Protein Level for Alfalfa and Corn Silage-Based Diets: II. Nitrogen Balance and Manure Characteristics

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ABSTRACT

This N balance study was completed with 48 multiparous Holstein cows (body weight [BW] = 653 kg; days in milk = 89) blocked by calving date and assigned to a 2 × 2 factorial arrangement of dietary treatments. The total mixed ration included alfalfa silage (AS) or corn silage (CS) as the primary forage source (41 and 14% vs. 14 and 41% of diet dry matter (DM), respectively) and were formulated for recommended (RP) or excessive (HP) amounts of rumen degradable protein (RDP) and rumen undegradable protein (RUP) according to the guidelines of the National Research Council (NRC). Crude protein (CP) averaged 16.5, 18.0, 16.4, and 17.3% for the AS-RP; AS-HP; CS-RP; and CS-HP diet, respectively (DM basis). Regardless of primary forage source, the reduction in dietary CP to the NRC guidelines tended to improve milk yield (43.4 vs. 41.0 kg/d) but did not alter 3.5% fat-corrected milk (37.0 kg/d) or milk true protein yield (1167 g/d). In this trial, cows fed the CS-based diets consumed less DM than those fed the AS-based diets in part because of rumen acidosis. The adverse effect of low rumen pH was accompanied by an increase in urinary N (UN) as a percentage of N intake, but did not alter milk yield. Notwithstanding partial confounding, fecal N (FN) was 49 g/d lower (213 vs. 164 g/d), UN was unchanged (229 g/d), but milk N tended to be higher (194 vs. 206 g/d) when cows were fed the CS-based diets compared with AS-based diets. Compared with the HP diets, cows fed the RP diets had similar FN (189 g/d) and milk N (200 g/d), but UN and urine urea N were reduced by 41 g/d (249 vs. 208 g/d) and 40 g/d (210 vs. 171 g/d), respectively. Fecal N concentration was higher for CS-based diets, but urinary N concentration was higher for AS-based diets. The reduction in dietary CP did not influence these concentrations but lowered urine volume. The metabolic relationships between energy and protein in determining the fate of excess dietary N (primarily in the

form of excess RUP in this trial) was illustrated by a 17% increase in the UN to FN ratio for cows fed AS-HP compared with the AS-RP diet and a 42% increase in the UN to FN ratio for CS-HP compared with CS-RP diet, when cows' energy status was compromised because of rumen acidosis. In this trial, UN ranged from 150 to 320 g/d, and was best predicted as $UN (g/d) = 0.0283 \times BW (kg) \times \text{milk urea N} (mg/dL)$. The NRC protein guidelines should not be exceeded to avoid unnecessary losses of manure N and, in particular, urine urea N.

(**Key words:** dairy cow, forage source, nitrogen balance, manure)

Abbreviation key: AS = alfalfa silage, CS = corn silage, FN = fecal N, HP = high protein, MaN:MkN = manure N to milk N ratio; MUN = milk urea N, NI = N intake, RP = NRC (2001) recommended protein, TP = true protein, UN = urinary N.

INTRODUCTION

The dairy industry is under increasing pressure to adjust feeding and management of dairy cattle to decrease nutrient losses to the environment. In a recent survey, Jonker et al. (2002) found that, on average, producers fed 6.6% more N than recommended. Typically, the excess of dietary N is lost as urea in urine, which is the primary source of volatile N to the environment (Paul et al., 1998). Recent research focused on the prediction of urinary N (UN) output (Kauffman and St-Pierre, 2001; Kohn et al., 2002) and dietary manipulations that may shift N excretion from urine to feces (Castillo et al., 2001a). On many Midwest commercial farms, the formulation and composition of rations depend on the proportion of alfalfa silage (AS) and corn silage (CS) available as home-grown crops. In general, efficiency of N use in AS-based diet is low (Castillo et al., 2001b) because of high concentration of RDP (Nagel and Broderick, 1992). Conversely, CS is rich in starch and, thus, provides a key source of fermentable energy to the rumen microbial population. Although Broderick (1985) and Dhiman and Satter (1997) showed that the combination of AS and CS in the diet improved effi-

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ciency of N use for milk production, results were difficult to interpret because the effects of primary forage source were confounded with dietary protein concentration in both trials.

Using midlactation cows, Broderick (2003) demonstrated that UN output was reduced from 236 to 193 g/d and further to 140 g/d as dietary CP concentration was lowered from 18.4 to 16.7% and to 15.1%, respectively, with no impact on milk true protein (TP) yield. Other factors influencing UN included the amount and proportion of RUP and RDP in the diet (Davidson et al., 2003), the intestinal digestibility of RUP (Noftsker and St-Pierre, 2003), the dietary concentration of NDF (Broderick, 2003), and the postruminal supply of starch (Reynolds et al., 2001). These very factors were, however, ineffective in altering fecal N (FN) output. But, FN was reduced as a result of decreased dietary CP (Broderick, 2003) and with the use of monensin (Ruiz et al., 2001).

Using diets balanced for RDP and RUP according to NRC (2001), Wattiaux and Karg (2004) demonstrated that dietary CP may be reduced from 17.1 to 16.2% on CS-based diets and from 18.0 to 16.5% (DM basis) on AS-based diets with no yield penalty for cows producing at least 45 kg of 3.5% FCM. In this companion study, our objectives were to study the impact of reducing NRC-predicted excess in RDP and RUP, on FN, UN, and urine urea N outputs and the prediction of UN when AS or CS was the primary forage source in the diet.

MATERIALS AND METHODS

Experimental Design, Diets, and Cows

The data collection for the N balance measurements of this study was performed on 48 multiparous Holstein cows during wk 13 and 14 of lactation, following a 12-wk lactation trial (Wattiaux and Karg, 2004). At the start of this 10-d study, cows were 84 ± 3 DIM (mean $\pm$ SE), weighed 653 ± 3.0 kg, and had been consuming treatment diets since d 21 postpartum. The experiment was designed as a randomized complete block with calving date as the blocking criterion. Cows were assigned randomly within blocks to 1 of 4 dietary treatments that were arranged as a 2×2 factorial. Main effects included dietary protein concentration and primary forage source. The AS-based diets consisted of 41.2% AS (DM basis) and 13.8% CS, and the CS-based diets consisted of 13.8% AS and 41.2% CS. The NRC (2001) model was used to formulate recommended protein (RP) diets with predicted RDP and RUP balance and high protein (HP) diets in excess of predicted RDP and RUP balance for a 650-kg multiparous Holstein cow producing 45 kg/d 3.5% FCM, as outlined in Wattiaux

and Karg (2004). The predicted RDP and RUP balances were 100 and 101%, 105 and 116%, 100 and 103%, and 105 and 118% of requirements for the AS-RP; AS-HP; CS-RP; and CS-HP diets, respectively. The proportion of ground corn grain, molasses, tallow, and the source and proportion of supplemental protein (soybean meal expeller, soybean meal, corn gluten meal, and urea) in each diet can be found in Wattiaux and Karg (2004). Cows were fed their 55% forage (DM basis) TMR once daily at 1000 h, allowing for ad libitum intake (5 to 10% orts), and they were milked in a parlor twice daily at approximately 0300 and 1500 h. After each milking, cows were left unrestrained in an outdoor paddock for about an hour (except during the urine collection period) before returning to their individual tie stall bedded with rubber mattress and wood shavings. The Research Animal and Resource Committee of the University of Wisconsin-Madison approved cow care and experimental procedures of this study. Generally, cow availability allowed for the formation of at least one block per week. But occasionally, measurements of cows in the same block were performed over a period of 2 wk, and at other times, measurements of cows in separate blocks were performed the same week. Measurements began in early April and continued until mid July 2001. Sampling and analyses of feeds and concentrate mixes were described in Wattiaux and Karg (2004). Orts were measured daily, sampled on 4 consecutive d (d 1 to 4 and 7 to 10) and composited separately according to the amount refused. Dry matter of forages was determined weekly, and amounts added to the mixer were adjusted accordingly. Average DMI and intake of N (NI), OM, NDF, and starch were calculated daily, but values were averaged separately for d 1 to 4 and d 7 to 10, to enable statistical analysis of changes in nutrient intake over the course of the study.

Rumen Sampling

Rumen fluid samples were collected by rumenocentesis (Nordlund and Garrett, 1994) on all cows, approximately 4 h after feeding on d 2 and 9. Cows were partially sedated with an intramuscular injection of xylazine (Xyla-ject; Phoenix Pharmaceutical Inc., St. Joseph, MO) at 20 mg/kg BW. The puncture site was closely monitored for abscess development, and cows' rectal temperatures were measured 7 and 31 h after the procedure to detect possible infection. 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).

Milk, Feces, and Urine

Milk and milk composition. Milk yield was recorded daily, and milk samples were collected starting on d 1 and 8 for 4 consecutive milkings. Samples were analyzed by AgSource (Menomonie, WI) using the MilkoScan 4000 (Foss Electric, Hillerød, Denmark) for determination of fat, TP, lactose (AOAC, 1990), and milk urea N (MUN) by infrared analysis using the differential pH method as a standard. To convert milk TP to milk N from TP, 6.38 was used as the conversion factor (DePeters and Cant, 1992). In addition, it was assumed that the MUN free NPN fraction of the milk averaged 0.203 g/kg as observed by Broderick (2003) when cows were fed diets ranging from 15.1 to 18.4% CP and 28 to 36% NDF (DM basis) with the same major feed ingredients as in this trial. Thus, total milk N (g/d) was calculated as milk TP/6.38 + MUN + (milk yield $\times$ 0.203), where milk TP and MUN were expressed as g/d, and milk yield was expressed as kg/d. Milk yield and milk component percentages and yields were averaged over the 10-d data collection period.

Fecal excretion and digestibility. Ytterbium chloride in solution (Rhodia, Phoenix, AZ) was used as an external marker to measure total tract digestibility and was dosed orally at 12-h intervals for 10 d starting d -5 of the trial to provide 1.131 g/d of ytterbium per cow. On the first day of sampling, fecal grab samples were collected 8 h apart at 0200, 1000, and 1800 h. On the subsequent 3 d, sampling hours were staggered such that fecal samples were taken from every 2 h of a 24-h period by the end of the 4-d collection period. Fecal samples were dried using a forced-air oven at 60°C for 48 h, composited, ground to pass through a 2-mm screen, and stored for further analysis. Also, at each sampling time of d 1, approximately 5 g of the feces were mixed with an equal weight of tap water, and the pH was measured using a hand-held pH meter within 10 min of collection. Fecal N was determined by the Kjeldahl method (AOAC, 1990), and concentration of ytterbium was determined by direct current plasma emission spectroscopy (Spectra Metrics, Inc., subsidiary of Beckman Instruments, Inc., Andover, MA) after dry-ashing fecal samples at 500°C for 16 h (Combs and Satter, 1992). Total tract apparent digestibility of DM was calculated for each cow as $[100 - (100 \times (Yb_i/Yb_f))]$, where Yb_i is the concentration of ytterbium in ingested DM, and Yb_f is the concentration of ytterbium in fecal DM. Total tract apparent digestibility of N was calculated for each cow as $[100 - (100 \times ((Yb_i \times N_f)/Yb_f \times N_i))]$, where Yb_i and Yb_f are as indicated previous, and N_f and N_i are the concentrations of N in fecal DM and ingested DM, respectively.

Urine collection and analysis. On d 7, cows were fitted with indwelling Foley catheters (28 French, 100 cc; Harvet, Spring Valley, WI) through the urethra for a 72-h total urine collection. During this period, catheters were clamped as cows were moved around the facilities for milking. Cows were returned to their stall immediately after milking. Urine was collected in containers with 500 mL of 1.5 N H₂SO₄. After recording the volume of urine excreted every 12 h, the acidified urine was mixed thoroughly, and subsamples (20 mL) were taken, diluted to 100 mL with tap water, and frozen (-20°C) until later analysis. Upon thawing, subsamples were composited and analyzed for Kjeldahl N and urea N (Crocker, 1967). Urinary N excretion (g/d) was calculated as $N_u \times$ daily urine volume (L/d), where N_u is the concentration of N in urine (g/L). Similarly, urine urea N excretion was calculated as the product of urea N concentration and daily urine volume. On d 1, mid-stream urine samples were collected at 0200, 1000, and 1800 h as cows were made to urinate by rubbing gently the region around the vulva. Urine pH was measured within 5 min of collection using a hand-held pH meter.

Statistical Analysis

Data were analyzed using procedure MIXED of SAS (SAS, 1998) with 3 models depending on type of data collected. The first model was used for DMI, nutrient intake, and rumenocentesis data. These data were analyzed as repeated measurements with a model including the fixed effects of treatment, week, and treatment $\times$ week interaction. The second model, used for analysis of urine pH and fecal pH data, included the fixed effects of treatment, sampling time, and sampling time $\times$ treatment interaction. The third model was used for measurements related to milk production, milk components concentration and yield, digestibility, FN, UN, and urine urea N and included treatment as fixed effect and block as a random effect.

The data from 4 cows were removed from the analysis because these cows experienced a drop in milk yield >25% relative to the month prior to the trial, compared with an average decline of approximately 10% for the 48 cows on this trial. Cows that suffered this decline were fed the AS-RP (n = 1); CS-RP (n = 1); and CS-HP (n = 2) diets; they were in blocks that completed the trial in early April (n = 1), early June (n = 1), and late June (n = 2). These occurrences were not entirely specific to a treatment or a block, but reflected in part individual response of high-producing cows to the combination of intensive fecal sampling, urinary catheterization, and heat stress in the early summer. As a result, the original blocks were no longer complete, and 2 adjacent blocks were combined into one to provide at

least one observation per treatment in all blocks. This adjustment halved the number of blocks and expanded slightly the range of calving dates of cows within a block. Given this redefinition of blocks, 2 random effects were considered, one including block only, and one including block and treatment by block interaction. For each of the 3 models, the difference between the -2 log likelihood values always yielded nonsignificant χ^2 (Littell et al., 1996); hence, the simpler random statement was used. Therefore, treatment $\times$ block interaction and cow within treatment $\times$ block interaction were pooled as the error term to test significance of treatment effects.

Single degree of freedom orthogonal comparisons were used to test main effects and interaction between primary forage source and concentration of dietary protein. Least square means were reported throughout; significance was declared at $P \leq 0.05$, and tendencies at $0.05 < P \leq 0.10$. When the interaction between primary forage source and dietary protein concentration was significant or tended to be significant, individual means were separated by Fisher's LSD. Individual mean differences were declared significant for $P \leq 0.05$.

RESULTS AND DISCUSSION

Nutrient Supply, Metabolic and Production Responses

Diet composition. Composition of concentrate mix was not different from that reported in the companion paper, but average forage composition was slightly different. Crude protein and NDF averaged 19.8, 41.8, 7.7, and 38.3% (DM basis) for AS and CS, respectively, compared with corresponding values of 19.8, 43.7, 7.3, and 37.1% in the companion paper (Wattiaux and Karg, 2004). In this trial, dietary CP and NDF averaged 16.5 and 27.5%, 18.0 and 27.1%, 16.4 and 26.4%, and 17.3 and 26.3% of diet DM for the AS-RP; AS-HP; CS-RP; and CS-HP diets, respectively. Other chemical components were only marginally different from those reported in the companion paper. Given actual feed ingredient composition and individual cow DIM, BW, DMI, and 3.5% FCM yields in this trial, NRC-predicted RDP averaged 9.8, 10.2, 10.0, and 10.7% of diet DM for AS-RP; AS-HP; CS-RP; and CS-HP diets, respectively, and, correspondingly, predicted RUP averaged 6.7, 7.5, 6.3, 6.9% of diet DM. Dietary NE_L concentration averaged 1.60, 1.62, 1.61, and 1.66 Mcal/kg DM for the AS-RP; AS-HP; CS-RP; and CS-HP diets, respectively. For all diets, the metabolizable protein allowable milk was higher than the NE_L allowable milk.

DMI and nutrient intake. In this trial, DMI averaged 24.1 ± 0.5 kg/d or $3.68 \pm 0.08\%$ of BW, a value lower than the 4% of BW predicted by NRC (2001) for

a cow producing milk at the level observed here. Dry matter intake and nutrient intake did not differ between weeks, and there were neither treatment $\times$ week interactions (data not shown) nor primary forage source $\times$ dietary protein concentration interactions, except for NI (Table 1). The intake of N was lower in CS-based diets relative to AS-based diets and tended to be reduced in the RP diets relative to the HP diets, but these effects were almost exclusively due to a NI reduction of 73 ± 26 g/d (mean $\pm$ SE) for cows fed the AS-based diets. There was no difference in NI when cows consumed the CS-RP diet or the CS-HP diet. Dietary concentration of protein did not influence DMI, OM intake, and NDF intake, but cows fed the RP diets consumed 0.69 ± 0.2 kg/d more starch than cows fed the HP diets (5.98 vs. 5.29 kg/d) because corn grain replaced protein sources in the RP diets. Intake of DM, OM, and NDF was 2.1 ± 0.8 , 1.8 ± 0.7 , and 0.8 ± 0.2 kg/d lower for cows fed the CS-based diets compared with the AS-based diets. Although primary forage source did not influence DMI in the 12-wk companion study (Wattiaux and Karg, 2004), other researchers have found a reduction in DMI in CS-based diets relative to AS-based diets (Onetti et al., 2002; Ruppert et al., 2003).

NRC predictions. The NRC (2001)-predicted balances of RDP, RUP, metabolizable protein, and NE_L intake were calculated with individual cow performance data (Table 1). The RDP balance among treatments averaged 0.8 ± 1.4 g of N/d, a value lower than the overall average of 10 g of N/d excess calculated for the formulated diets (Wattiaux and Karg, 2004). The lower-than-anticipated RDP balance may be related directly to the lower-than-predicted DMI discussed previously. Differences in RDP balance caused by forage source $\times$ protein level interaction and forage source were significant but numerically small (Table 1). However, as intended by design, both predicted RDP and RUP balances were reduced on RP diets compared with HP diets. On average, RDP balance was 19 ± 0.6 g of N/d lower (-7 vs. 12 g of N/d), and RUP balance was 49 ± 14 g of N/d lower (56 vs. 104 g of N/d), for the RP compared with the HP diets, respectively. In the formulated diets, the overall NRC-predicted RUP balance was 26 g of N/d, but when animal performance data were included in the model, the NRC predicted an overall RUP balance of 78 ± 8 g of N/d. The greater-than-anticipated RUP balance was likely due to the lower-than-expected RUP demand for milk TP synthesis because 3.5% FCM was less than anticipated (37 vs. 45 kg/d).

Apparent digestibility. In this study, dietary treatments had no influence on either the apparent total tract DM digestibility ($69.6 \pm 0.8\%$) or the amount of DM apparently digested (16.6 ± 1.0 kg/d). However,

Table 1. Least square means for DMI, nutrient intake, NRC predictions, apparent total tract digestibility, and rumen measurements.

Item	Treatment ¹				SEM	Significance (<i>P</i>)		
	AS-RP	AS-HP	CS-RP	CS-HP		Forage (F)	Protein (P)	F × P
DMI and nutrient intake								
DMI, kg/d	24.8	25.3	23.7	22.2	1.1	<0.01	0.53	0.21
OM intake, kg/d	23.1	23.5	22.1	20.8	1.0	0.02	0.53	0.22
N intake, g/d	656 ^b	729 ^a	620 ^b	616 ^b	31	<0.01	0.10	0.07
NDF intake, kg/d	6.83	6.86	6.25	5.83	0.31	<0.01	0.37	0.29
Starch intake, kg/d	5.91	5.34	6.06	5.23	0.28	0.92	<0.01	0.45
NRC (2001) predictions ²								
RDP balance, g of N/d	−9 ^c	11 ^a	−5 ^b	12 ^a	1	0.01	<0.01	0.02
RUP balance, g of N/d	62	123	51	84	15	0.06	<0.01	0.29
MP balance, g of N/d	50	101	42	71	12	0.10	<0.01	0.31
NE _L intake, Mcal/d	39.9	40.9	38.3	37.2	1.5	0.01	0.94	0.30
Apparent total tract digestibility								
DM, %	69.5	68.1	68.9	71.8	1.9	0.24	0.60	0.11
DM digested, kg/d	17.1	17.2	16.4	16.0	1.1	0.11	0.81	0.63
N, %	68.1	69.8	71.3	75.3	1.8	<0.01	0.04	0.40
N digested, g/d	442	509	444	463	29	0.18	0.01	0.14
Rumen measurements								
NH ₃ N, mg/dL	17.6	17.2	15.4	17.8	2.0	0.65	0.55	0.43
pH	6.06	6.03	5.70	5.77	0.1	<0.01	0.73	0.43

a, b, c Least square means within a row without a common superscript differ ($P \leq 0.05$).

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

²Calculated based on NRC (2001) using actual feed chemical composition, DMI, BW, and yield of 3.5% FCM, and assuming 90 DIM for each cow in this trial. The NRC predictions of the formulated diets can be found in Wattiaux and Karg (2004).

protein concentration in the diet influenced significantly the apparent N digestibility, which was 2.6 ± 1.4 percentage units higher for cows fed HP diets compared with RP diets (Table 1). Using wider ranges in dietary CP, some workers have shown a decrease in apparent N digestibility with a decrease in dietary CP (Wright et al., 1998; Kauffman and St-Pierre, 2001; Broderick, 2003), but not others (Noftsker and St-Pierre, 2003). Also in this trial, the amount of N apparently absorbed (digested) was higher for cows fed the HP diets than for cows fed the RP diets (488 vs. 434 g/d). The estimate of N apparently absorbed has varied with the concentration of dietary CP (Kauffman and St-Pierre, 2001), with the dietary concentration of RUP (in the study of Wright et al. [1998], but not in the study of Castillo et al. [2001b]), with the digestibility of RUP (Noftsker and St-Pierre, 2003), and with the postruminal availability of starch (Reynolds et al., 2001).

In this trial, primary forage source influenced the apparent digestibility of N, which was 4.4 ± 1.4 percentage units higher for cows fed the CS-based diets compared with the AS-based diets. This difference reflected variations in N digestibility of both primary forage source and other dietary ingredients. According to NRC (2001), the intestinal digestibility of RUP in CS is 5 percentage units higher than in AS (70% vs. 65%). Also, in comparison to the AS-based diets, the CS-based diets contained no corn gluten meal (intestinal RUP digest-

ibility = 90%), but more soybean meal (intestinal RUP digestibility = 93%), and 0.5% urea. Primary forage source did not influence the amount of N apparently absorbed (digested), indicating that the higher apparent digestibility of N on the CS-based diets compensated for the lower NI described earlier.

Rumen measurements. Concentration of NH₃ N averaged 17.0 ± 1.9 mg/dL and did not differ among treatments. This result was not surprising because differences in dietary CP were relatively small, and all diets were relatively close to zero RDP balance. Values reported for this trial with samples obtained by rumenocentesis 4 h after feeding were in agreement but, on average, were 3.0 mg/dL higher than those reported for samples obtained throughout the day via a ruminal cannula (Wattiaux and Karg, 2004).

Four hours after feeding, rumen pH averaged 5.90 ± 0.04 and was lower for cows fed CS compared with AS-based diets (5.73 vs. 6.04). When cows were fed the same diets earlier in lactation (DIM = 64), rumen pH obtained via a rumen cannula averaged 6.41 ± 0.14 and did not differ among treatments (Wattiaux and Karg, 2004). Garrett et al. (1999) showed that ruminal pH was 0.28 units lower for fluid collected by rumenocentesis than for fluid collected through a ruminal cannula. Those researchers also defined subacute ruminal acidosis when 25% of sampled cows have a ruminal pH below a cut point of 5.5. In this trial, 5 of 24 cows (21%)

Table 2. Least square means for BW and milk yield responses.

Item	Treatment ¹				SEM	Significance (<i>P</i>)		
	AS-RP	AS-HP	CS-RP	CS-HP		Forage (F)	Protein (P)	F × P
BW, kg	652	668	632	668	19	0.57	0.14	0.57
Milk yield, kg/d	41.7	40.9	45.1	41.1	1.7	0.14	0.06	0.20
3.5% FCM, kg/d	38.9	37.9	37.8	34.4	3.0	0.16	0.17	0.46
Fat, %	3.12	3.02	2.50	2.53	0.30	0.004	0.82	0.71
Fat yield, g/d	1.30	1.29	1.12	1.05	0.16	0.02	0.58	0.72
True protein, %	2.75 ^{ab}	2.66 ^b	2.68 ^{ab}	2.89 ^a	0.08	0.27	0.34	0.04
True protein yield, g/d	1.16	1.12	1.20	1.20	0.06	0.12	0.61	0.56
Lactose, %	4.85	4.80	4.77	4.83	0.08	0.66	0.89	0.34
Lactose yield, g/d	2.04	2.00	2.14	2.03	0.09	0.35	0.29	0.58
MUN ² , mg/dL	11.2	13.4	10.8	12.3	0.6	0.15	0.001	0.47
MUN, g/d	4.7	5.5	4.9	5.1	0.3	0.84	0.09	0.26

^{a, b} Least square means within a row without a common superscript differ ($P \leq 0.05$).

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

²MUN = Milk urea N.

fed the CS-based diets had an average rumen pH <5.5 (but only 1 of these 5 cows was among those removed from the analysis as described earlier). As in this trial, other reports have shown a reduction in DMI with decreased rumen pH (Krajcarski-Hunt et al., 2002; Krause et al., 2002a). Thus, as a consequence of the expected reduction in ruminal fiber digestibility with low rumen pH (Krajcarski-Hunt et al., 2002), the NRC-predicted NE_L values reported in Table 1 may be biased upward for the CS-based diets.

In this trial, diets contained at least 0.4% sodium bicarbonate, NDF content averaged 26 and 27%, and starch content averaged 25 and 23% (DM basis) for the CS- and AS-based diets, respectively. Starch intake did not differ with primary forage source in the diet (but rather with protein level), excluding excess starch as an explanation for rumen acidosis (Krajcarski-Hunt et al., 2002). However, using diets containing 24% NDF and 27% starch, Krause et al. (2002b) observed a decrease in rumen pH from 6.02 to 5.81 when the forage was finely chopped. Unfortunately, neither silage particle size nor cow chewing activity was measured here, but results are consistent with a lack of effective fiber in the CS-based diets. However, results are consistent also with an interaction between primary forage source and dietary tallow (which averaged 2% of diet DM in this trial) in contributing to lower ruminal pH (Onetti et al., 2002; Ruppert et al., 2003).

Milk yield responses. Lactational responses to dietary treatments were studied and reported for the preceding 12-wk period of this trial in Wattiaux and Karg (2004). As in the companion study, the interaction between treatment main effects influenced milk TP percentage, the primary forage source influenced milk fat percentage and milk fat yield, MUN was higher for HP than for RP diets, but 3.5% FCM was not altered by treatments (Table 2). However, in this trial, cows fed

the RP diets produced 2.4 ± 1.3 kg/d more milk than cows fed the HP diets (43.4 vs. 41.0 kg/d; $P = 0.06$). Although the difference in dietary CP was small between the RP and HP diets (16.4% vs. 17.7%), the trend for higher milk yield with reduced CP was unusual. No difference in milk yield was reported when CP in ration DM was lowered from 19.4 to 16.5% (Davidson et al., 2003) or from 18.4 to 16.7% (Broderick, 2003). In the latter study, however, milk yield was reduced with the further reduction in dietary CP from 16.7 to 15.1%. In these reports, actual milk yield averaged (34 kg/d compared with 42.2 kg/d in this trial. This additional 8 kg/d of milk should be considered in interpreting results of this trial, as cows were presumably closer to their full potential for milk production. The trend for higher milk yield of cows fed lower CP diets observed here might have reflected in part the energy diverted away from milk production as a result of the energy cost associated with urea synthesis and increased energy loss in urine (Tyrrell et al., 1970). This contention might have been particularly true for cows fed the AS-based diets. Despite numerically higher DMI (Table 1), cows fed the AS-HP diet produced 0.8 kg less milk (Table 2), but excreted 43 g/d urea N more than cows fed the AS-RP diet. Using estimates of 7.3 kcal/g of N for the energy used in the formation of urea and 4.75 kcal/g of N for the dietary energy (carbon) loss in urine (Tyrrell et al., 1970), cows fed the AS-HP diet had 0.52 Mcal/d ($43 \times [7.3 + 4.75]$) less energy available for milk production compared with cows fed the AS-RP diet. Given the observed milk composition of cows fed the AS-based diets (Table 2), the NE_L required per kg of milk was 0.63 Mcal/kg (NRC, 2001). Thus, the amount of energy lost with the carbon and the energy needed to synthesize urea amounted to about 0.8 kg/d of milk (0.52/0.63). In the case of the CS-based diets, the same

Table 3. Least square means for N balance measurements.

Item	Treatment ¹				SEM	Significance (<i>P</i>)		
	AS-RP	AS-HP	CS-RP	CS-HP		Forage (F)	Protein (P)	F × P
N excretion and retention	(g/d)							
Fecal	205 ^{ab}	220 ^a	177 ^{bc}	150 ^c	12	<0.01	0.60	0.08
Urinary	211	251	206	247	11	0.65	<0.01	0.99
Milk	197	190	208	203	9	0.07	0.34	0.87
Apparently retained	39	68	31	16	19	0.06	0.64	0.15
	(% of N intake)							
Fecal	31.9	30.2	28.7	24.7	1.8	<0.01	0.04	0.40
Urinary	32.4 ^b	34.7 ^b	33.1 ^b	39.9 ^a	1.3	0.02	<0.01	0.09
Milk	30.1 ^b	26.2 ^c	33.8 ^a	33.1 ^a	1.1	<0.01	0.01	0.07
Apparently retained	5.8	8.9	4.3	2.2	2.6	0.08	0.82	0.25
	(% of N apparently absorbed)							
Urinary	46.7	50.2	46.7	53.2	2.3	0.53	0.03	0.52
Milk	45.1	37.8	47.5	44.3	2.0	<0.01	<0.01	0.15
Apparently retained	8.2	12.0	5.6	2.3	3.6	0.05	0.93	0.25

a, b, cLeast square means within a row without a common superscript differ ($P \leq 0.05$).

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

limitation might have occurred, but differences in DMI and energy intake might have contributed also to the observed difference in milk yield.

N Partitioning

Daily N excretion and apparent retention. In this trial NI, FN, UN, milk N, and apparently retained N averaged 658 ± 14 , 188 ± 7 , 229 ± 6 , 200 ± 4 , and 40 ± 9 g/d, respectively. Fecal N excretion tended to be affected by the interaction between forage source and dietary protein concentration with the highest and lowest FN excretion observed for the AS-HP and CS-RP diets, respectively (Table 3).

In this study, dietary protein concentration influenced UN, which was 40.9 ± 9.1 g/d lower for cows fed RP compared with HP diets, but FN and milk N were not affected by concentration of dietary protein. Each of these 3 results is in agreement with the report of Noftsgger and St-Pierre (2003), who worked with dietary CP in the range of 17 to 18.3% (DM basis). Urinary N decreased also, but no difference in milk N or FN was found when dietary CP was reduced from 18 to 16.5% (Broderick, 2003) or from 19.4 to 16.5% (Davidson et al., 2003). However, when dietary CP was reduced further from 16.5 to 15% (Broderick, 2003) and from 17 to 13% (Kauffman and St-Pierre, 2001), all 3 routes of excretion (FN, UN, and milk N) were reduced. As reported by others (Kauffman and St-Pierre, 2001; Noftsgger and St-Pierre, 2003), the amount of apparently retained N was not influenced by dietary protein in this study.

Primary forage source did not influence UN output, but cows fed the AS-based diets excreted 49 ± 12 g/d

more FN and 12 ± 6 g/d less milk N compared with those fed the CS-based diets. These findings were in agreement with those reported by Ruppert et al. (2003), but contrasted those of Santos (2003), who did not detect an effect of primary forage source on FN, UN, and milk N for cows in mid to late lactation producing 27.8 kg/d of milk. Primary forage source also tended to influence the apparently retained N. Values were higher for cows fed AS-based diets compared with CS-based diets, a result similar to that reported by Ruppert et al. (2003).

As a common practice, apparently retained N is calculated as the N unaccounted after milk N, FN, and UN were subtracted from NI. Yet, the true amount of retained N is likely less than the apparent values usually reported. Assuming body tissue contains 17% protein (NRC 2001), the 40 g/d of retained N found here would amount to an accretion of 1.5 kg/d of tissue gain ($40 \times 6.25/0.17$), a value that contrasts to the minimal changes in BW observed in this trial (data not shown). Accepted methodology may be implicated in the discrepancy between apparent and true N retention because balance trials tend to underestimate N excretion relative to intake (Spanghero and Kowalski, 1997). Based on an analysis of 35 published balance studies, those researchers reported a positive bias of 39 g of N/d.

N excretion and retention as a percentage of NI. In this trial, there was a tendency for a differential response to decreasing dietary CP depending on primary forage source when UN was expressed as a percentage of NI. This percentage was reduced with a reduction in CP in CS, but not in AS-based diets (Table 3). Similarly, there was a tendency for milk N as a

percentage of NI to be influenced by the interaction between primary forage source and dietary CP. This percentage was higher for cows fed the CS-based diets than for those fed the AS-based diets (33.8% vs. 28.1%) and increased as a result of a reduction in CP in AS-based diets but not in CS-based diets (Table 3). In separate experiments, others had reported that both UN and milk N expressed as percentages of NI were influenced by the main effect of primary forage source (Ruppert et al., 2003) and the main effect of dietary protein concentration (Kauffman and St-Pierre, 2001; Broderick, 2003). However, the interaction between primary forage source and dietary CP found in this trial had not been reported before. Notwithstanding the partial confounding caused by rumen acidosis on the CS-based diets, these interactions deserve further attention.

Fecal N represented a greater proportion of NI when cows were fed the RP compared with the HP diets (31.2% vs. 27.8%), a result found also in Kauffman and St-Pierre (2001) and Castillo et al. (2001b). This decline was found to be linear for diets varying from 18.4 to 15.1% of CP (Broderick, 2003). Fecal N represented a greater proportion of NI when cows were fed the AS-based diets compared with CS-based diets (31.8% vs. 27%). Primary forage source also tended to influence apparently retained N expressed as a percentage of NI (7.4% vs. 3.4%). Ruppert et al. (2003) found no difference in FN expressed as a percentage of NI between AS- and CS-based diets, but found the same tendency as reported here for the apparently retained N.

N excretion and retention as a percentage of N apparently absorbed. Cows fed the RP diets used 5.7 ± 1.6 units more of the apparently absorbed N for milk, and, correspondingly, the percentage lost in the urine decreased (4.8 ± 2.2). This pattern of change was also found in Kauffman and St-Pierre (2001). In this trial, feeding CS-based diets increased the partition of apparently absorbed N for milk, but lowered the apparently retained N. Ruppert et al. (2003) reported no difference between AS- and CS-based diets on the partition of apparently absorbed N toward milk, but reported a trend similar to our results for the apparently retained N.

Manure Production and Composition

Feces. The average cow on this trial produced 48.8 ± 1.7 kg/d of feces (as-is basis) with an average DM content of $15.3 \pm 0.4\%$ and a pH of 6.45 ± 0.03 . There were no effects of dietary CP concentration on output of fecal DM and concentration of N in feces (Table 4). In contrast, the output of fecal DM and the concentration of N in fecal matter (as-is basis) and in fecal DM were 0.96 ± 0.5 kg/d higher, 0.069 ± 0.015 percentage

units higher, and 0.30 ± 0.05 percentage units higher when cows were fed AS-based diets compared with CS-based diets. Delaquis and Block (1995) also reported higher concentrations of N in feces of cows fed AS- vs. CS-based diets (2.45% vs. 2.33%).

Urine. The average cow on this trial produced 27.9 ± 1.2 L/d of urine with a pH of 8.12 ± 0.02 . Dietary protein did not influence concentration of N in urine, but urine volume was 4.5 ± 2.2 kg/d lower for cows fed RP diets compared with HP diets (25.6 vs. 30.4 L/d). Broderick (2003) and Sannes et al. (2002) have also reported higher urine output with increasing dietary CP. Primary forage source did not influence urine volume, but N concentration in urine was 1.05 ± 0.38 percentage units higher when cows were fed the CS- compared with the AS-based diets (9.00% vs. 7.96%). These results are opposite those reported by Delaquis and Block (1995), who found higher urine output (28.9 vs. 21.5 L/d) but no difference in N concentration (6.12 vs. 6.38 g/L) when cows consumed AS- vs. CS-based diets. Daily urine output depends on the intake of K, Na, and N (Bannink et al., 1999) and can be predicted also from the proportion of concentrates in diet DM (Valadares et al., 1999).

Manure N. Primary forage source influenced total manure N (FN + UN) output, which was 58 ± 20 g/d higher when cows were fed AS- vs. CS-based diets (448 vs. 390 g/d). In this trial, manure N expressed as a percentage of NI was not influenced by dietary treatment and averaged $64.3 \pm 1.1\%$. A value of 69% was reported by Wilkerson et al. (1997) for cows producing 29 kg/d of milk and consuming diets averaging 16.1% CP. Table 4 reports also the UN:FN and the manure N to milk N ratio (**MaN:MkN**) that were calculated as measures of N use efficiency in the context of manure N management on a farm and efficiency of N use by a dairy herd, respectively. When less N is found in the urine relative feces (lower UN:FN), less ammonia loss from manure is expected because UN is more vulnerable to environmental losses than FN. Similarly, a lower MaN:MkN is more desirable because it indicates that less manure N must be managed per unit of milk N produced by the herd. In this trial, feeding the RP diets lowered the UN:FN for both AS- and CS-based diets, but the extent of the reduction was much greater for the CS-based diets. The elevated values for CS-based diets and the particularly high value observed for the CS, HP diet might have reflected, in part, changes in digestives and metabolic processes that resulted from rumen acidosis as discussed previously. Also, in this trial, MaN:MkN was 0.24 ± 0.11 units lower when cows were fed the RP relative to the HP diets and 0.44 ± 0.10 unit lower when fed CS- compared with AS-based diets.

Table 4. Least square means for manure output and composition.

Item	Treatment ¹				SEM	Significance (<i>P</i>)		
	AS-RP	AS-HP	CS-RP	CS-HP		Forage (F)	Protein (P)	F × P
Feces								
Output as excreted, kg/d	51.8	50.6	48.5	43.0	3.8	0.11	0.29	0.51
DM, kg/d	7.4	8.1	7.3	6.2	0.4	0.02	0.61	0.03
N, % of DM	2.78	2.71	2.44	2.44	0.06	<0.01	0.59	0.56
N, % of as excreted	0.43	0.44	0.37	0.37	0.03	<0.01	0.98	0.70
Urine								
Output, L/d	28.0	30.8	23.2	29.9	2.6	0.22	0.04	0.38
N, g/L	7.72	8.18	9.03	8.99	0.46	0.01	0.59	0.52
Manure N								
Manure N ² , g/d	409	471	384	398	18	0.01	0.04	0.19
UN:FN ³	1.02 ^c	1.19 ^b	1.17 ^b	1.66 ^a	0.08	<0.01	<0.01	0.01
MaN:MkN ⁴	2.10	2.49	1.86	1.96	0.10	<0.01	0.01	0.12

a, b, ^cTreatment means within a row without a common superscript differ ($P \leq 0.05$).

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

²Manure N (g/d) = urinary N output (g/d) + fecal N output (g/d).

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

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

Urine urea N. Reducing dietary CP did not influence urine urea N concentration, but influenced daily excretion of urine urea N, which was 39.5 ± 9.0 g/d lower for cows on RP relative to HP diets (Table 5). Urine urea N accounted for 96% of the difference in total UN excretion observed between the RP diets and the HP diets. Corresponding values reported by Sannes et al. (2002) and Broderick (2003) were 96 and 100%, respectively. Also in this trial, urine urea N excretion, expressed as a percentage of UN, manure N, apparently absorbed N, and NI, were significantly lower when cows were fed the RP compared with the HP diets. Primary forage source influenced concentration of urea N in urine, which was 1.05 ± 0.40 g/L lower when cows were fed the AS- compared with CS-based diets. Primary forage source also influenced urine urea N expressed as a percentage of manure N and as a percentage of NI (Table 5).

Prediction of urine N excretion. Recent reports suggested that UN may be predicted from the equation $0.0259 \times \text{MUN (mg/d/L)} \times \text{BW (kg)}$ (Kauffman and St-

Pierre, 2001). Figure 1 shows the predicted and residual UN using MUN concentration and BW of the 48 cows on this trial. Overall, the slope of the regression (0.89 ± 0.03) was different from 1.0, indicating a significant linear bias. The mean UN output was underpredicted by 22 g/d (203 vs. 225 g/d). When the model was applied to the 12 cows on each treatment, the slopes of the regression were 0.90 ± 0.05 , 0.91 ± 0.05 , 0.92 ± 0.06 , and 0.83 ± 0.05 , and the root mean square error was 33, 39, 49, and 39 for AS-RP; AS-HP; CS-RP; and CS-HP diets, respectively. Sannes et al. (2002) reported that the equation of Kauffman and St-Pierre (2001) significantly improved the earlier models, but a mean and linear bias were still present. The residual plot showed that the equation underpredicted UN excretion values >260 g/d and tended to overpredict UN excretion less than approximately 170 g/d (Figure 1). The range in UN excretion was 50 to 250 g/d in the report of Kauffman and St-Pierre (2001), but 150 to 320 g/d in this trial. To test whether the biases could be reduced,

Table 5. Least square means for urine urea N (UUN) excretion.

UUN	Treatment ¹				SEM	Significance (<i>P</i>)		
	AS-RP	AS-HP	CS-RP	CS-HP		Forage (F)	Protein (P)	F × P
Excretion, g/d	169	212	172	208	10	0.94	<0.01	0.70
g/L of urine	6.26	6.95	7.65	7.62	0.47	0.02	0.42	0.38
% urine N	81.2	85.8	81.7	84.8	1.9	0.92	0.05	0.68
% manure N	41.0	45.7	43.8	52.3	1.5	<0.01	<0.01	0.20
% digested N	38.2	43.3	40.1	45.3	2.4	0.41	0.03	0.97
% N intake	26.2	29.7	28.0	33.7	1.3	0.03	<0.01	0.38

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

Figure 1. Predicted vs. observed urinary N (UN) excretion for cows fed the AS-RP (■), AS-HP (□), CS-RP (●), and CS-HP (○) diets, and residual UN excretion (observed minus predicted; +). Predicted UN excretion was calculated as in Kauffman and St-Pierre (2001). The vertical dotted line is the upper limit of the data set used in generating the Kauffman and St-Pierre (2001) equation and shows that observed UN excretion >260 g/d of this trial were underpredicted. The slope of the regression (solid line) was 0.89 and differed ($P < 0.001$) from the expected slope of 1.0 (dashed line) in the absence of a linear prediction bias.

the $\text{MUN} \times \text{BW}$ interaction was regressed against actual UN excretion, forcing the intercept through zero as in Kauffman and St-Pierre (2001). The resulting regression coefficient was 0.0283 ± 0.00076 . Using this coefficient to predict UN yielded a regression with a slope that was not different from 1.0 (0.97 ± 0.03) and a mean prediction that was only 4 g/d lower than the actual value (221 vs. 225 g/d). In addition to the difference in the range of UN excretion, the analytical method used to measure MUN may also be implicated in the differences reported here. All MUN data in this trial were from infrared spectroscopy, which may be biased upward compared with MUN concentration values obtained by wet chemistry as discussed by Kauffman and St-Pierre (2001).

CONCLUSION

Comparing NRC-predicted RDP and RUP balances with and without actual cow performance in the model demonstrated that a lower-than-expected milk yield (41 vs. 45 kg/d) increased predicted RUP balance (78 vs. 28 g of N/d), and a lower-than-expected DMI (24.2 vs. 26.1 kg/d) reduced predicted RDP balance (1 vs. 10 g of N/d). Nevertheless, our attempt to follow NRC guidelines led to a reduction in CP from 18.0 to 16.5% and from 17.3 to 16.4% when AS and CS were the primary forage sources in the diet, respectively. Concentration

of dietary protein had no effect on milk N output; thus, there were no advantages to deviate upward from NRC protein recommendations in formulating diets. Following NRC guidelines lowered urine volume and outputs of UN and urine urea N but did not influence FN output. When cows were fed CS- compared with AS-based diets, milk N and UN outputs were unchanged, but FN was lowered. The amount of manure N and concentration of N in urine and feces were influenced by primary forage source. Results suggested a compounding effect of excess dietary N with low rumen pH as illustrated by significant increases in UN as a percentage of NI and UN:FN when cows were fed the CS-HP diet compared with the CS-RP diet. The tendency for higher milk yield when cows were fed the RP diets, regardless of primary forage source, suggested that excretion of excess dietary N (primarily as excess RUP in this trial) in the form of urea may represent a biologically meaningful energy burden in high-producing cows and, in effect, lowers the net energy of lactation of a diet.

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