was 1.5 kg/d lower than predicted by NRC (24.6 vs. 26.1 kg/d), but 3.5% fat-corrected milk (FCM) was higher than expected (46.1 vs. 45.0 kg/d). The responses to a reduction in dietary protein were independent of primary forage source, except for milk true protein (TP) percentage. Primary forage source did not influence DMI, 3.5% FCM, TP yield, or MUN. However, compared with the AS-based diets, cows fed CS-based diets produced more milk (49.0 vs. 46.4 kg/d), less fat (3.07% vs. 3.54% and 1500 vs. 1651 g/d), and tended to gain more body weight. There were no benefits to feeding diets above NRC protein recommendations, regardless of forage source. Reducing CP from 17.5 to 16.4% of diet DM did not alter milk yield (47.7 kg/d) or milk TP yield (1293 g/d), but lowered N intake by 65 g/d (700 vs. 635 g/d) and lowered MUN by 1 unit (12.7 vs. 11.7 mg/dL). A positive correlation between MUN and production efficiency (3.5% FCM/DMI) on wk 3 of lactation sug-

gested that body protein mobilization might impact MUN in early lactation. The correlation between MUN and DMI tended to be negative in wk 3, but was positive in wk 6 to 12 of lactation. The same was true for the correlation between MUN and somatic cell score. Regression analysis of the postpeak lactation data of this study indicated that the expected MUN was essentially 12 mg/dL when NRC-predicted RDP and RUP balances were 0 g/d, with a linear deviation of 0.1 and 0.03 mg/dL per 10 g of change in RDP and RUP balance, respectively.

(Key words: dairy cow, forage source, nitrogen, environment)

Abbreviation key: AS = alfalfa silage, CS = corn silage, EE = ether extract, FA = fatty acid, HP = high protein, NI = N intake, RP = NRC (2001) recommended protein, TP = true protein.

INTRODUCTION

Managers of livestock operations are becoming more aware of, and concerned about, the contributions of their industry to nonpoint N pollution of air and water. From a whole-farm perspective, abatement of excess N can be achieved by integrating crop rotations with the herd feeding strategy as a single unit of management. For example, the proportion of alfalfa silage (AS) and corn silage (CS) in the farm's crop rotation alters the "home-grown" production of digestible energy, RDP and RUP; the need for purchased feed N; the manure management options; the requirements for purchased fertilizers; and ultimately the overall efficiency of N use on the farm (Wattiaux, 2001). Fortunately, despite low efficiency of alfalfa CP use (Broderick, 1985), high milk production can be achieved with a wide range of dietary AS:CS ratio (Dhiman and Satter, 1997). Additional improvements in efficiency of N use by a dairy herd can be obtained by feeding diets balanced for RUP and RDP (St-Pierre and Thraen, 1999) and by increasing milk production (Dunlap et al., 2000; Jonker et al., 2002b). The work of Wu and Satter (2000) showed that milk yield was not penalized when CP was reduced to 16% of dietary DM in the last one-third of the lactation,

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but the data suggested that CP of at least 17.5% was necessary during the first two-thirds of the lactation to maintain lactation performance >11,000 kg. Since then, the recommendations put forth by the revised NRC (2001) indicated that high-producing cows could be fed reduced CP diets compared with the recommendations of the previous edition (NRC, 1989).

Although not widely adopted by producers (Jonker et al., 2002a), milk urea N (MUN) has been suggested as a monitoring tool to evaluate protein level and efficiency of use in dairy diets (Broderick and Clayton, 1997; Jonker et al., 1998). This potential usage on commercial farms was demonstrated in a recent field study, indicating that an average excess of 6.6% N relative to NRC (1989) recommendations was associated with an average MUN that was 1.8 mg/dL higher than recommended (Jonker et al., 2002b).

Thus, our main objective was to compare responses of early lactation dairy cows fed AS- or CS-based diets formulated near RDP and RUP balance or in excess of RDP and RUP according to NRC (2001). In essence, we hypothesized that dietary CP may be lowered, regardless of primary forage source, without penalizing milk yield when the NRC (2001) model was used to formulate diets. Our second objective was to determine whether MUN would reflect relatively small differences in dietary protein fed in early lactation diets.

MATERIALS AND METHODS

Cows and Experimental Design

This lactation study was conducted as a complete randomized block design with 48 multiparous Holstein cows. The 12-wk experimental period was divided into a pretreatment period (wk 1 to 3) and a treatment period (wk 4 to 12), in which 4 dietary treatments were arranged as a 2 × 2 factorial. Cows were blocked by calving date and were assigned to a dietary treatment randomly within each block; 3 of the 12 blocks included cows fitted with a rumen cannula. During the trial, cows were housed in individual tie stalls bedded with rubber mattresses and wood shavings and were free to exercise unrestrained in an outdoor paddock for 1 to 2 h daily after being milked at approximately 0300 and 1500 h. Cows were cared for according to the guidelines of the Research Animal and Resource Committee of the University of Wisconsin-Madison, and all experimental procedures performed were approved. The experiment was conducted from early January to the end of May 2001.

Diet Formulation

Initially, the Spartan Ration Evaluator/Balancer for dairy cattle (ver. 2.02b; Michigan State University) was

used to formulate diets based on CS or AS as the primary forage source and containing 16.5 or 18% CP and 28% NDF (DM basis). Diets included 55% forages (DM basis), and the primary forage contributed three-fourths of the total forage. Thus, AS diets consisted of 41.2% AS (DM basis) and 13.8% CS, and CS diets consisted of 13.8% AS and 41.2% CS. Ground corn grain and molasses were used as sources of ruminally available energy, tallow was included in all diets, and soybean meal (48% CP) and soybean meal expeller were used as main sources of supplemental RDP and RUP. Then, the rations were evaluated with the NRC (2001) model, using feed ingredients CP and forage NDF values adjusted on the basis of analyses performed prior to the start of the trial. The sources and proportions of protein supplements were altered to formulate diets near RDP and RUP balance (recommended protein [RP] diets) or in excess of RDP and RUP recommendations (high protein [HP] diets) for an early lactation, 650-kg multiparous Holstein cow producing 45 kg/d of 3.5% FCM. Plant protein sources were chosen in an attempt to maintain similar levels of Lys and Met in all diets. Soybean meal was used as a source of Lys in the CS diets, and corn gluten meal was used as a source of Met in the AS diets. Diet ingredients and predicted concentration of NE_L, RDP, RUP, and CP are presented in Table 1. Lysine and Met contents were 5.7, 5.6, 5.6, and 5.7% and 2.0, 2.1, 2.6, and 2.5% of metabolizable protein for the AS-RP; AS-HP; CS-RP; and CS-HP diets, respectively. The NRC (2001) predicted NE_L allowable milk was 45 kg/d for all experimental diets, but the metabolizable protein allowable milk was 45 and 50 kg/d for the RP and HP diets, respectively. The pretreatment diet was formulated to support 38 kg of milk with all ingredients of the treatment diets. Final adjustments for mineral supplementation were made with the "auto-balance" function of the Spartan Ration Evaluator/Balancer.

Diet Composition

Diets were offered as TMR for ad libitum intake (10%orts on an as-fed basis) once daily at 0800 h. Dry matter (60°C for 48 h) of forages was determined weekly, and diet formulations (as-fed basis) were adjusted accordingly to account for changes in forage DM content. Nutrient composition of the diets was determined on 3-wk composites of forage samples collected weekly and on samples of concentrate mixes collected approximately every 4 wk and composited bimonthly. Orts were measured daily, but sampled biweekly on 3 consecutive d and composited in proportion to the amount refused. Individual cow refusal data were used for DMI calculations.

Table 1. Dietary ingredients and concentration of selected nutrient as predicted by NRC (2001).

Ingredient	Treatment ¹				
	Pretreatment	AS-RP	AS-HP	CS-RP	CS-HP
	(g/100g of DM)				
Alfalfa silage	27.5	41.2	41.2	13.8	13.8
Corn silage	27.5	13.8	13.8	41.2	41.2
Corn grain, ground	21.8	30.2	26.7	23.7	20.1
Soybean meal, expeller	8.6	3.0	5.3	3.8	6.1
Soybean meal, 48% CP	4.6	2.0	3.3	10.3	11.6
Corn gluten meal	1.5	3.0	3.0	...	...
Urea	0.2	...	...	0.5	0.5
Cane molasses, dehydrated	3.0	3.0	3.0	3.0	3.0
Tallow	2.0	2.0	2.0	2.0	2.0
Limestone	0.10	...	...	0.27	0.27
Sodium bicarbonate	0.77	0.46	0.46	0.39	0.39
Phosphate monosodium	0.26	0.39	0.39	...	...
Dicalcium phosphate, dibasic	0.77	0.19	0.19	0.46	0.46
Magnesium oxide	0.10	0.12	0.12	0.04	0.04
Mineral vitamin mix ²	1.24	0.92	0.92	0.92	0.92
NRC predicted nutrient concentration ³					
NE _L , Mcal/kg, DM	1.66	1.57	1.58	1.58	1.60
RDP balance, g/d	9	-11	127	-2	137
RUP balance, g/d	3	17	280	55	299
MP balance, g/d	-27	14	229	47	256
RDP, % of DM	10.3	9.8	10.3	9.9	10.4
RUP, % of DM	7.4	6.8	7.7	6.5	7.4
CP, % of DM	17.7	16.6	18.0	16.4	17.8

¹AS = Alfalfa silage, RP = NRC (2001) recommended protein, HP = high protein, and CS = corn silage.

²Contained 0.55% Mn, 0.55% Zn, 0.35% Fe, 0.14% Cu, 0.008% I, 0.006% Se, 0.002% Co, 3304 IU/g of vitamin A, 1101 IU/g of vitamin D, and 11 IU/g of vitamin E.

³Calculated based on NRC (2001) values for individual feedstuffs adjusted for analysis of diet ingredients prior to start of trial for a 650-kg nonpregnant lactating Holstein dairy cow, 80 DIM, with a milk yield of 45 kg/d, 3.5% fat and 3.0% true protein (pretreatment diet values were calculated for a cow 30 DIM and a milk yield of 38 kg/d).

Forage and concentrate mix samples were ground to pass a 2-mm screen (Wiley mill; Arthur H. Thomas, Philadelphia, PA). Dry matter content was determined by drying at 135°C for 2 h, and ash was determined by drying at 500°C for 16 h. Organic matter content was calculated as 100 – ash. Crude protein was determined by microKjeldahl (AOAC, 1990) and NDF was determined according to Van Soest et al. (1991) using α -amylase (Sigma no. A3306; Sigma Chemical Co., St. Louis, MO) and sodium sulfite. Calculation of NDF concentration included a correction for ash content according to Mertens (1999) adapted for Ankom²⁰⁰ Fiber Analyzer (Ankom Technology, Fairport, NY). Nitrogen bound to the neutral detergent residues obtained from the Ankom²⁰⁰ Fiber Analyzer was determined by microKjeldahl (AOAC, 1990) and expressed as neutral detergent insoluble CP. Fatty acid (FA) content was determined by gas chromatography (Sukhija and Palmquist, 1988). Nonfiber carbohydrate content was calculated as 100 – (CP + (NDF – neutral detergent insoluble CP) + (FA + 1) + ash); where all values are percentages of DM, and FA + 1 is an estimate of ether extract (EE) content (NRC, 2001).

The aforementioned forage samples were composited further to obtain 3 samples, each representative of what was fed during each one-third of the trial. Starch was determined by a colorimetric assay including a pure cornstarch sample as outlined by Bal et al. (2000). Forage RUP was determined by near infrared reflectance spectroscopy, assuming a ruminal rate of passage of 0.06/h as outlined by Hoffman et al. (1999). Digestibility of NDF was determined in a 48-h in vitro system (Goering and Van Soest, 1970), adhering to the conditions described by Grant and Mertens (1992) and Grant and Weidner (1992).

Performance and Rumen Measurements

Starting at wk 2 of lactation, milk yield was recorded daily, and milk samples were collected for 4 consecutive milkings once weekly. Samples were analyzed by Ag-Source (Menomonie, WI) using the combiFoss 5000 (Foss Electric, Hillerød, Denmark) that included the MilkoScan 4000 for determination of fat, true protein (TP), lactose, SNF (AOAC, 1990), and MUN by infrared analysis using the differential pH method as a standard

and the Fossomatic 5000 for analysis of SCC by flow cytometry (AOAC, 1999). Somatic cell scores were calculated using a natural logarithmic transformation of the SCC (cells/mL) using the formula $SCS = (\ln(SCC/100,000)/0.6931) + 3$, where SCC is expressed as cells/mL (Shook, 1993). As a parameter, SCS is more suitable than SCC for statistical analysis because the transformed data better fit the assumptions of normal distribution and homogeneity of variance (Shook, 1993). Cows were weighed at 1400 h on 2 consecutive d, and body condition was scored independently by the same 3 persons on a scale of 1 to 5 (Wildman et al., 1982) during wk 3, 6, 9, and 12 postpartum.

Diurnal variation in rumen pH and ammonia concentration during wk 8 postpartum was measured on 3 blocks of cows ($n = 12$) fitted with rumen cannula. Samples were collected from 5 different locations in the rumen with a metal filter probe shortly before feeding (0 h) and every 2 h for a 24-h period. Rumen fluid pH was determined within 3 min of collection using a handheld pH meter (Twin pH meter model B-213; Spectrum Technologies Inc., Plainfield, IL). Also, 1 mL of rumen fluid was acidified with 20 μ L of 50% trichloroacetic acid and frozen until analysis for NH_3 N concentration (Chaney and Marbach, 1962).

Statistical Analysis

Data were analyzed using the MIXED procedure of SAS (1998). Milk yield, DMI, N intake (NI), production efficiency (3.5% FCM/DMI), milk component yield and percentage, MUN, SCC, SCS, BW, and BCS were analyzed with week as repeated measurements. The model included the effects of treatment, week, and treatment by week interaction, with block and treatment by block interaction included in the random statement. Each variable was adjusted by analysis of covariance, utilizing data collected during the pretreatment period. Compound symmetry and first-order autoregressive covariance structures were compared, and the first-order autoregressive covariance structure was chosen because it provided the best fit for all variables based on Akaike's information criterion (Littell et al., 1998). Except for fat yield and SCC, the covariate measurements were significant, but for consistency, all reported values were adjusted least square means. For changes in BW and BCS from the beginning to the end of the trial, the model included the effects of treatment and block. Ruminal pH and NH_3 N concentration were analyzed as repeated measurements with a model similar to the one described previously. Single degree of freedom orthogonal comparisons were used to test the main effects of primary forage source (AS vs. CS), dietary protein level (RP vs. HP), and their interaction. Significance was

declared at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$. Fisher's LSD test was used to separate treatment means ($P \leq 0.05$) when an interaction was significant.

The MUN measurements obtained on wk 3 of lactation were considered appropriate to study factors associated with MUN in early lactation, other than diet composition, because all cows were fed the same dry cow diet, prepartum diet, and pretreatment diet. First, the CORR procedure of SAS was used to correlate MUN with parity and wk 3 measurements including BW, DMI, NI, starch intake, starch intake/NI, milk yield, 3.5% FCM, production efficiency (3.5% FCM/DMI), milk component yields and concentrations, SCC, SCS, and BW change from wk 3 to 6. Single factor correlations were reported for $P \leq 0.10$. Then, the stepwise option of Proc REG was used to identify variables in the aforementioned list that made significant contributions to predicting MUN. The levels of significance for a variable to enter and to stay in the model, respectively, were the default value of $P < 0.15$.

Factors associated with variation in MUN postpeak lactation (wk 6 to 12 of lactation) were examined both with individual cow data and weekly averages of dietary treatments (diet data). Correlation and regression analyses were performed as described previously with the following measurements: BW, BW change from wk 6 to 12, DMI, NI, starch intake, starch intake/NI, NDF intake, dietary CP, dietary starch, dietary NDF, dietary NFC, milk yield, 3.5% FCM, production efficiency, milk component yields and concentrations, SCC, SCS, RDP balance, RUP balance, and (RDP balance + RUP balance). These balances were calculated according to the NRC (2001) model based on diet ingredient composition and weekly treatment averages for BW, DMI, milk yield, and milk composition.

RESULTS AND DISCUSSION

Chemical Composition of Feed and Diets

Chemical composition of dietary ingredients is reported in Table 2. The AS was a second cut of different cultivars harvested from different fields on the same date, and the CS was from a late maturity grain hybrid (Lemke 6060; Lemke Seed Farms, Inc., Mequon, WI). Overall, nutrient content of silages was relatively constant during the trial; however, the CP and NDF content of CS was slightly variable than that for AS (Table 2). For unforeseen reasons, CS NDF in DM increased progressively from 34% in the first half of the trial to about 39% in the second half. The 48-h NDF digestibilities were in the lower quartile and about 10 units below the respective population average for CS and AS samples analyzed by the Marshfield laboratory in the last 2 yr (P. Hoffman, personal communication 2002). The

Table 2. Chemical composition of dietary ingredients.

Item	Forage ¹		Concentrate mixes in				
	AS	CS	PRTRT ²	AS-RP ²	AS-HP ²	CS-RP ²	CS-HP ²
DM, %	39.7 (3.8)	31.8 (2.4)	88.8	88.2	88.3	88.6	88.6
OM, % of DM	91.2 (0.4)	96.1 (0.3)	91.0	93.7	93.5	92.2	92.3
CP, % of DM	19.8 (0.9)	7.3 (0.6)	20.3	16.1	19.3	23.3	25.3
NDF, % of DM	43.7 (2.8)	37.1 (3.0)	11.2	11.0	10.2	10.8	10.6
NDICP, ³ % of DM	2.9 (0.4)	0.6 (0.1)	1.3	1.6	1.1	1.4	1.0
FA, ⁴ % of DM	2.2 (0.7)	2.1 (0.3)	4.4	4.1	4.3	4.4	4.5
NFC, ⁵ % of DM	27.4 (1.7)	50.0 (3.1)	55.2	63.1	59.9	54.1	51.9
Starch, ⁶ % of DM	0.2 (0.1)	24.9 (1.2)	35.0	44.8	38.7	34.0	29.2
NDFD, ⁷ % of NDF	39.0 (0.7)	50.4 (3.9)	...	...	...	...	...
RUP, ⁸ % CP	20.1 (0.3)	29.2 (0.5)	...	...	...	...	...

¹Means (SD) for alfalfa silage (AS) and corn silage (CS) (n = 9, except for starch, NDFD, and RUP [n = 3]).

²PRTRT = Pretreatment diet, AS = alfalfa silage, RP = NRC (2001) recommended protein, HP = high protein, and CS = corn silage.

³NDICP = Neutral detergent insoluble CP.

⁴FA = Fatty acids, according to Sukhija and Palmquist (1988).

⁵NFC = Non fiber carbohydrate = 100 - (CP + (NDF - NDICP) + (FA + 1) + ash).

⁶According to Bal et al. (2000).

⁷NDFD = Neutral detergent fiber digestibility according to Goering and Van Soest (1970) adhering to in vitro conditions described by Grant and Mertens (1992) and Grant and Weidner (1992).

⁸According to Hoffman et al. (1999).

NE_L content of AS and CS averaged 1.24 and 1.35 Mcal/kg DM, respectively, when computed with the summative equation and the energy discount factors (10 to 11%) associated with the actual DMI (around 4× maintenance) observed in this trial (NRC, 2001). The CP in concentrate mixes was as expected for the AS diets, but somewhat lower than expected for the CS-based diets (24.3 vs. 25.1% of DM), most likely because of lower than expected CP in the soybean meal. Overall, the CP of concentrate mixes was 2.6 percentage units lower in RP diets than in HP diets and 6.7 percentage units lower in AS diets than in CS diets. Starch content in the concentrate mixes was higher for the AS diets than for the CS diets (41.8 vs. 31.6% of DM), reflecting higher contents of corn grain (Table 1).

Dry matter content and chemical composition of the DM in diets are presented in Table 3. Dietary CP was as formulated for the AS diets, but was somewhat lower than expected for the CS diets. Over the course of the trial, the CP of the RP and HP diets averaged 16.4 and 17.5%, respectively, and the CP of the AS- and CS-based diets averaged 17.3 and 16.7%, respectively. Dietary NDF concentration was near expectation (i.e., 28%) for the AS diets, but 2.1 percentage units lower than expected for the CS diets (27.8% vs. 25.9%). Diets exceeded the minimum recommendations for NDF from forage (NRC, 2001). Starch concentration was 2.0 percentage units higher in CS diets than in AS diets (24.5% vs. 22.5%) and 2.4 percentage units higher in RP diets than in HP diets (24.7% vs. 22.3%). On the average, starch was 50% of NFC, but ranged from 46.5% for AS,

HP diet to 52.5% for CS, RP diet. Dietary NE_L computed on the basis of forage composition, individual concentrate feed ingredient composition, and individual cow traits (BW and DIM) and performance (DMI, milk yield, and composition) throughout the trial was 1.61, 1.61, 1.62, and 1.63 Mcal/kg for the AS-RP; AS-HP; CS-RP; and CS-HP diet, respectively.

DM and Nutrient Intake

Intake of DM and nutrients increased during the course of the trial, but there were no diet by week interactions ($P = 0.14$ to 0.19), nor were there any primary forage source by dietary protein level interactions ($P = 0.23$ to 0.90 ; Table 4). Overall DMI (means $\pm$ standard

Table 3. Chemical composition of diets.

	Treatment ¹				
	PRTRT	AS-RP	AS-HP	CS-RP	CS-HP
DM, % (as-fed)	59.5	60.7	60.8	58.5	58.6
OM, % of DM	92.4	93.0	92.9	93.7	93.7
CP, % of DM	16.9	16.5	18.0	16.2	17.1
NDF, % of DM	27.4	27.9	27.5	26.1	26.0
Forage NDF, % of DM	22.4	22.9	22.9	21.2	21.2
Ether extract, ² % of DM	4.2	4.1	4.2	4.2	4.2
Starch, % of DM	22.7	23.8	21.1	25.6	23.5
NFC, % of DM	46.3	46.8	45.4	48.8	47.8

¹PRTRT = Pretreatment diet, AS = alfalfa silage, RP = NRC (2001) recommended protein, HP = high protein, and CS = corn silage.

²According to NRC (2001): ether extract = fatty acids (% of ration DM) + 1.

Table 4. Least square means for nutrient intake.

Item	Treatment ¹				SEM	Significance (<i>P</i>)		
	AS-RP	AS-HP	CS-RP	CS-HP		Forage (F)	Protein (P)	F × P
DMI, kg/d	24.4	25.3	24.2	24.5	0.62	0.41	0.30	0.63
OM intake, kg/d	22.6	23.5	22.7	23.0	0.57	0.69	0.25	0.57
N intake, g/d	643	729	626	670	17.1	0.03	<0.001	0.23
NDF intake, kg/d	6.80	6.96	6.30	6.37	0.168	0.002	0.49	0.78
Starch intake, kg/d	5.80	5.33	6.18	5.76	0.145	0.007	0.003	0.90
EE ² intake, kg/d	1.00	1.06	1.01	1.03	0.026	0.73	0.13	0.34
NE _L intake, Mcal/d	39.8	42.6	39.2	39.6	1.36	0.17	0.23	0.36

¹AS = Alfalfa silage, RP = NRC (2001) recommended protein, HP = high protein, and CS = corn silage.

²EE = Ether extract according to NRC (2001): EE = fatty acids (% of ration DM) + 1.

deviation) averaged 24.6 ± 0.19 kg/d, which was 1.5 kg/d lower than the NRC (2001) predicted values used to balance the rations (26.1 kg/d). This difference was somewhat surprising, because BW, milk yield, and week of lactation, which are the animal traits that determine DMI according to NRC (2001), were a posteriori quite similar to the anticipated values. The fact that NRC (2001) assumes that DMI is animal-dependent only conflicts with data, indicating major effects of diet composition (Allen, 2000) and environmental factors (Ingvarsen and Andersen, 2000). In this trial, the change in relative proportion between AS and CS as the primary forage source in the diet did not influence DMI or intake of OM, EE, or NE_L. However, when AS was the primary forage source in the diet, cows consumed 38 g/d more N (686 vs. 648 g/d), 0.55 kg/d more NDF (6.88 vs. 6.34 kg/d), and 0.41 kg/d less starch (5.57 vs. 5.97 kg/d) than did cows fed the CS-based diets. The absence of a forage effect on DMI agrees with results of Broderick (1985), who compared AS and CS as sole source of forage in 60% forage diets, and of Dhiman and Satter (1997), who reported no change in DMI when the proportion of CS increased from 0 to 17 and 33% of diet DM, at the expense of AS in 50% forage diets. In contrast, Onetti et al. (2002) reported a quadratic increase in DMI and NE_L intake as the proportion of AS in the diet increased from 0 to 13 and 25% of diet DM at the expense of CS in 50% forage diets. Similarly, Ruppert et al. (2003) reported a higher DMI for cows fed diets containing 40% AS and 10% CS (DM basis) compared with the reverse proportions in 50% forage diets.

In this trial, dietary protein level did not influence the intake of DM, OM, NDF, EE, or NE_L. However, intake of N was 65 g/d higher (700 vs. 635 g/d), and intake of starch was 0.44 kg/d lower (5.55 vs. 5.99 kg/d), for cows fed HP diets compared with cows fed RP diets. Broderick (2003) showed a linear increase in DMI from 21.2 to 22.1 and 22.6 kg/d as dietary CP increased from 15.1 to 16.7 and 18.4% of diet DM. However, in

the report of Davidson et al. (2003), DMI was not influenced by dietary CP ranging from 16.5 to 19.4% of diet DM. In the work of Wu and Satter (2000), differences were not significant, but DMI was 1.9 kg/d higher when CP level increased from 15.4 to 17.4% of diet DM and decreased by 0.5 kg/d when CP level was increased further to 19.3% in the first 16 wk of lactation.

Performance and Rumen Measurements

Yield of milk and milk components. Rations were formulated assuming cows would produce an average of 45 kg/d of 3.5% FCM. Overall milk yield and 3.5% FCM averaged 47.7 ± 0.32 and 46.2 ± 0.45 kg/d (means $\pm$ standard deviation). Production of milk, fat, TP, lactose, SNF, and concentration of these major milk components increased significantly over the course of the trial, except for fat yield and lactose concentration. There was no primary forage source by dietary protein level interaction for milk yield measurements and milk components, except for milk TP percentage ($P = 0.03$; Table 5). Overall, the average milk TP was $2.70 \pm 0.01\%$. Values were not influenced by dietary CP for the AS diets, but varied from 2.60% in the CS-RP diet to 2.79% in CS-HP diet ($P < 0.05$; Table 5). This interaction is difficult to interpret because it was accompanied also by a tendency ($P = 0.09$) for milk TP percentage to change differently among dietary treatments over the course of the trial. The plotted data (not shown) indicated that milk TP percentage was lower for the CS-RP diet in the early parts of the trial, but increased faster than for the other 3 treatments between wk 6 and 12 of lactation. None of the other diet by week interactions for milk yield and milk component measurements were significant.

Primary forage source had no effect on 3.5% FCM and production efficiency, but influenced milk yield, fat percentage, and, to a lesser statistical significance, fat yield. When cows were fed CS diets compared with AS diets, milk yield was 2.6 kg/d higher (49.0 vs. 46.4 kg/

Table 5. Least square means for lactation performance.

Item	Treatment ¹				SEM	Significance (P)		
	AS-RP	AS-HP	CS-RP	CS-HP		Forage (F)	Protein (P)	F × P
Milk, kg/d	46.1	46.7	49.3	48.7	1.29	0.03	0.97	0.60
3.5% FCM, ² kg/d	46.1	46.4	46.0	45.9	1.66	0.84	0.95	0.87
Production efficiency ³	1.90	1.91	1.89	1.91	0.060	0.98	0.83	0.94
Milk components								
Fat, %	3.59	3.48	3.03	3.11	0.152	<0.001	0.93	0.46
True protein, %	2.73 ^a	2.70 ^a	2.60 ^b	2.79 ^a	0.049	0.72	0.12	0.03
Lactose, %	4.81	4.76	4.81	4.82	0.052	0.56	0.71	0.60
SNF, %	8.47	8.40	8.40	8.55	0.084	0.68	0.67	0.18
Fat, g/d	1708	1593	1523	1476	106.9	0.08	0.35	0.69
True protein, g/d	1274	1269	1315	1311	38.5	0.20	0.88	0.99
Lactose, g/d	2246	2238	2426	2278	68.8	0.09	0.23	0.28
SNF, g/d	3955	3939	4242	4034	115.8	0.07	0.28	0.35
SCS ⁴	3.06	3.36	3.23	2.90	0.448	0.73	0.97	0.47
MUN, mg/dL	11.69	12.21	11.48	12.80	0.338	0.35	<0.001	0.77

¹AS = Alfalfa silage, RP = NRC (2001) recommended protein, HP = high protein, and CS = corn silage.

²3.5% FCM (kg/d) = (0.4255 × milk yield [kg/d]) + (16.425 × fat yield [kg/d]).

³Production efficiency = average daily 3.5% FCM (kg/d)/average daily DMI (kg/d).

⁴Calculated according to Shook (1993): SCS = 3 + (ln [SCC/100,000]/0.6931).

^{a, b}Means within row with different superscript differ ($P < 0.05$).

d; $P < 0.03$), milk fat percentage was 0.47 percentage unit lower (3.07% vs. 3.54%; $P < 0.001$), and fat yield was 151 g/d lower (1500 vs. 1651 g/d; $P = 0.08$). Also, there was a tendency for cows to produce more lactose and more SNF when fed the CS diets compared with the AS diets. The fat-depressing effect of CS-based diets compared with AS-based diets is in agreement with a series of experiments that have compared various ratios of AS and CS in 50 to 55% forage diets (Dhiman and Satter, 1997; Onetti et al., 2002; Ruppert et al., 2003). Milk fat depression has been recently associated with ruminal accumulation of *trans*-10, *cis*-12 C_{18:2} FA isomer (Baumgard et al., 2000), a transient intermediate in the process of biohydrogenation of unsaturated FA under normal rumen conditions. However, as suggested by Ruppert et al. (2003), when diets tend to induce lower rumen pH and include a substantial amount of unsaturated FA (as in the form of supplemental tallow in this study), conditions appear to be met for possible accumulation of *trans*-10, *cis*-12 C_{18:2} FA isomer in the rumen and milk fat depression. Onetti et al. (2002) speculated that including AS in the diet might lead to a more stable rumen environment and a more complete rumen hydrogenation of unsaturated FA. This contention is consistent with our results because the higher milk fat percentage observed with the AS-based diets was associated with less ruminal pH fluctuation compared with CS-based diets (see subsequent).

Under the conditions of this trial, primary forage source did not influence MUN, which averaged 12.0 and 12.1 mg/dL for cows fed AS- and CS-based diets, respectively. This result contrasted with those of Dhi-

man and Satter (1997), who reported a decrease in MUN with an increased proportion of CS in the diet, and those reported by Broderick (1985), who found the reverse. The values reported here were much lower than those found by Dhiman and Satter (1997) or Broderick (1985), which averaged 21.4 and 15.9 mg/dL, respectively, despite comparable dietary CP. Comparison of MUN values across experiments should be cautioned, however, because of the variation caused by laboratory technique (Broderick, 2003) and a change in near infrared spectroscopy calibration in September 1998, which brought about an average reduction of 4 mg/dL in the near infrared MUN readings (Kohn et al., 2002).

In this trial, the average difference of 1.1 percentage units of CP between HP and RP diets did not influence early lactation milk yield, 3.5% FCM, or production efficiency (Table 5). A meta-analysis of 38 studies (NRC, 2001) indicated a positive quadratic relationship between milk yield and CP in diet DM in the range of 16 to 21%; however, increasing CP with RDP was less effective than with RUP. A recent midlactation study showed no differences in milk and milk component yields when cows were fed a 16.7 or 18.4% CP in the diet; however, yields were penalized when dietary CP was reduced to 15.1% of diet DM (Broderick, 2003). Wu and Satter (2000) did not find any benefits from increasing dietary CP above 17.5% during the first 6 wk of lactation, but those researchers suggested that higher dietary CP between wk 7 and 16 of lactation could be beneficial to maximize whole lactation performance.

Figure 1. Milk urea N concentration (mg/dL) during the pretreatment diet (wk 3) and for cows fed the high protein diets (■; 17.5% CP) and the recommended protein diets (□; 16.4% CP) from wk 4 to 12 of lactation. (SE of the difference between treatments within a week from wk 4 to 12 = 0.56 mg/dL).

Level of dietary protein did not influence yields or percentages of milk fat, TP, lactose, and SNF, nor did it influence SCC or SCS (Table 5). These results are in agreement with those reported by Wu and Satter (2000), working with early lactation cows fed diets of either 17.4 or 19.3% CP, and those of Broderick (2003), working with midlactation cows fed diets of either 16.7 or 18.4% CP.

Cows fed the HP diets had higher MUN than did cows fed the RP diets (12.5 vs. 11.6 mg/dL). This result was consistent with other works (Frank and Swensson, 2002; Broderick, 2003; Davidson et al., 2003), all of which used dietary CP differences between treatments of 2 to 4 percentage units, compared with a difference of only 1.1 percentage units in this trial. Although the week by diet interaction did not reach significance ($P = 0.18$), the difference in MUN between the RP and HP diets appeared to widen progressively over a period of weeks (Figure 1). This observation suggests that, in addition to short-term excesses or deficiencies in rumen ammonia (Gustafsson and Palmquist, 1993), MUN may also reflect a long-term metabolic adaptation to dietary protein level, at least in early lactation.

BW and BCS. Overall, BW averaged 656 ± 5 and 654 ± 5 kg (means $\pm$ SD) on wk 3 and from wk 6 to 12 of lactation, respectively. There was a tendency for a diet by week interaction for BW ($P = 0.08$). Although treatments did not influence BW in wk 6 or 12, in wk 9, cows fed the CS-RP diet weighed more than those fed the AS-based diets ($P = 0.07$).

Primary forage source influenced average BW and the change in BW from wk 3 to 12 of lactation, which were 648, -9.6 and 660 , 2.5 kg for cows fed AS and CS diets, respectively (Table 6). By wk 12, cows fed 3 of the 4 treatments had not entirely regained the BW lost earlier in the trial. Average daily BW change was

-0.131 , -0.141 , $+0.189$, and -0.128 kg/d for cows fed AS-RP; AS-HP; CS-RP; and CS-HP diets, respectively. Dhiman and Satter (1997) did not find differences in BW change when cows were fed AS and CS in different proportions, but our observations agreed with results indicating that cows gained more BW when CS, rather than AS, was the sole forage source in the diet (Broderick, 1985). Dietary CP level had no effects on BW or BW change, a result similar to that found by Wu and Satter (2000). Neither primary forage source nor dietary CP level influenced BCS or the change in BCS throughout the trial.

Ruminal pH and NH_3 N Concentration. Although ruminal pH and NH_3 N concentration changed with time after feeding, there was no treatment by time interaction and no treatment effects (Table 6). Nevertheless, a data plot (not shown) indicated a distinct pattern of changes with a greater 24-h pH fluctuation for the CS-based diets than for the AS-based diets. Peak and nadir pH were observed immediately before feeding and 12 h after feeding, and respective pH values were 6.9 and 5.9 for CS-based diets, but 6.7 and 6.1 for AS-based diets. From 4 to 14 h after feeding, pH values were consistently lower for CS than AS diets, but the converse was true starting 16 h after feeding. Although some reports failed to detect differences in ruminal pH when cows were fed diets based on AS and CS (Dhiman and Satter, 1997), others found a lower ruminal pH with increasing proportion of CS in the diet (Onetti et al., 2002; Ruppert et al., 2003).

Treatments did not influence ruminal NH_3 N concentration, but values were higher for HP diets than for RP diets. Differences were not expected because these diets were formulated with similar predicted excess of RDP (Table 1). Based on actual feed composition and cow data, the predicted RDP balances were -52 and 74 g/d (i.e., -8 and 12 g N/d) for cows fed RP and HP diets, respectively; thus, the predicted deficit of RDP in RP diets and the excess RDP in HP diets were both within 2% of observed NI.

MUN Correlations and Predictions

Pretreatment MUN. The average MUN of individual cows fed the pretreatment diet on wk 3 of lactation was 12.8 mg/dL, but values ranged from 5.9 to 15.9 mg/dL (Figure 2). In the data analysis, the record of one cow with a SCC of 10 million cells/mL was removed. Single factors correlated significantly with MUN are reported in Table 7. There was a tendency for weak negative correlations ($-0.4 < r < 0$) between MUN and BW, DMI, and NI. As diet composition was constant during this phase of the trial, the correlation between MUN and NI was a direct reflection of individual cow

Table 6. Least square means for BW, BCS, and rumen measurements.

Item	Treatment ¹				SEM	Significance (P)		
	AS-RP	AS-HP	CS-RP	CS-HP		Forage (F)	Protein (P)	F × P
BW, ² kg	645	651	663	657	5.40	0.04	0.99	0.28
Change in BW, ³ kg	-8.3	-10.8	11.9	-6.1	7.56	0.09	0.16	0.29
BCS ⁴	2.66	2.60	2.62	2.66	0.05	0.82	0.81	0.30
Change in BCS ⁴	-0.13	-0.32	-0.13	-0.14	0.08	0.15	0.14	0.16
Ruminal pH ⁵	6.37	6.43	6.42	6.43	0.14	0.76	0.69	0.71
Ruminal NH ₃ N, ⁵ mg/dL	10.12	15.68	11.25	13.37	3.02	0.85	0.25	0.59

¹AS = Alfalfa silage, RP = NRC (2001) recommended protein, HP = high protein, and CS = corn silage.

²Average BW for wk 6, 9, and 12 of lactation covariately adjusted for BW on wk 3, which averaged 659, 679, 624, and 667 kg for cows fed AS, RP; AS, HP; CS, RP; and CS, HP diets, respectively.

³Week 12 – wk 3 measurements.

⁴Average BCS on a scale of 1 = thin to 5 = obese (Wildman et al., 1982) for wk 6, 9, and 12 of lactation covariately adjusted for BCS on wk 3, which averaged 2.79, 2.93, 2.61, and 2.83 for cows fed AS, RP; AS, HP; CS, RP; and CS, HP diets, respectively.

⁵Samples were collected on wk 8 of lactation from 12 cows fitted with a rumen cannula.

DMI. These results differ from those obtained from diet averages during postpeak lactation (Figure 2) and those reported by Broderick and Clayton (1997) and Broderick (2003). Interestingly, there was a strong positive correlation between MUN and production efficiency (3.5% FCM/DMI), which averaged 2.02 ± 0.06 , but ranged from 1.07 to 3.44 during the pretreatment period. Hence, the higher MUN of cows with higher milk yield relative to DMI suggested that energy balance and protein mobilization might be important determinants of MUN in the first weeks of lactation. Unfortun-

nately, we did not have good estimates of BW loss during the first 3 wk of lactation. However, assuming cows mobilize 21 kg of body protein between wk 2 prepartum and wk 5 postpartum (Komaragiri and Erdman, 1997), tissue mobilization may supply an average of 68 g/d of N (21 kg CP/6.25)/49 d $\times$ 1000 g/kg), or the equivalent of 7 to 21% of observed NI of the cows in the pretreatment period of this study. Thus, MUN in early lactation may reflect in part the metabolic partitioning of amino acids for either milk protein synthesis in the udder or energy generation after hepatic deamination. Although a recent report failed to detect an impact of prepartum diet CP level or postpartum RDP to RUP ratio on MUN in early lactation (Greenfield et al., 2000), additional studies should focus on the impact of peripartum diet composition and BW loss on early lactation MUN.

Also, wk 3 MUN was negatively correlated with SCC and SCS (Table 7). The SCC data were skewed to the right, with a mean of 318,000 but a median of 77,000 cells/mL. In contrast, SCS data were closer to a normal distribution, with a mean of 3.1, a median of 2.6, and a range of 0.3 to 7.9. Analyses of test-day DHI data from laboratories in Germany (Richardt et al., 2002), Ontario (Godden et al., 2001) and Ohio (Rajala-Schultz and Saville, 2003) have also reported a negative correlation between MUN and SCS. Most of the urea enters the milk by diffusion from the blood (Gustafsson and Palmquist, 1993), but changes in concentration of milk constituents as a result of bacterial infections have been documented (Wheelock et al., 1966; Berning and Shook, 1992). Thus, a change in mammary gland physiology as a result of bacterial infection could affect MUN. However, artifacts of measurement techniques cannot be ruled out as another possible explanation for this correlation.

Figure 2. Relationship between milk urea N (MUN) concentration (mg/dL) and N intake (NI) when cows were fed the 16.9% CP pretreatment diet on wk 3 of lactation (●); each data point represents one cow. The (dashed) regression line was described as $\text{MUN (mg/dL)} = -0.0053 \times \text{NI (g/d)} + 15.92$ ($r^2 = 0.06$; $P = 0.08$). This same relationship is also depicted during the postlactation peak period, wk 6 to 12 of lactation (■); each data point represents the average of 12 cows per treatment/wk. The (solid) regression line was described as $\text{MUN (mg/dL)} = 0.0148 \times \text{NI (g/d)} + 2.16$ ($r^2 = 0.62$; $P < 0.001$).

Table 7. Pearson correlation coefficients between MUN, (mg/dL) and selected measurements collected in wk 3 (pretreatment) and in wk 6 to 12 (postpeak lactation).

Item	Pretreatment		Postpeak lactation			
	Cow data ¹		Cow data ²		Diet data ³	
	r	P	r	P	r	P
BW, kg	-0.24	0.10	...	NS	0.76	<0.001
Diet composition and nutrient intake						
Dietary CP, % of DM	NA ⁴	NA	0.35	<0.001	0.84	<0.001
DMI, kg/d	-0.25	0.08	0.12	0.03	0.46	0.02
NI, kg/d	-0.25	0.08	0.20	<0.001	0.79	<0.001
RDP balance, ⁵ g/d	NA	NA	NA	NA	0.72	<0.001
RUP balance, ⁵ g/d	NA	NA	NA	NA	0.74	<0.001
Lactation performance						
3.5% FCM yield, kg/d	0.20	NS	0.12	0.03	0.02	NS
Production efficiency ⁶	0.36	0.01	0.03	NS	-0.15	NS
Fat yield, kg/d	0.12	NS	0.12	0.03	0.06	NS
Protein yield, kg/d	-0.06	NS	0.10	NS	-0.37	0.06
SCC, cells/mL	-0.42	0.003	-0.27	<0.001	0.06	NS
SCS ⁷	-0.52	<0.001	-0.32	<0.001	0.37	0.05

¹Individual cow measurements were performed on wk 3 of lactation (n = 47).

²Individual cow measurements performed from wk 6 to 12 of lactation (n = 336), except for BW, which was measured on wk 6, 9, and 12 (n = 144).

³Dietary treatment means from wk 6 to 12 of lactation (n = 28).

⁴NA = Not applicable.

⁵Balances were predicted according to NRC (2001) using means of BW, DMI, milk yield, and milk composition observed for each experimental diet.

⁶Production efficiency = average daily 3.5% FCM (kg/d)/average daily DMI (kg/d).

⁷Calculated according to Shook (1993): $SCS = 3 + (\ln [SCC/100,000]/0.693)$.

Regression analysis showed that, in combination with each other, production efficiency and SCS were the best predictors of MUN in wk 3 of lactation. However, the regression accounted for only 32% of total variation (mean square error = 2.95 mg/dL).

Postpeak MUN. In contrast to the correlation obtained in wk 3 of lactation, during the postpeak lactation period (wk 6 to 12), cow MUN data were no longer correlated with production efficiency, the correlation between MUN and DMI had become positive, albeit weakly ($0 < r \leq 0.4$), and MUN was found to be correlated strongly ($r \geq 0.8$) with dietary CP (Table 7). In addition, in this data set, MUN was correlated weakly with dietary starch ($r = -0.33$; $P < 0.001$), NDF intake ($r = 0.13$; $P = 0.02$), lactose yield ($r = 0.15$; $P = 0.01$), and lactose concentration ($r = 0.26$; $P < 0.001$; data not shown).

When the individual cow data were averaged to obtain weekly treatment means for wk 6 to 12 of lactation, MUN became positively correlated with SCS, and intermediate ($0.5 \leq r < 0.8$) to strong ($r \geq 0.8$) positive correlations were found between MUN and BW, DMI, NI, dietary CP, and predicted RDP and RUP balances (Table 7). The relationship between MUN and NI during this phase of the lactation contrasted with results found with individual cow data during the pretreatment pe-

riod (Figure 2) but was in agreement with other reports (Broderick, 2003; Nousiainen et al., 2004).

In this data set, predicted RDP balance and RUP balance averaged 7.7 ± 12 and 116 ± 43 g/d. The linear relationship between MUN and RDP balance was: $MUN \text{ (mg/dL)} = 0.010 (\pm 0.002) \times RDP \text{ balance (g/d)} + 12.1 (\pm 0.1)$ ($r^2 = 0.51$). Thus, under the conditions of this trial, MUN was 12.1 mg/dL when RDP balance was 0 g/d and increased or decreased by 0.1 mg/dL for every 10 g of RDP excess or deficit, respectively. Similarly, the linear relationship between MUN and RUP balance yielded a regression with an r^2 of 0.61, an intercept of $11.9 (\pm 0.13)$ mg/dL, and a slope of 0.0028 (± 0.0005). The ratio of the slopes of these 2 regressions indicated that the magnitude of change in MUN was 3.6 ($0.010/0.0028$) times greater per unit of change in RDP than in RUP. Alternatively, if an excess RDP of 100 g/d is expected to raise MUN by 1 mg/dL, it would take an excess RUP of 350 g/d to yield the same rise in MUN.

Also, in this data set, MUN was negatively correlated with dietary starch ($r = -0.80$; $P < 0.001$) and starch intake ($r = -0.57$; $P < 0.02$), but positively correlated with NDF intake ($r = 0.42$; $P = 0.03$; data not shown). The best multiple factor regression equation to predict MUN with this data set included dietary CP ($P < 0.001$),

milk yield ($P = 0.05$), TP yield ($P = 0.04$), and BW ($P = 0.13$), which together explained 81% of total MUN variation (mean square error = 0.17 mg/dL). The equation was: $\text{MUN (mg/dL)} = -10.83 (\pm 5.2) + 0.737 (\pm 0.272) \times \text{dietary CP (\%)} + 0.194 (\pm 0.06) \times \text{milk yield (kg/d)} - 8.26 (\pm 3.2) \times \text{TP yield (kg/d)} + 0.018 (\pm 0.017) \times \text{BW (kg)}$. In the study of Richardt et al. (2002), SCC, milk protein yield, and DIM were found to be the best predictors of MUN. Broderick and Clayton (1997) found as many as 12 factors that together contributed significantly to the regression of MUN.

CONCLUSION

Results confirm that reducing dietary CP from 17.1 to 16.2% on CS-based diets and 18.0 to 16.5% (DM basis) on AS-based diets as a result of balancing RDP and RUP according to NRC (2001) does not penalize cows producing at least 45 kg of 3.5% FCM in early lactation. Provided rations are carefully balanced, producers may choose substantially different proportions of AS and CS in TMR of high-producing cows, allowing flexibility to design cropping systems intended to improve manure use and whole-farm N efficiency. Results also supported the contention that during the first 3 wk of lactation, MUN is indicative of the metabolic status of the cow (BW loss and presumably body protein mobilization). Interpretation of MUN as an indicator of dietary N adequacy should be cautioned during the first few weeks of lactation. However, postpeak lactation, MUN was a sensitive tool that reflected relatively small dietary differences in RDP and RUP balances.

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