Effect of Dietary Crude Protein Concentration on Milk Production and Nitrogen Utilization in Lactating Dairy Cows¹

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ABSTRACT

Forty lactating Holstein cows, including 10 with ruminal cannulas, were blocked by days in milk into 8 groups and then randomly assigned to 1 of 8 incomplete 5 × 5 Latin squares to assess the effects of 5 levels of dietary crude protein (CP) on milk production and N use. Diets contained 25% alfalfa silage, 25% corn silage, and 50% concentrate, on a dry matter (DM) basis. Rolled high-moisture shelled corn was replaced with solvent-extracted soybean meal to increase CP from 13.5 to 15.0, 16.5, 17.9, and 19.4% of DM. Each of the 4 experimental periods lasted 28 d, with 14 d for adaptation and 14 d for data collection. Spot sampling of ruminal digesta, blood, urine, and feces was conducted on d 21 of each period. Intake of DM was not affected by diet but milk fat content as well as ruminal acetate, NH₃, and branched-chain volatile fatty acids, urinary allantoin, and blood and milk urea all increased linearly with increasing CP. Milk and protein yield showed trends for quadratic responses to dietary CP and were, respectively, 38.3 and 1.18 kg/d at 16.5% CP. As a proportion of N intake, urinary N excretion increased from 23.8 to 36.2%, whereas N secreted in milk decreased from 36.5 to 25.4%, as dietary protein increased from 13.5 to 19.4%. Under the conditions of this study, yield of milk and protein were not increased by feeding more than 16.5% CP. The linear increase in urinary N excretion resulted from a sharp decline in N efficiency as dietary CP content increased.

Key words: dietary crude protein, milk production, nitrogen utilization

INTRODUCTION

Producers often feed high CP diets to ensure a sufficient supply of the MP required for maximal milk and protein production of dairy cows. A survey of 6 Wisconsin dairy farms with mean rolling herd averages of 14,140 kg indicated lactating cows were fed diets averaging 19.1% CP of DM, with a range from 18.0 to 21.5% (Gunderson et al., 1998). However, several studies have reported no improvement in milk and protein production when dietary CP was increased from 16.1–16.7% to 18.4–18.9% (Cunningham et al., 1996; Broderick, 2003; Leonard et al., 2003).

It is well established that, as the CP content of the diet increases, the amount of protein degraded in the rumen also increases. If RDP exceeds microbial needs, then large amounts of NH₃ are produced, absorbed into the blood, converted to urea in the liver, and excreted in the urine. In the manure, urinary urea can be rapidly hydrolyzed to NH₃ and lost by volatilization to the environment (Muck, 1982). Overfeeding CP also reduces profit margins because of the relatively high cost of protein supplements and the poor efficiency of N use by dairy cows fed high protein diets (Broderick, 2003).

Although a wide range of RDP values have been reported for solvent-extracted soybean meal (SSBM), the most common protein source fed to dairy cows in the United States, an overall mean of about 65% is given by NRC (2001). Substitution of high RUP sources in dairy diets often has been used to increase MP flow to the small intestine. However, reviews by Santos et al. (1998) and Ipharraguerre and Clark (2005) summarized data showing that adding RUP to the diet, at the expense of SSBM, often had little effect on MP supply because of depressed ruminal formation of microbial NAN. A recent trial indicated that omasal flow of bacterial and total NAN decreased linearly, despite increased flow of nonmicrobial NAN, when lignosulfonate-treated soybean meal replaced SSBM in the diet (Reynal and Broderick, 2005).

Therefore, the objective of this study was to determine the optimum CP content of the diet required to maximize production of milk and protein at minimal N excretion by cows fed diets formulated from typical US ingredients.

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Table 1. Composition of feeds¹

Item	Alfalfa silage		Corn silage		RHMSC		SSBM		RSB	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
DM, %	44.2	5.8	41.2	2.7	72.4	1.6	91.1	0.9	97.7	0.5
CP, % of DM	22.9	1.2	7.23	0.60	8.44	0.62	52.0	2.7	40.0	0.7
Ash, % of DM	10.7	0.8	4.13	0.36	1.95	0.28	7.52	0.19	5.40	0.58
NDF, % of DM	35.8	4.4	36.1	2.1	8.32	0.75	8.06	0.46	23.9	0.9
ADF, % of DM	27.0	3.8	18.3	0.8	1.97	0.18	4.57	0.41	4.04	1.40
Neutral detergent insoluble CP, % of DM	1.41	0.76	0.28	0.03	0.18	0.08	0.26	0.10	3.30	0.50
pH	4.71	0.22	3.89	0.08	4.38	0.07	—	—	—	—
NPN, % of total N	46.3	6.9	55.9	7.6	31.3	4.6	—	—	—	—
NH ₃ N, % of total N	6.48	3.55	8.69	1.76	3.64	1.27	—	—	—	—
Free AA N, % of total N	25.9	4.7	36.3	6.3	20.3	2.9	—	—	—	—
Unidentified NAN, % of total NPN	14.0	1.9	10.9	1.6	7.32	1.18	—	—	—	—

¹RHMSC = Rolled high-moisture shelled corn; RSB = roasted soybeans; and SSBM = solvent-extracted soybean meal.

MATERIALS AND METHODS

Experimental Procedure

Thirty-eight multiparous and 2 primiparous Holstein cows (8 multiparous and 2 primiparous cows were ruminally cannulated) averaging 120 DIM (SD 76), 2.5 parity (SD 1.1), 589 kg of BW (SD 58), and 41 kg of milk/d (SD 6) were blocked by DIM into 8 groups (2 groups of ruminally cannulated animals) then randomly assigned to 1 of 8 diet sequences in an incomplete 5 × 5 Latin square (5 diets and 4 periods). This design, rather than a complete 5 × 5 Latin square, was used because there was insufficient alfalfa and corn silage to complete a fifth period. The duration of each experimental period was 28 d with 14 d for diet adaptation and 14 d for collection of data. Cows were held in tie stalls for the duration of the experiment, had free access to water, and were weighed on 3 consecutive days at the beginning and at the end of each period. All animals were injected with recombinant bST (500 mg of Posilac; Monsanto, St. Louis, MO) every 14 d starting on the first day of the experiment. Animal care and experimental procedures met the requirements of the Institutional Animal Care and Use Committee of the UW-Madison (Research Animal Resources Center protocol # A-07-3400-A00286). Two intact cows had sharp drops in milk yield during the last 2 wk of period 1 and were removed from the experiment; one ruminally cannulated cow was removed from the trial after period 3 for health reasons unrelated to the experiment.

The experimental diets were fed as TMR and contained 25% alfalfa silage, 25% corn silage, and 50% of a concentrate (DM basis) formulated principally from rolled high-moisture shelled corn (RHMSC), SSBM, and roasted soybeans. Dietary CP was increased in increments of approximately 1.5 percentage units, from 13.5 to 19.4%, by replacing RHMSC with SSBM. Cows were fed once daily at about 1600 h and feed offered was

adjusted daily to yield 5 to 10%orts. Samples of individual feeds and orts (about 0.5 kg) were taken daily, and stored at −20°C. Weekly composite samples from feeds and orts were dried at 60°C for 48 h and the as-fed composition of the diets was adjusted every week. Weekly feed composites were ground through a 1-mm screen (Wiley mill, Arthur H. Thomas, Philadelphia, PA) and analyzed for DM at 105°C (AOAC, 1980) and for total N (Leco 2000; Leco Instruments, Inc., St. Joseph, MI) to adjust diets to the desired CP content (total N × 6.25) every week. Intake of DM was corrected for orts and recorded daily throughout the experiment. Feed samples were analyzed sequentially for NDF and ADF (Van Soest et al., 1991) using heat-stable amylase and sodium sulfite (Hintz et al., 1995) in an Ankom Fiber Analyzer (Ankom Technology Corp., Fairport, NY). The N content of NDF residues was analyzed by combustion assay (Leco Instruments Inc.). Ash and OM contents of feeds were also measured (AOAC, 1980). Weekly samples of alfalfa silage, corn silage, and RHMSC were thawed, water extracts were prepared (Muck, 1987), and pH was measured. Extracts were then deproteinized and analyzed for NPN as described by Muck (1987), and for NH₃ and total free AA by flow-injection analysis as described by Broderick et al. (2004). Chemical composition of the principal dietary ingredients and the composition of the diets, reported in Tables 1 and 2, are averages from wk 3 and wk 4 of all 4 experimental periods.

Cows were milked twice daily and milk weights were recorded at each milking. On d 20 and 27 of each period, a.m. and p.m. milk samples were collected, preserved with 2-bromo-2-nitropropane-1,3-diol, and analyzed for fat, true protein, lactose, SNF, and MUN content by infrared methods (AgSource Laboratory, Verona, WI). On d 22 of each period, samples of rumen fluid were collected from 2 locations in the midventral ruminal sac of cannulated cows starting at 0 (just before feeding), 1, 2, 4, 8, 12, 18, and 24 h after feeding. Samples were

Table 2. Composition of diets

Item ¹	Diets				
	A	B	C	D	E
	(% of DM)				
Alfalfa silage	25.0	25.0	25.0	25.0	25.0
Corn silage	25.0	25.0	25.0	25.0	25.0
RHMSC	44.0	40.6	37.2	33.8	30.4
SSBM	2.4	5.8	9.2	12.6	16.0
Roasted soybeans	2.5	2.5	2.5	2.5	2.5
Sodium bicarbonate	0.6	0.6	0.6	0.6	0.6
Salt	0.2	0.2	0.2	0.2	0.2
Dicalcium phosphate	0.2	0.2	0.2	0.2	0.2
Vitamin-mineral premix ²	0.1	0.1	0.1	0.1	0.1
Chemical composition					
DM, %	54.1	54.3	54.6	54.9	55.2
CP	13.5	15.0	16.5	17.9	19.4
Ash	5.97	6.16	6.35	6.54	6.73
NDF	22.4	22.4	22.4	22.4	22.4
ADF	12.4	12.5	12.6	12.7	12.8
NDICP	0.50	0.55	0.59	0.63	0.68
RDP ³	9.3	10.2	11.0	11.9	12.7
RUP ³	4.2	4.8	5.5	6.0	6.7
NFC ³	55.0	53.5	51.9	50.4	48.8
Discounted total digestible nutrients ³	66.2	65.9	65.5	65.8	65.4
NE _L , ³ Mcal/kg of DM	1.59	1.59	1.60	1.62	1.62

¹NDICP = Neutral detergent insoluble CP; RHMSC = rolled high-moisture shelled corn; and SSBM = solvent-extracted soybean meal.

²Provided per kilogram of DM: 56 mg of Zn, 46 mg of Mn, 22 mg of Fe, 12 mg of Cu, 0.9 mg of I, 0.4 mg of Co, 0.3 mg of Se, 6,440 IU of vitamin A, 2,000 IU of vitamin D, and 16 IU of vitamin E.

³Computed using NRC (2001) model based on actual composition of feeds, least squares means of actual DMI, milk yield, and milk composition for each diet, and overall average BW (614 kg).

withdrawn using a 60-mL plastic syringe with a regular Luer tip using a metal filter probe. The filter of the probe, a cylinder 7.5 cm long and 1.8 cm in diameter with 1.0-mm diameter holes, was welded to a 1 m long metal tube with internal diameter of 0.5 cm. The pH of the samples was measured immediately, and 20 mL of rumen fluid was acidified with 0.4 mL of 50% (vol/vol) sulfuric acid and stored at -20°C for later analysis of VFA (Brotz and Schaefer, 1987), total free AA, and NH_3 (Broderick et al., 2004). Blood was sampled into heparinized test tubes from the coccygeal artery or vein of each cow at 4 h after feeding on d 21 of each period; heparinized blood was held on ice until returning to the laboratory. Blood was deproteinized by mixing 1.25 mL of 25% (wt/vol) TCA with 5 mL of whole blood and then centrifuging ($15,000 \times g$, 4°C , 15 min); supernatants were stored at -20°C until analyzed for urea (Broderick and Clayton, 1997).

Spot urine and fecal samples were collected from every cow on d 21 of each period at about 6 h before and after feeding. Previously, this spot-sampling scheme yielded estimates of purine derivative excretion that were similar to total urine collection (Valadares et al., 1999). Fecal samples were dried in a forced-draft oven at 60°C for 72 h and ground through a 1-mm screen (Wiley mill). Equal amounts of DM from the a.m. and p.m. samples from

each cow were composited to yield 29 to 32 separate samples per diet, and analyzed for DM (105°C), ash, OM, NDF, ADF, and N as described earlier for feeds. Total tract apparent digestibility of nutrients was estimated using the indigestible ADF content (ADF remaining after 12-d in situ incubations; Huhtanen et al., 1994) in feces and TMR as an internal marker. Urine samples (15 mL) were acidified with 60 mL of 0.072 N H_2SO_4 , and immediately stored at -20°C . After thawing, urine samples were analyzed for total N (Mitsubishi TN-05 Nitrogen Analyzer; Mitsubishi Chemical Corp., Tokyo, Japan), and for urea (Broderick and Clayton, 1997) and creatinine (Oser, 1965) by flow injection (Lachat Quick-Chem 8000 FIA; Zellweger Analytical, Milwaukee, WI). Urine volume was computed using creatinine as a marker assuming a creatinine excretion of 29 mg/kg of BW per day (Valadares et al., 1999). Allantoin (Vogels and van der Drift, 1970) and uric acid (kit no. 1830, Thermo DMA, Waltham, MA) were also determined in urine using assays adapted to a 96-well plate reader.

Statistical Analyses

Average intake and milk production data from each cow over the last 14 d of each period were analyzed as a Latin square design using the mixed procedures of

SAS (SAS Institute, 1999). Because of missing data, there were 31 observations each for diets B and E, 30 observations for diets A and D, and 29 observations for diet C. Model sums of squares were separated into overall mean, cow (within square), square, period, diet (treatment effect), and square $\times$ treatment interaction. All variables were considered fixed, except cow (within square) and overall error, which were considered random. The interaction term square $\times$ treatment was removed from the model when $P \geq 0.25$. Linear and quadratic effects of treatments were also estimated. Eight complete sets of ruminal samples were obtained on diets A, B, D, and E, but only 7 sets for diet C because one ruminally cannulated cow did not complete period 4. Model sums of squares for data collected at different times after feeding (ruminal pH and concentrations of VFA, NH_3 , and total free AA) were separated into overall mean, cow (within square), square, period, diet (treatment effect), square $\times$ treatment interaction, whole-plot error, hours postfeeding (repeated measures), hours postfeeding $\times$ treatment interaction, and subplot error. Repeated measures analyses were performed using the SP(POW) structure of SAS. All variables were considered fixed, except cow (within square), whole-plot error, and subplot error, which were considered random. The interaction term square $\times$ treatment was removed from the model when $P \geq 0.25$. Linear and quadratic effects of treatments were also estimated. Significance was declared at $P \leq 0.05$ and trends at $P \leq 0.10$. Variables showing quadratic effects that were significant at $P \leq 0.10$ were regressed on dietary CP concentration using the mixed procedures of SAS (SAS Institute, 1999) to obtain intercept and linear and quadratic coefficients of the regression model. These equations were solved for dietary CP concentrations at which these variables were maximal or minimal.

RESULTS AND DISCUSSION

Composition of Diets

The composition of dietary ingredients is presented in Table 1. Low NDF and ADF contents of the alfalfa silage and corn silage (Table 1) indicated that both forages were of very high quality. Mean NPN content of alfalfa silage was 46.3% of total N, which was lower than the average of 54% from 19 experiments reported by Broderick (1995). The lower NPN content may have improved CP use in the current trial (Nagel and Broderick, 1992). The RHMSC, roasted soybeans, and SSBM contained 8.4, 40.0, and 52.0% CP (DM basis), respectively. Low variation in CP content of all dietary ingredients (Table 1) made it possible to maintain the 5 experimental diets close to target CP concentrations throughout the 16 wk of the trial (Table 2). The NDF and ADF contents of the

diets were similar among treatments but probably did not meet the requirements of these cows because the fiber levels in both the alfalfa and corn silages were about 10 percentage units below the averages reported in NRC (2001). Conversely, the estimated NFC contents (NRC, 2001) of all diets were quite high (49 to 55% of DM) compared with NRC (2001) recommendations (42% DM). However, no apparent detrimental effects of diets were observed either on ruminal pH or on cow health.

Milk Yield and Composition

Although CP content of diets fed in this trial covered a wide range (13.5 to 19.4% of DM), only a few production traits were affected (Table 3). Intake of DM, yield of milk, and FCM were not significantly affected. However, milk and FCM yields showed trends for quadratic ($P = 0.10$) and linear ($P = 0.10$) responses to dietary CP, respectively. Milk yield increased from 36.3 kg/d at 13.5% CP to 38.3 kg/d at 16.5% CP, then declined to 36.6 and 37.0 kg/d at 17.9 and 19.4% CP, respectively. Protein and fat yields also showed quadratic ($P = 0.09$) and linear ($P = 0.06$) trends; both traits reached maxima (1.18 and 1.24 kg/d) at 16.5% CP, with no further improvement at higher dietary CP. Fat content of milk increased linearly ($P < 0.01$), and SNF showed a linear trend ($P = 0.08$), with increasing CP content of the diet but there was no effect of dietary CP on milk protein content, lactose content and yield, SNF yield, BW change, or feed efficiency (milk/DMI).

In agreement with our findings, Cunningham et al. (1996) and Leonardi et al. (2003) observed no effect of dietary CP content on DMI and milk yield of dairy cows when dietary CP was increased from 16.5 to 18.5% and from 16.1 to 18.9%, respectively. On the other hand, Broderick (2003) reported a linear increase in DMI when dietary CP was increased from 15.1 to 16.7 and 18.3%; however, milk yield increased from 33.0 to 34.1 kg/d only with the first CP increment, with no further change at 18.3% CP, resulting in lower feed efficiency (milk/DMI) at the highest CP. Broderick (2003) also reported that yields of fat and protein improved when the dietary CP increased from 15.1 to 16.7 but with no further increase at 18.4% CP. Leonardi et al. (2003) found that protein yield was unaffected (1.35 and 1.34 kg/d) and milk protein content actually decreased (3.25 and 3.18%) when dietary CP was increased from 16.1 to 18.9%; however, fat content and yield increased significantly in response to dietary CP. These reports and the current trial are consistent with the pattern shown in the much larger databases of NRC (2001) and the reviews of Huhtanen and Shingfield (2005) and Ipharraguerre and Clark (2005), indicating that milk yield increased at a substantially lower rate at higher dietary CP than at lower

Table 3. Effect of dietary CP content on milk production and composition

Item	Dietary CP, % of DM					SE ²	<i>P</i> > F ¹		
	13.5	15.0	16.5	17.9	19.4		Diet	L	Q
DM intake, kg/d	22.3	22.2	23.0	22.3	22.9	0.5	0.25	0.22	0.93
Milk yield, kg/d	36.3	37.2	38.3	36.6	37.0	1.01	0.17	0.65	0.10
3.5% FCM, kg/d	34.2	35.6	36.7	35.7	36.1	1.09	0.22	0.10	0.16
BW change, kg/d	0.49	0.46	0.70	0.55	0.64	0.11	0.49	0.24	0.74
Milk/DMI	1.71	1.71	1.71	1.71	1.72	0.04	1.00	0.89	0.96
Milk composition and yield									
Fat, %	3.14 ^b	3.27 ^{ab}	3.27 ^{ab}	3.47 ^a	3.44 ^a	0.14	0.02	<0.01	0.91
Fat yield, kg/d	1.14	1.20	1.24	1.23	1.24	0.05	0.29	0.06	0.29
True protein, %	3.09	3.15	3.09	3.18	3.16	0.05	0.13	0.12	0.98
True protein yield, kg/d	1.10	1.15	1.18	1.13	1.15	0.03	0.10	0.24	0.09
Lactose, %	4.91	4.89	4.94	4.91	4.92	0.04	0.71	0.58	0.95
Lactose yield, kg/d	1.78	1.81	1.91	1.78	1.82	0.06	0.13	0.64	0.15
SNF, %	8.92	8.96	8.93	9.01	9.00	0.07	0.33	0.08	0.94
SNF yield, kg/d	3.21	3.30	3.44	3.24	3.31	0.09	0.11	0.47	0.11

^{a,b}Means in the same row without common superscripts differ ($P < 0.05$).

¹Probability of a significant effect of dietary CP or of a linear (L) or quadratic (Q) effect of dietary CP.

²Standard error of the least squares means.

dietary CP concentrations. Therefore, it appears that, under some circumstances, feeding diets with about 16.0% or less CP (DM basis) provides insufficient MP for maximal milk synthesis, but feeding diets with more than 17.0% CP does not improve milk yield.

Intake, Digestibility, and Ruminal Metabolism

Intake of ADF was significantly altered by diet ($P = 0.02$), including a linear increase ($P < 0.01$) with increasing CP content (Table 4), which probably reflected the replacement of RHMSC (2.0% ADF) with SSBM (4.6% ADF). Apparent total tract digestibility of DM, OM, NDF, and ADF all showed quadratic responses ($P < 0.01$) to increasing dietary CP (Table 4). Digestibilities were

lowest at 13.5%, intermediate at 17.9 and 19.4%, and maximal at 15.0 and 16.5% CP. If RDP supply is lower than the minimum required for microbial growth, intake may be restricted because of depressed ruminal digestion, especially of fiber. Feeding more RDP increases the deamination of AA in the rumen and the supply of branched-chain VFA, which may improve fiber digestion (Misra and Thakur, 2001). The linear increase in acetate concentration in the rumen with increasing dietary CP (Table 5) also suggested that cellulolytic activity was increased and may be related to the linear increase in milk fat content (Table 3). Ruminal VFA concentrations are related but not equivalent to rates of VFA production and absorption (Sutton et al., 2003) and acetate supply may not directly influence milk fat secretion (Bauman

Table 4. Effect of dietary CP content on intake and estimated apparent digestibility

Item	Dietary CP, % of DM					SE ²	<i>P</i> > F ¹		
	13.5	15.0	16.5	17.9	19.4		Diet	L	Q
Intake, kg/d									
DM	22.3	22.2	23.0	22.3	22.9	0.5	0.25	0.22	0.93
OM	21.0	20.8	21.5	20.8	21.3	0.4	0.35	0.42	0.94
CP	3.02 ^c	3.32 ^d	3.78 ^c	4.00 ^b	4.44 ^a	0.08	<0.01	<0.01	0.76
NDF	5.01	4.97	5.15	4.99	5.13	0.11	0.27	0.25	0.92
ADF	2.77 ^b	2.77 ^b	2.90 ^a	2.83 ^{ab}	2.92 ^a	0.06	0.02	<0.01	0.91
Apparent total tract digestibility, ³ %									
DM	71.2 ^c	74.6 ^a	74.0 ^a	72.6 ^b	72.4 ^b	0.5	<0.01	0.75	<0.01
OM	72.1 ^c	75.3 ^a	74.9 ^a	73.5 ^b	73.4 ^b	0.5	<0.01	0.61	<0.01
CP	59.7 ^c	67.1 ^b	68.0 ^{ab}	69.5 ^a	70.4 ^a	1.0	<0.01	<0.01	<0.01
NDF	39.8 ^c	46.8 ^a	46.3 ^a	42.8 ^b	43.6 ^b	0.8	<0.01	0.16	<0.01
ADF	38.1 ^c	45.4 ^a	44.5 ^a	40.8 ^b	42.2 ^b	0.8	<0.01	0.15	<0.01

^{a-c}Means in the same row without common superscripts differ ($P < 0.05$).

¹Probability of a significant effect of dietary CP or of a linear (L) or quadratic (Q) effect of dietary CP.

²Standard error of the least squares means.

³Estimated using the indigestible ADF content in TMR and feces as an internal marker.

Table 5. Effect of dietary CP content on ruminal metabolites

Item	Dietary CP, % of DM					SE ²	P > F ¹		
	13.5	15.0	16.5	17.9	19.4		Diet	L	Q
pH	6.42	6.41	6.39	6.40	6.44	0.06	0.93	0.86	0.34
NH ₃ N, mg/dL	6.1 ^d	7.7 ^c	9.2 ^b	12.7 ^a	12.8 ^a	0.7	<0.01	<0.01	0.56
Free AA, mM	4.69	4.63	5.13	5.36	4.66	0.50	0.71	0.64	0.40
Acetate (A), mM	45.1	48.4	49.0	49.9	50.9	2.2	0.29	0.03	0.56
Propionate (P), mM	18.5	20.1	20.9	19.0	18.5	1.4	0.28	0.74	0.05
A:P	2.63 ^c	2.58 ^c	2.50 ^c	2.77 ^b	2.91 ^a	0.14	<0.01	<0.01	<0.01
Butyrate, mM	9.7	9.5	9.9	10.6	10.1	0.5	0.58	0.21	0.90
Isobutyrate, mM	1.03 ^b	1.10 ^{ab}	1.13 ^{ab}	1.17 ^a	1.20 ^a	0.05	0.02	<0.01	0.58
Valerate, mM	2.12	2.06	2.15	1.88	1.76	0.22	0.35	0.06	0.44
Isovalerate, mM	1.54	1.70	1.72	1.74	1.74	0.09	0.19	0.04	0.24
Total VFA, mM	78.0	83.0	84.7	84.1	84.3	4.0	0.58	0.17	0.33

^{a-d}Means in the same row without common superscripts differ ($P < 0.05$).

¹Probability of a significant effect of dietary CP or of a linear (L) or quadratic (Q) effect of dietary CP.

²Standard error of the least squares means.

and Griinari, 2001). However, there was a linear trend for increased fat yield with increasing dietary CP (Table 3) that paralleled the linear increases in ADF digestibility (Table 4) and ruminal acetate concentration (Table 5). Cunningham et al. (1996) found linear increases in NDF and ADF intake when increasing the CP content of the diet from 14.4 to 16.4 and 18.4% but no effects on ruminal digestibility of fiber or OM. Christensen et al. (1993) did not detect improvement in intake or apparent ruminal digestibility of OM, NDF, and ADF when increasing the CP content of the diet from 16.4 to 19.6% of DM. Total tract digestibility of CP showed a linear and quadratic response to dietary CP and its maximum was observed on the 19.4% CP diet. This probably reflected the progressively greater dilution of metabolic fecal N with increasing dietary SSBM, thus increasing apparent N digestibility on the higher CP diets (Holter et al., 1982).

Ruminal pH, and concentrations of total free AA, butyrate, and total VFA were not affected by the percentage of CP in the diet. However, concentrations of NH₃, acetate, isobutyrate, and isovalerate all increased linearly, and that of propionate increased quadratically, in response to dietary CP (Table 5). Concentration of valerate showed a trend ($P = 0.06$) for a linear decline. Acetate:propionate ratio also showed a quadratic response to dietary CP level; however, the square $\times$ treatment interaction was significant ($P = 0.02$) for this variable. Linear increases in ruminal concentration of NH₃ and branched-chain VFA were not unexpected because SSBM, which was added to increase CP content of diets, is high in RDP (NRC, 2001; Reynal and Broderick, 2003). As dietary CP and RDP increased, there would be greater deamination of AA released from protein degradation, which would elevate concentrations of these metabolites. Sannes et al. (2002) observed higher concentrations of NH₃ and branched-chain VFA when the CP content of

the diets was increased from 17.2 to 19.1%. Cunningham et al. (1996) and Christensen et al. (1993) also found higher concentrations of NH₃ in the rumen in response to higher CP supplementation; however, concentrations of branched-chain VFA were not altered in those studies.

Nitrogen Metabolism and Excretion

Data on variables related to N metabolism and use are in Table 6. Concentrations of BUN and MUN, urine volume, and urinary excretion of total N and urea N all increased significantly in response to dietary CP content. Purine derivative (allantoin plus uric acid) excretion showed a linear trend in response to increasing CP content of the diets, whereas excretion of N in feces as a proportion of N intake showed linear and quadratic declines.

High concentrations of blood urea, the end product of N metabolism, are indicative of inefficient use of dietary CP by dairy cows (Broderick and Clayton, 1997). Blood urea rapidly equilibrates with milk (Gustafsson and Palmquist, 1993), resulting in a high correlation between these 2 variables (Broderick and Clayton, 1997). In the present study, blood urea N increased from 10.7 to 24.0 mg/dL, and MUN increased from 7.7 to 15.6 mg/dL, when CP content of the diet increased from 13.5 to 19.4%. The correlation between these 2 variables was high ($r = 0.83$).

Secretion of milk protein N (milk true protein/6.38; Table 6), of course, showed the same quadratic trend ($P = 0.09$) in response to dietary CP as milk protein yield (Table 3). There was a highly significant linear decline in apparent N efficiency (milk protein N/N intake) as dietary CP increased, decreasing from 36.5% at 13.5% CP to 25.4% at 19.4% CP. Increasing CP from 16.5 to 19.4% depressed N efficiency by 5.4 percentage units. Broderick (2003) found that N efficiency decreased from

Table 6. Effect of dietary CP content on N metabolism

Item	Dietary CP, % of DM					SE ²	<i>P</i> > F ¹		
	13.5	15.0	16.5	17.9	19.4		Diet	L	Q
N intake, g/d	483 ^e	531 ^d	605 ^c	641 ^b	711 ^a	13	<0.01	<0.01	0.75
BUN, mg/dL	10.7 ^e	13.4 ^d	17.1 ^c	21.2 ^b	24.0 ^a	0.7	<0.01	<0.01	0.69
MUN, mg/dL	7.7 ^d	8.5 ^d	11.2 ^c	13.0 ^b	15.6 ^a	0.5	<0.01	<0.01	0.08
Milk protein N, ³ g/d	173	180	185	177	180	5	0.11	0.25	0.09
Milk protein N, % of N intake	36.5 ^a	34.0 ^b	30.8 ^c	27.5 ^d	25.4 ^e	0.8	<0.01	<0.01	0.80
Urinary excretion									
Urine volume, L/d	17.3 ^{bc}	15.4 ^c	17.9 ^b	19.4 ^{ab}	21.7 ^a	1.0	<0.01	<0.01	0.02
Total N, g/d	113 ^e	140 ^d	180 ^c	213 ^b	257 ^a	7	<0.01	<0.01	0.15
Total N, % of N intake	23.8 ^e	26.6 ^d	29.8 ^c	33.2 ^b	36.2 ^a	0.9	<0.01	<0.01	0.79
Urea N, g/d	63 ^e	91 ^d	128 ^c	174 ^b	208 ^a	5	<0.01	<0.01	0.19
Urea N, % of total urinary N	55.4 ^d	64.7 ^c	72.1 ^b	81.8 ^a	81.8 ^a	1.3	<0.01	<0.01	<0.01
Allantoin, mmol/d	286	303	315	312	315	11	0.12	0.02	0.21
Uric acid, mmol/d	29.0 ^a	29.8 ^a	26.3 ^{ab}	24.8 ^b	24.0 ^b	1.5	<0.01	<0.01	0.77
Purine derivatives, mmol/d	314	333	341	336	339	12	0.30	0.08	0.22
Fecal excretion									
DM, kg/d	6.50 ^a	5.65 ^c	6.03 ^{bc}	6.12 ^{ab}	6.31 ^{ab}	0.21	<0.01	0.82	<0.01
N, g/d	196 ^a	176 ^b	196 ^a	197 ^a	210 ^a	8	<0.01	0.01	0.04
N, % of N intake	40.3 ^a	32.9 ^b	32.0 ^{bc}	30.5 ^{cd}	29.6 ^d	1.0	<0.01	<0.01	<0.01

^{a-e}Means in the same rows without common superscripts differ ($P < 0.05$)

¹Probability of a significant effect of dietary CP or of a linear (L) or quadratic (Q) effect of dietary CP.

²Standard error of the least squares means.

³Milk protein N = milk true protein/6.38.

30.3 to 27.0 and 23.4% when the CP content of the diet was increased from 15.1 to 16.7 and 18.4%.

Estimated urine volume increased from 17.3 to 21.7 L/d in response to higher CP supplementation. This has also been observed in a number of total collection studies. For instance, Sannes et al. (2002) reported that urinary excretion increased from 22.2 to 25.6 L/d when dietary CP was increased from 17.2 to 19.1%. These data indicated that greater urine volume was required for excreting the excess of N consumed by the cows (Holter et al., 1982). As the CP content of the diet increased, the proportion of N intake excreted in the urine also increased, going from 23.8% of N intake at 13.5% CP to 36.2% at 19.4% CP. Moreover, an elevated proportion of the urinary N was excreted as urea: urea N increased from 55.4% of total urinary N at 13.5% CP to 81.8% at 19.4% CP. These results clearly showed that any increase on N intake in diets higher than 16.5% CP was lost mainly as urinary urea.

Castillo et al. (2001), from an extensive review of published studies, reported that on average, 72% of the N consumed by dairy cows was excreted in feces and urine and that there was a linear relationship between N intake and N excreted in feces and urine. Moreover, above 400 g/d of N intake, the proportion of N excreted in urine increased exponentially whereas proportionate N output in feces and milk declined linearly. Castillo et al. (2001) suggested that a reduction in dietary CP from 19.0 to 15.0% of diet DM would reduce urinary N excretion from 225 to 151 g/d without significantly altering milk production.

Urinary excretion of purine derivatives, of which allantoin is the major component, reflects microbial nucleic acid absorption from the small intestine and is related to microbial protein formation in the rumen (Stangas-singer et al., 1995). There was a linear increase ($P = 0.02$) in urinary allantoin excretion, and a trend ($P = 0.09$) for a linear increase in excretion of total purine derivatives, with increasing dietary CP (Table 6). However, excretion of allantoin and total purine derivatives did not increase numerically above 16.5% dietary CP, suggesting no elevation in bacterial CP formation in the rumen beyond this point. Ruminal NH_3 N concentrations less than 5 mg/dL may limit microbial protein formation (Satter and Slyter, 1974). Over the course of the day, ruminal NH_3 N was less than 5 mg/dL for several hours on both 13.5 and 15.0% CP diets but was never lower than this concentration on the other 3 diets (Figure 1). Direct determinations of microbial protein flow from the rumen in this trial, measured using ^{15}N as microbial marker and omasal sampling, are reported in the companion paper (Olmos Colmenero and Broderick, 2006).

Optimal Dietary CP

Regression coefficients as well as estimated optima of dietary CP concentration for the 13 variables showing quadratic responses ($P \leq 0.10$) to dietary CP level are in Table 7. Dietary CP optima ranged from 14.7 to 20.9% with an overall mean of 17.0%. However, optima for milk and protein yield, the most economically important responses, were 16.7 and 17.1% CP, respectively. Quad-

Figure 1. Effect of dietary CP content on ruminal ammonia concentrations after feeding (error bars are ± 1 SEM).

ratic responses in yield of milk and protein to increasing dietary CP were obtained using large databases by NRC (2001), Huhtanen and Shingfield (2005), and Ipharreguerre and Clark (2005). However, all 3 sources reported regressions indicating that milk and protein production was maximal when the diet contained from 21 to 23% CP. The optima for apparent total tract digestibility of DM, OM, NDF, and ADF averaged 16.6% CP. The optima (minima) for the variables related to N excretion in feces were, on average, 16.8% CP.

CONCLUSIONS

When feeding diets based on alfalfa and corn silages, with RHMSC as the main source of NFC and SSBM as

the main supplemental protein, 16.5% CP was sufficient for maximizing production of milk and protein. A linear increase in urinary N excretion resulted in a substantial decline in N efficiency as dietary CP content was increased from 13.5 to 19.4%. Results from this study indicated that diets containing 16.5% CP supported maximal production in dairy cows with minimal N excretion to the environment compared with diets with higher CP content.

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Table 7. Regression coefficients and optimal dietary CP concentrations for traits with significant quadratic effects ($P \leq 0.10$)

Item	Intercept	SE	Linear		Quadratic		Dietary CP, % of DM		P^1
			Coefficient	SE	Coefficient	SE	Maximum	Minimum	
Milk yield, kg/d	-1.26	20.09	4.16	2.45	-0.12	0.07	16.7		0.10
True protein yield, kg/d	-0.17	0.69	0.15	0.08	-0.004	0.003	17.1		0.09
Ruminal propionate, mM	-43.0	30.2	7.27	3.72	-0.22	0.11	16.3		0.05
Total tract DM digestibility, %	-0.15	15.05	8.55	1.85	-0.26	0.06	16.5		<0.01
Total tract OM digestibility, %	3.24	14.30	8.24	1.75	-0.25	0.05	16.6		<0.01
Total tract NDF digestibility, %	-96.6	26.5	16.8	3.2	-0.50	0.10	16.7		<0.01
Total tract ADF digestibility, %	-89.4	26.9	16.0	3.3	-0.48	0.10	16.7		<0.01
Total tract CP digestibility, %	-75.7	27.5	14.9	3.4	-0.40	0.10	18.5		<0.01
Urine volume, L/d	72.2	27.9	-7.3	3.4	0.20	0.10		14.7	0.02
Urinary urea N, % of total urinary N	-155	41	22.2	5.1	-0.53	0.15	20.9		<0.01
Fecal DM, kg/d	23.5	5.1	-1.95	0.62	0.059	0.019		16.4	<0.01
Fecal N, g/d	655	212	-50.8	26.0	1.65	0.79		15.4	0.04
Fecal N, % of N intake	176	28	-14.9	3.4	0.40	0.10		18.5	<0.01

¹Probability of quadratic effect of dietary CP content.

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