Lactation response to soybean meal and rumen-protected methionine supplementation of corn silage-based diets¹

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ABSTRACT

Corn silage, an important forage fed to dairy cows in the United States, is energy rich but protein poor. The objectives of this experiment were to investigate the effects on production of milk and milk components of feeding corn silage-based diets with 4 levels of dietary crude protein (CP) plus rumen-protected methionine (RPM). Thirty-six cows were blocked by days in milk into 9 squares and randomly assigned to 9 balanced 4 × 4 Latin squares with four 4-wk periods. All diets were formulated to contain, as a percent of dry matter (DM), 50% corn silage, 10% alfalfa silage, 4% soyhulls, 2.4% mineral-vitamin supplement, and 30% neutral detergent fiber. Supplemental RPM (Mepron, Evonik Corp., Kennesaw, GA) was added to all diets to maintain a Lys:Met ratio of 3.1 in digested AA. Ground high-moisture corn was reduced and soybean meal (SBM) plus RPM increased to give diets containing, on average, 11% CP (28% corn, 31% starch, 6% SBM, 4 g of RPM/d), 13% CP (23% corn, 29% starch, 10% SBM, 8 g of RPM/d), 15% CP (19% corn, 26% starch, 15% SBM, 10 g of RPM/d), and 17% CP (14% corn, 24% starch, 19% SBM, 12 g of RPM/d). Data from the last 14 d of each period were analyzed using the mixed procedures in SAS (SAS Institute Inc., Cary, NC). With the exception of milk fat and milk lactose content, we found no significant effects of diet on all production traits. We did note linear responses to dietary CP concentration for intake, production of milk and milk components, and MUN. Cows fed the 11% CP diet had

reduced DM intake, lost weight, and yielded less milk and milk components. Mean separation indicated that only true protein yield was lower on 13% CP than on 17% dietary CP, but not different between 15 and 17% CP. This indicated no improvement in production of milk and milk components above 15% CP. Quadratic trends for yield of milk, energy-corrected milk, and true protein suggested that a dietary CP concentration greater than 15% may be necessary to maximize production or, alternately, that a plateau was reached and no further CP was required. Although diet influenced apparent digestibility of DM, organic matter, and neutral detergent fiber, digestibility did not increase linearly with dietary CP. However, we observed linear and quadratic effects of dietary CP on acid detergent fiber digestibility. As expected, we found a linear effect of dietary CP on apparent N digestibility and on fecal and urinary N excretion, but no effect of diet on estimated true N digestibility. Ruminal concentrations of ammonia, total AA, peptides, and branched-chain volatile fatty acids also increased linearly with dietary CP. Quadratic responses indicated that 14.0 to 14.8% CP was necessary to optimize digestion and energy utilization. Overall results indicated that, when RPM was added to increase Lys:Met to 3.1, 15% CP was adequate for lactating dairy cows fed corn silage diets supplemented with SBM and secreting about 40 kg of milk/d; N excretion was lower than at 17% CP but with no reduction in yield of milk and milk components.

Key words: corn silage, soybean meal, rumen-protected methionine, dietary crude protein

INTRODUCTION

Energy and protein in the diet must be properly matched to optimize milk production in dairy cows. Corn silage, an important forage fed to dairy cows in the United States, is rich in energy but low in CP. Solvent-extracted soybean meal (SBM), which is high in Lys but low in Met and the most common protein supplement fed to US dairy cows, is estimated to con-

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tain 43% RUP in its CP (DMI = 4 times maintenance; NRC, 2001). Of the 3 protein concentrates tested by Brito et al. (2007), RUP flow measured using omasal sampling was greatest in cottonseed meal, intermediate in canola meal, and lowest in SBM; however, milk and protein yield were highest on canola meal, intermediate on SBM, and lowest on cottonseed meal. Milk and protein yield were greater on canola meal than on SBM (Huhtanen et al., 2011; Martineau et al., 2013); this difference was attributed partly to greater Met content in RUP supplied by canola meal (Broderick et al., 2015).

Conversion of feed N into milk N ranges from 23 to 34% in dairy cows with daily production of 30 to 50 kg of milk (Kalscheur et al., 2006; Arriola Apelo et al., 2014). Low N efficiency often makes it necessary to feed additional supplemental protein to improve yield of milk and milk components, further lowering efficiency and increasing cost of dairy production. Nitrogen not captured in milk is excreted in feces and especially in urine. Excreted urinary N is unstable, contributing to volatile losses of ammonia to the atmosphere and nitrate contamination of ground water, making dairy production less environmentally sustainable (Kaswari, 2004; Powell and Broderick, 2011). In addition, excessive dietary protein may also lead to increased incidence of health and reproductive problems (Jordan et al., 1983; Canfield et al., 1990). Increasing N efficiency in dairy cows without reducing yield is best achieved by reducing the total amount of CP fed to the animal and balancing the metabolizable AA supply (Lapierre et al., 2005; Brito and Broderick, 2007). Methionine is the EAA most often limiting in lactation diets based on legume and corn silages supplemented with SBM, and positive responses in milk and protein yields to rumen-protected Met (**RPM**) have been consistently observed (Patton, 2010; Zanton et al., 2014).

Previous work indicated that, on dairy diets based on SBM plus typical forages, about 16.5% CP appears to be adequate for mid-lactation cows (Broderick, 2003). Colmenero and Broderick (2006b) fed SBM-supplemented diets ranging from 13.5 to 19.4% CP and found that milk and true protein yield were optimal at, respectively, 16.7 and 17.1% CP. Lowering dietary CP from 16.6 to 15.6% while replacing a portion of the solvent SBM with expeller SBM tended to reduce milk yield (Colmenero and Broderick, 2006a). Because Met is the first-limiting EAA in SBM CP, we hypothesized that supplementation of RPM rather than RUP would reduce the dietary CP content required to maintain secretion of milk and milk components. Therefore, the objective of our experiment was to determine the optimal CP concentration in lactation diets supplemented with SBM plus RPM.

MATERIALS AND METHODS

Animals and Diets

Thirty-six multiparous Holstein cows (8 fitted with permanent, 10-cm ruminal cannulas; Bar Diamond, Inc., Parma, ID) with mean (SD) parity 2.6 (0.7), 623 (54) kg of BW, 91 (37) DIM, and 48 (5) kg of milk/d at the start of the trial were blocked by DIM into nine 4 × 4 Latin squares (2 with ruminally cannulated cows) with four 4-wk periods (total 16 wk). Cows were randomly assigned within squares to 1 of 4 balanced dietary treatment sequences. The 4 diets were fed as TMR for ad libitum intake and were formulated from corn silage, alfalfa silage, ground high-moisture and dry corn, SBM, soyhulls, minerals, and vitamins to contain 11, 13, 15, and 17% CP (DM basis). The 4 diets were supplemented with RPM (Mepron, Evonik Corp., Kenosaw, GA), which provided 4.2, 8.1, 10.3, and 12.4 g/d of absorbed Met (assuming 85% chemical DL-Met content with 72% bioavailability; Lee et al., 2012) in the diets containing, respectively, 11, 13, 15, and 17% CP. At the mean DMI observed on each dietary treatment, diets were estimated to provide 1,703 to 2,438 g/d of MP with Lys:Met ratios of about 3.1 in the MP of each diet (NRC, 2001). To improve distribution of RPM in the TMR, 2 premixes were prepared containing (DM basis) only 98% ground shelled corn plus 2% molasses (control premix) or 91% ground shelled corn, 2% molasses, plus 7% of the RPM supplement (RPM premix); depending on batch size and treatment, 0 to 2.1 kg/batch of control premix or 1.4 to 3.7 kg/batch of RPM premix was weighed out to the nearest 0.05 kg on a top-loading balance before blending into specific batches of TMR. Mean compositions of the major feed ingredients fed during the trial are in Table 1. Compositions of experimental diets actually fed during the trial (based on daily as-fed weights and weekly mean DM contents of each ingredient mixed into the TMR) are in Table 2.

All cows of the experiment were injected with bST (500 mg of Posilac, Elanco Animal Health, Greenfield, IN) beginning on d 1 of the trial and at 14-d intervals throughout. Cows were housed in a tiestall barn and had ad libitum access to fresh water during the trial. Care and handling of the animals on the experiment were applied as outlined in the guidelines of the University of Wisconsin institutional animal care and use committee.

Each of the 4 experimental periods lasted 28 d and consisted of 14 d for adaptation after switching diets and 14 d for collection of intake and production data. Diets were offered once daily at 1000 h and Orts were

collected and weights recorded at 0900 h. Feeding rate was adjusted daily to yield orts of about 5 to 10% of intake. Weekly composites of corn silage, alfalfa silage, and high-moisture shelled corn, plus the 4 different TMR and orts were obtained from daily subsamples of about 0.5 kg of each material and stored at -20°C . Weekly samples also were collected of SBM, soyhulls, and the 2 premixes and stored at room temperature. Dry matter was determined in weekly composites of corn silage, alfalfa silage, and high-moisture shelled corn by drying at 60°C for 48 h and in weekly samples of SBM, soyhulls, and the 2 premixes at 105°C (AOAC, 1980). These DM contents were used to make weekly adjustments of DM composition of TMR over the course of the trial. Intake of DM was computed based on 60°C DM determinations of weekly composites of TMR and orts. Dried (60°C) samples of corn silage, alfalfa silage, and high-moisture shelled corn and undried samples of SBM, soyhulls, and the 2 premixes from wk 3 and 4 of each period (8 samples of each ingredient over the trial) were ground to pass a 1-mm screen (Wiley mill, Arthur H. Thomas, Philadelphia, PA). These were analyzed for total N (Leco FP-2000 N Analyzer, Leco Instruments, Inc., St. Joseph, MI), DM at 105°C , ash and OM (AOAC, 1980), sequentially for NDF, ADF and ADIN using heat-stable α -amylase and Na_2SO_3 (Van Soest et al., 1991; Hintz et al., 1996), and for NDIN omitting α -amylase and Na_2SO_3 during extraction (Licitra et al., 1996). The 8 samples each of frozen composites of alfalfa and corn silages were thawed, extracted, and analyzed for pH, ammonia, and NPN (Leco FP-2000 N Analyzer) as described by Muck (1987). The 32 TMR samples from wk 3 and 4 of each period were analyzed

for total lipid according to AOAC International (1997; method 920.39; Dairyland Laboratories, Arcadia, WI). The 16 TMR samples collected during wk 4 of each period also were analyzed for indigestible ADF (ADF remaining after 288 h of in situ incubation) by the method of Huhtanen et al. (1994).

Cows were milked twice daily at 0500 and 1700 h and milk yield was recorded at each milking in all experimental periods. Milk samples from a.m. and p.m. milkings were collected on d 17 to 18 and 24 to 25 of each period and analyzed for fat, true protein, lactose, SNF, and MUN by infrared analysis (AgSource, Verona, WI) with a Foss FT6000 (Foss North America Inc., Eden Prairie, MN) using AOAC (1990) method no. 972.16. Concentrations and yields of fat, true protein, lactose and SNF, and MUN concentration were computed as weighted means based on a.m. and p.m. milk yields on each test day. Yields of ECM also were computed as described by Krause and Combs (2003). Efficiency of conversion of feed DM was calculated for each cow over the last 2 wk of each period by dividing mean yield of actual milk and ECM by mean DMI. Apparent N efficiency (assuming no retention or mobilization of body N) was also computed for each cow by dividing the period mean for milk N secretion (milk true protein/6.38) by mean N intake. For computation of BW change, BW of all cows was measured on 3 consecutive days just before beginning the experiment and at the end of each period.

On d 28 of each period, spot urine and fecal samples were collected 6 h before and 6 h after feeding. Urine was immediately diluted by mixing 15 mL of sample with 60 mL of 0.072 *N* H_2SO_4 and storing at -20°C

Table 1. Composition of principal dietary ingredients

Component	Corn silage		Alfalfa silage		HMSC ¹		Soybean meal	
	Mean ²	SEM	Mean	SEM	Mean	SEM	Mean	SEM
DM, % as fed	39.1	1.69	39.3	2.24	84.0	1.57	89.5	0.17
CP, % of DM	6.1	0.38	20.9	0.31	7.7	0.22	52.6	0.63
Ash, % of DM	4.2	0.09	9.8	0.28	1.4	0.06	7.5	0.16
OM, % of DM	95.8	0.09	90.2	0.28	98.6	0.06	92.5	0.16
NDF, % of DM	40.9	1.49	42.4	0.66	8.8	0.23	9.8	0.60
ADF, % of DM	20.5	0.68	31.8	0.73	1.8	0.06	5.2	0.31
NFC, ³ % of DM	49.7	1.35	29.4	0.77	83.1	0.42	31.7	0.64
NDIN, % of total N	14.2	0.70	11.6	0.71	13.3	0.84	3.0	0.16
ADIN, % of total N	1.6	0.16	4.0	0.17	0.0	0.25	0.0	0.02
Fraction B2, ⁴ % of total N	12.7	0.57	8.4	0.91	13.3	0.73	2.9	0.15
NPN, % of total N	49.5	4.7	52.5	8.0	—	—	—	—
Ammonia-N, % of total N	1.9	0.2	4.4	0.8	—	—	—	—
pH	4.06	0.04	4.46	0.55	—	—	—	—

¹HMSC = high-moisture shelled corn.

²Means from 8 replicate samples of each feed ingredient.

³Nonfiber carbohydrates computed according to NRC (2001) model.

⁴Fraction B2 = NDIN – ADIN (Higgs et al., 2015).

Table 2. Composition of diets ranging from 11 to 17% CP

Item	Dietary CP, % of DM			
	11	13	15	17
Ingredient, % of DM				
Corn silage	50.5	50.5	50.5	50.5
Alfalfa silage	10.4	10.4	10.4	10.4
High-moisture shelled corn	26.0	21.5	17.1	12.8
Ground shelled corn	1.01	1.03	1.05	1.06
Soybean meal	5.6	10.0	14.3	18.5
Soy hulls	4.0	4.0	4.0	4.0
Molasses	0.02	0.02	0.02	0.02
RPM product ¹	0.029	0.052	0.067	0.079
Calcium sulfate	1.37	1.38	1.38	1.38
Biophos ²	0.22	0.22	0.22	0.22
Sodium chloride	0.18	0.18	0.18	0.18
Magnesium oxide/sulfate	0.51	0.51	0.51	0.51
Vitamins-trace minerals ³	0.13	0.13	0.13	0.13
Composition				
CP, % of DM	10.9	12.9	14.8	16.8
Soybean meal CP, % of total CP	27	41	51	58
Ash, % of DM	4.2	4.4	4.7	4.9
NDF, % of DM	30.3	30.4	30.5	30.5
ADF, % of DM	16.1	16.3	16.4	16.6
NDIN, % of total N	11.2	9.7	8.6	7.7
ADIN, % of total N	1.6	1.3	1.1	1.0
Fraction B2, ⁴ % of total N	9.7	8.4	7.4	6.7
Ether extract, % of DM	3.2	3.1	3.0	2.9
Starch, ⁵ % of DM	30.6	28.5	26.4	24.4
NFC, ⁶ % of DM	49.8	47.7	45.7	43.8
NE _L , ⁶ Mcal/kg of DM	1.50	1.52	1.53	1.55
MP, ^{6,7} g/d	1,703	2,122	2,294	2,438
NRC-predicted ECM, ^{6,7} kg/d	34	37	37	38
NRC-predicted true protein, ^{6,7} kg/d	0.55	0.81	0.94	1.03
NorFor-predicted ECM, ⁸ kg/d	35	38	38	39
NorFor-predicted true protein, ⁸ kg/d	1.10	1.21	1.21	1.25
Lys in MP, ^{6,7} g/d	111	142	155	163
Met in MP, ^{6,7} g/d	32	38	40	41
RPM (Mepron), ⁷ g/d	4.2	8.1	10.3	12.4
Total Met supply, ^{6,7} g/d	36	46	50	53
Lys in MP, ^{6,7} %	6.4	6.7	6.8	6.8
Met in MP, ^{6,7} %	2.1	2.2	2.2	2.2
Lys:Met ratio (without RPM) ^{6,7}	3.5	3.7	3.9	4.0
Lys:Met ratio (total) ^{6,7}	3.1	3.1	3.1	3.1

¹Rumen-protected Met fed as Mepron, Degussa Corp., Kennesaw, GA.

²Biophos obtained from Mosaic Global Sales, Lithia, FL.

³Provided (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; and 12 mg Monensin.

⁴Fraction B2 = NDIN – ADIN (Higgs et al., 2015).

⁵Starch computations based on Table 4-2 of NRC (2001).

⁶Computed according to NRC (2001) model at mean DMI observed on each diet (Table 3). ECM yield computed based on NRC (2001) estimates of fat, true protein, and lactose yields using the equation of Krause and Combs (2003).

⁷Computed assuming rumen-protected Met (RPM) product contained 85% Met with 72% bioavailability (Lee et al., 2012) at mean DMI observed for each dietary treatment.

⁸Computed according to NorFor (2011) model. ECM computed using the factor 4.184 MJ/Mcal and assuming 0.951 kg of milk true protein/kg of total milk protein (Cerbulis and Farrell, 1975).

until analysis. Fecal samples were dried for 72 h at 60°C, ground through a 1-mm screen (Wiley mill), and composited on an equal DM basis to obtain 1 fecal sample/cow per period. All fecal samples were analyzed for DM, ash, OM, NDF, ADF, total N, and indigest-

ible ADF using the assays described above for feeds. Indigestible ADF was used as an internal marker to estimate apparent nutrient digestibility and fecal output (Cochran et al., 1986). Excretion of metabolic fecal N (MFN), estimated as 4.8 g/kg of DMI (NRC,

2001), was used to compute true N digestibility from apparent N digestibility for each diet. Urine samples were thawed and analyzed for total N by elemental analysis (Leco FP-2000 N Analyzer), for urea using an automated colorimetric assay (Broderick and Clayton, 1997) adapted to flow-injection (Lachat Quick-Chem 8000 FIA, Lachat Instruments, Loveland, CO), and for creatinine (Valadares et al., 1999). Daily urine volume and excretion of urea N were estimated from mean urinary concentrations in each period and assuming a creatinine excretion rate of 29 mg/kg of BW (Valadares et al., 1999).

On d 27 to 28 of each period, about 100 to 200 mL of fluid digesta was collected from 4 locations in the ventral rumen at 0 (just before feeding), 1, 2, 4, 6, 8, 12, 18, and 24 h after feeding in the 8 lactating Holstein cows fitted with ruminal cannulas using the probe described by Colmenero and Broderick (2006a). At each sampling, mixed fluid digesta was strained through 2 layers of cheesecloth and pH was measured immediately in strained fluid using a glass electrode. Two 10-mL aliquots of ruminal fluid were then preserved in scintillation vials by addition of 0.2 mL of 50% H₂SO₄ and stored at -20°C; the remaining fluid and digesta were returned to the rumen. Just before analysis, samples were thawed and centrifuged (15,300 × *g* for 20 min at 4°C) and flow-injection analyses (Lachat QuikChem 8000) applied to supernatants to determine ammonia using a phenol-hypochlorite method (Lachat Method 18-107-06-1-A). Supernatants were also analyzed using the *o*-phthaldialdehyde reaction (Roth, 1971) for total AA by fluorescence and for total AA plus oligopeptide concentrations by absorbance (Colombini et al., 2011). Leucine was the standard in both *o*-phthaldialdehyde assays and total AA and total AA plus oligopeptides are reported as Leu equivalents. The other ruminal samples were thawed and centrifuged (28,000 × *g* for 30 min at 4°C) before VFA determination using the method described by Broderick et al. (2015). This method did not resolve isovalerate and 2-methyl butyrate. Time-weighted means were computed for all ruminal traits (pH and concentrations of ammonia, total free AA, and individual and total VFA) using the equation

$$\begin{aligned} \text{Time-weighted mean} = & [\text{avg}(0-1 \text{ h}) + \text{avg}(1-2 \text{ h}) \\ & + 2 \times \text{avg}(2-4 \text{ h}) + 2 \times \text{avg}(4-6 \text{ h}) + 2 \\ & \times \text{avg}(6-8 \text{ h}) + 4 \times \text{avg}(8-12 \text{ h}) + 6 \\ & \times \text{avg}(12-18 \text{ h}) + 6 \times \text{avg}(18-24 \text{ h})]/24, \end{aligned}$$

where avg(*n*-*n* h) represent the mean value for each ruminal trait observed at the sampling times listed above.

Statistical Analysis

A single mean observation was computed for all production data for each cow over the last 2 wk of each period; single time-weighted means were computed for all ruminal traits measured in cannulated cows. Data were analyzed as replicated 4 × 4 Latin squares using the mixed procedures of SAS Institute Inc. (2013). Model sums of squares from the data were separated into overall mean, cow(within square), square, period, diet (dietary CP concentration), and overall error. All variables were considered fixed except cow(within square) and overall error, which were considered random. The PDIF option was used to test for treatment differences among least squares means. Orthogonal contrasts were used to test for linear and quadratic effects of dietary CP concentration. Least squares means are reported for all statistical analyses; significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Feed Quality and Diet Composition

Composition data on the major feed ingredients used to prepare the TMR fed in the trial are in Table 1. Contents of DM, CP, NDF, and ADF in alfalfa and corn silages and high-moisture corn indicated that these feedstuffs were of typical composition (NRC, 2001). The NPN in alfalfa silage represented 53% of total N; NPN normally accounts for 45 to 55% of total N in alfalfa silage (Broderick, 1995). Very low contents of ammonia-N in both alfalfa and corn silages indicated these feeds were well preserved (McDonald et al., 1991). Concentrations of ADIN in the 2 silages also were low and, on average, no ADIN was detected in the high-moisture corn and SBM, indicating essentially no heat damage to the major feed ingredients fed in this study.

Composition of the 4 diets fed in the study is in Table 2. Soybean meal, which was added at the expense of high-moisture corn, increased from about 6 to 19% of dietary DM; actual CP concentrations ranged from 10.9 to 16.8% (DM basis). Dietary alterations had little effect on NDF, ADF, and ADIN content; however, NDIN and N fraction B2 (NDIN - ADIN) as a proportion of total CP, as well as starch and NFC (as a proportion of DM), declined as SBM increased. Based on the NRC (2001) model, yield of ECM and milk true protein was predicted to increase by, respectively, 4 and 0.48 kg/d because estimated MP supply increased 735 g/d between the 11 and 17% CP diets (Table 2). However, the NRC (2001) model underestimated ECM yield by only 1 to 2 kg/d, but true protein yield by 0.53 to

0.27 kg/d as CP increased from 11 to 17% (Table 3). Yield predictions made using the NorFor (2011) model ranged from 33 to 39 kg of ECM/d and 1.10 to 1.25 kg of true protein/d (Table 2); the estimates of milk true protein averaged 99% of observed yields (Table 3). NorFor (2011) applies variable MP efficiencies that decline with increasing MP supply rather than a constant 67% MP efficiency as is used in NRC (2001). Neither model currently considers EAA pattern when estimating ECM and protein yield. Without RPM supplementation, Lys:Met ratios ranged from 3.5 to 4.0 on diets supplemented with SBM, implicating Met as the first-limiting AA in MP (NRC, 2001). Assuming 72% bioavailability of Met in the RPM source (Lee et al., 2012), RPM supplementation improved Lys:Met ratio to approximately 3.1 in all 4 diets. However, estimated Met and Lys concentrations in MP were not constant across diets (Table 2); Met and Lys increased from 2.1 and 6.4% of MP (on 11% CP) to 2.2 and 6.8% of MP (on 15 and 17% CP). Although NRC (2001) estimated Lys requirements when Met was about 2.0% of MP and estimated Met requirements when Lys was about 6.5% of MP, optimal Met and Lys concentrations were set equal to, respectively, 2.4 and 7.2% of MP. Schwab et al. (2003), however, suggested that Met and Lys were adequate in practical diets at 2.2 and 6.8% of MP.

Production Responses

The RUP in SBM is first-limiting in Met and several trials have shown that RPM supplementation of SBM-containing diets increases milk concentrations

of total protein (Armentano et al., 1997; Berthiaume et al., 2006), true protein (Berthiaume et al., 2006), and casein (Overton et al., 1998). Moreover, RPM supplementation elevated yield of milk (Schmidt et al., 1999), total protein (Armentano et al., 1997; Zanton et al., 2014), and true protein (Rulquin and Delaby, 1997; Patton, 2010). Therefore, we hypothesized that RPM supplementation would lower the dietary CP concentration needed to maintain production of milk and milk components. Lactation performance results are reported in Table 3. Except for milk fat and lactose concentrations, treatment and linear effects ($P \leq 0.05$) were observed for all production traits measured in our study. Quadratic effects were also detected for BW change and for MUN concentration and quadratic trends were detected for DMI, BW change, and for yield of milk, ECM, and true protein. Colmenero and Broderick (2006b) found yields of milk and milk true protein were maximal at, respectively, 16.7 and 17.1% CP when feeding diets composed of similar ingredients but with greater amounts of alfalfa silage and lower amounts of corn silage. The highly significant linear responses observed for most production traits were not surprising given the range of CP concentrations fed in our study. Although the quadratic responses will be discussed later, these strong linear responses suggested that mean separation should be applied to production traits to identify dietary CP concentrations above which there were no further improvements. Using this approach, production on 11% CP was clearly lower than on 13 to 17% CP; this was at least partly explained by DMI being 1.7 kg/d less than on the average of the other 3

Table 3. Production of lactating cows fed diets with increasing CP content but with constant estimated Lys:Met ratio of 3.1

Item	Dietary CP, % of DM				SEM ¹	Probability ²		
	11	13	15	17		Diet	Linear	Quadratic
DMI, kg/d	23.8 ^b	25.4 ^a	25.2 ^a	25.8 ^a	0.51	<0.01	<0.01	0.07
BW change, kg/d	-0.26 ^b	0.50 ^a	0.56 ^a	0.37 ^{ab}	0.229	0.04	0.05	0.04
Milk, kg/d	35.3 ^b	38.7 ^a	39.2 ^a	40.5 ^a	1.03	<0.01	<0.01	0.08
Milk/DMI	1.49 ^b	1.53 ^{ab}	1.56 ^a	1.58 ^a	0.038	<0.01	<0.01	0.40
ECM, kg/d	32.9 ^b	37.9 ^a	37.8 ^a	39.6 ^a	1.30	<0.01	<0.01	0.08
ECM/DMI	1.38 ^b	1.50 ^a	1.50 ^a	1.53 ^a	0.041	0.01	<0.01	0.19
Fat, %	3.81	4.08	3.91	3.97	0.155	0.40	0.56	0.35
Fat, kg/d	1.30 ^b	1.55 ^a	1.52 ^a	1.59 ^a	0.071	<0.01	<0.01	0.12
True protein, %	3.13 ^b	3.24 ^a	3.22 ^{ab}	3.27 ^a	0.047	<0.01	<0.01	0.27
True protein, kg/d	1.08 ^c	1.22 ^b	1.24 ^{ab}	1.30 ^a	0.035	<0.01	<0.01	0.10
Lactose, %	4.66	4.78	4.69	4.76	0.057	0.12	0.20	0.54
Lactose, kg/d	1.64 ^b	1.82 ^a	1.83 ^a	1.91 ^a	0.060	<0.01	<0.01	0.21
SNF, %	8.71 ^b	8.95 ^a	8.84 ^a	8.96 ^a	0.087	0.02	0.03	0.37
SNF, kg/d	3.04 ^b	3.39 ^a	3.42 ^a	3.59 ^a	0.102	<0.01	<0.01	0.16
MUN, mg/dL	5.7 ^d	8.0 ^c	10.6 ^b	14.9 ^a	0.48	<0.01	<0.01	0.01
Milk-N/N intake, %	40.8 ^a	36.5 ^b	32.7 ^c	29.5 ^d	0.82	<0.01	<0.01	0.37

^{a-d}Least squares means with different superscripts differ ($P < 0.05$).

¹Standard error of the LSM.

²Probability of significant effects of dietary treatment (diet) and linear and quadratic effects of dietary CP concentration.

diets. Interestingly, only true protein yield on 13% CP was lower than on the 17% CP diet. For no production trait was yield of milk, ECM and milk components, or milk composition different between diets containing 15 and 17% CP when supplemented with RPM. Colmenero and Broderick (2006a) reported that milk yield tended to be lower when CP was reduced from 16.6 to 15.6% CP, despite supplementation of RUP from expeller soybean meal on the lower CP diet. Swanepoel et al. (2015) reported that RPM supplementation increased milk yield and fat and protein concentration, but reduced milk lactose content and yield in Holstein cows fed 17% canola meal rations.

Linear responses of increasing MUN and decreasing apparent N efficiency ($P < 0.01$) with increasing dietary CP were anticipated. Milk urea concentration, which is an indicator of N inefficiency (Broderick and Clayton, 1997; Nousiainen et al., 2004), increased with each increment of dietary CP. Apparent N efficiency was more than 3 percentage units greater on 15 than on 17% dietary CP in our trial. The equation obtained by Nousiainen et al. (2004) by regressing MUN on dietary CP accurately predicted the MUN concentrations observed in the current study; the mean difference between observed and predicted values was less than 0.1 mg/dL.

Nutrient Digestibility and N Excretion

Nutrient digestibility and fecal and urinary N excretion, estimated using internal markers, are reported in Table 4. In our study, apparent digestibility of DM and OM and total-tract digestibility of NDF and ADF were affected by dietary treatment, with greatest digestibility for all 4 nutrients occurring at 13% CP. Linear effects of dietary CP content were found for ADF and apparent N digestibility, and quadratic effects on OM and ADF were also detected. The quadratic responses are discussed below. The BW loss on the 11% CP diet (Table 3) was surprising because intake of digested OM was 16.3 kg/d versus an average of 17.7 kg/d on the other 3 diets, which was only 9% lower than on 13 to 17% CP. Cows were in mid lactation during the trial and should have been gaining weight to replace the BW and BCS loss occurring in early lactation; hence, the weight loss observed on the 11% CP diet would not be sustainable. A possible explanation is that an inadequate MP supply may have impaired energy utilization on that diet. Effective dietary NE_L value was computed from milk ECM yield (assuming 0.749 Mcal/kg; Krause and Combs, 2003), plus BW change (6.98 Mcal/kg change in EBW; NRC, 2001) and maintenance (0.080 Mcal/kg of $BW^{0.75}$ (metabolic BW) at mean BW

of 640 kg; NRC, 2001), was 1.40 Mcal/kg of DM for the 11% CP diet versus an average 1.65 Mcal/kg of DM for the other 3 diets, which was 28% greater. Several literature reports document that improved MP status improved energy utilization; Brun-Lafleur et al. (2010) observed a linear increase in milk and fat yield as MP supply increased in diets formulated to be adequate and equal in energy supply. Auldist et al. (2014) reported that replacing starchy concentrates with canola meal in supplements fed to grazing dairy cows increased ECM yield.

As anticipated, apparent N digestibility increased linearly with increasing CP content of the diet (Table 4). This typically occurs because of dilution of MFN with elevated dietary CP (Nousiainen et al., 2009). We estimated true N digestibility by computing MFN as equivalent to 4.8 g of N/kg of DMI (NRC, 2001). Correcting observed fecal N excretion for this amount of MFN yielded true digestibility estimates ranging from 84 to 86%, which were not different among diets ($P = 0.34$). True N digestibility estimated from the data of Colmenero and Broderick (2006b) averaged 85.5%. Although NDF digestibility was influenced by diet no linear or quadratic effects were detected; NDF digestibility was greater on 13% CP than on the other 3 diets. Overall mean digestibility for NDF was 41% and for ADF was 44%. Chen et al. (2011) reported a mean NDF digestibility of 50% for cows consuming 25 kg of DM/d of a 60% forage diet, but with a 35:25 DM ratio of corn silage:alfalfa silage. Moreover, Faciola and Broderick (2014) observed NDF and ADF digestibilities of 44 and 49%, respectively, when cows were fed the control diet (i.e., with no added lipid) with a 10:50 ratio of corn silage:alfalfa silage DM. Although Lopes et al. (2015) found greater indigestible NDF in alfalfa silage versus corn silage, apparent total-tract NDF digestibility increased as the dietary proportion of alfalfa silage increased. This result was explained by a rate of ruminal digestion of digestible NDF in alfalfa silage that was twice as rapid as that for corn silage. This suggests that the relatively low fiber digestion observed in the current trial was due in part to the low level of alfalfa silage in the diet.

Urine volume ranged from 39 to 47 L/d and increased linearly with increasing dietary CP concentration (Table 4). Urine volume is influenced by both mineral and dietary CP intake. Valadares et al. (1999) reported that urine volume increased from 32 to 48 L/d as dietary alfalfa silage increased from 35 to 80% of dietary DM; alfalfa forage from first and second cutting was reported to average 3.5% potassium on a DM basis (Kume et al., 2001). Eriksson and Rustas (2014) observed urine volumes ranging from 14 to 40 L/d as potassium intake

Table 4. Apparent total-tract digestibility, true N digestibility, and N metabolism in lactating cows fed diets with increasing CP content but with constant estimated Lys:Met ratio of 3.1

Item	Dietary CP, % of DM				SEM ¹	Probability ²		
	11	13	15	17		Diet	Linear	Quadratic
Apparent digestibility, %								
DM	67.0 ^b	68.9 ^a	66.9 ^b	67.3 ^b	0.51	0.01	0.67	0.12
OM	68.4 ^b	70.6 ^a	68.7 ^b	69.1 ^b	0.49	0.01	0.96	0.05
Nitrogen	57.0 ^c	62.9 ^b	64.0 ^b	67.8 ^a	0.93	<0.01	<0.01	0.20
True N digestibility ³	84.5	86.1	84.3	85.7	0.94	0.34	0.63	0.89
NDF	40.6 ^b	43.8 ^a	39.2 ^b	40.5 ^b	0.88	<0.01	0.14	0.22
ADF	40.0 ^c	48.0 ^a	42.5 ^{bc}	45.3 ^{ab}	0.89	<0.01	<0.01	<0.01
N metabolism								
N intake, g/d	414 ^d	524 ^c	597 ^b	691 ^a	11.3	<0.01	<0.01	0.24
Fecal N, g/d	180 ^c	195 ^b	214 ^{ab}	222 ^a	7.0	<0.01	<0.01	0.49
Fecal N/N intake, %	43.0 ^a	37.1 ^b	36.0 ^b	32.2 ^c	0.93	<0.01	<0.01	0.20
Urine volume, L/d	38.5 ^b	40.7 ^b	45.0 ^a	47.3 ^a	1.74	<0.01	<0.01	0.96
Urinary urea N, g/d	24.3 ^d	66.9 ^c	106 ^b	176 ^a	7.5	<0.01	<0.01	0.03
Urinary urea N/TUN, ⁴ %	31.2 ^c	50.0 ^b	56.0 ^b	75.5 ^a	3.07	<0.01	<0.01	0.89
Total urinary N, g/d	86.3 ^d	136 ^c	206 ^b	231 ^a	16.4	<0.01	<0.01	0.22
Urinary N/N intake, %	20.8 ^b	26.0 ^{ab}	34.5 ^a	33.4 ^a	2.85	<0.01	<0.01	0.21
Excreted N, g/d	266 ^d	331 ^c	420 ^b	453 ^a	23.4	<0.01	<0.01	0.25
Excreted N/N intake, %	63.9	63.1	70.4	65.6	2.88	0.27	0.14	0.42

^{a-d}Least squares means with different superscripts differ ($P < 0.05$).

¹Standard error of the LSM.

²Probability of significant effects of dietary treatment (diet) and linear and quadratic effects of dietary CP concentration.

³True N digestibility computed assuming metabolic fecal n = 4.8 g/kg of DMI (NRC, 2001).

⁴TUN = total urinary N.

increased from 240 to 686 g/d; sodium intake has an effect on urine volume similar to potassium intake (Dijkstra et al., 2013b). Moreover, Dijkstra et al. (2013b) summarized data indicating that increasing ruminal balance of RDP from 0.1 to 1.0 kg/d increased urine volume from 32 to 51 L/d at approximately equal sodium and N intakes. Furthermore, Kume et al. (2008) observed a strong correlation between urine volume and urinary N excretion. Amounts of fecal and urinary N excretion also increased linearly with dietary CP but, as expected, fecal and urinary N excretion had different patterns when expressed as proportions of N intake; fecal N declined from 43 to 32% of N intake whereas urinary N increased from 21 to 35% of N intake over the range of CP fed in our trial. A similar pattern is commonly observed with increasing diet CP concentration (Kebreab et al., 2002). The proportion of urea-N in total urinary N, which increased with elevated dietary CP, is important because urea is the form of excretory N that is most rapidly degraded to ammonia and volatilized to the environment (Powell et al., 2014). Colmenero and Broderick (2006b) reported that urea increased from 55 to 82% of total urinary N when dietary CP ranged from 13.5 to 19.4% CP, whereas urinary urea N accounted for 31 to 76% to total urinary N in the present study when dietary CP ranged from 10.9 to 16.8% CP. A quadratic effect of dietary CP content on urinary urea

N excretion was also detected. Fecal and urinary N excretion were 36 and 35% of N intake on the 15% CP diet, indicating that about 29% of dietary N intake was secreted as milk true protein; based on N intake and milk true protein secretion, apparent N efficiency was estimated to be about 33% on the 15% CP diet (Table 3). Broderick et al. (2008) observed an apparent N efficiency of 34% on a 14.8% CP diet also supplemented with RPM; however, milk and true protein yields were lower than on a RPM-supplemented diet containing 16.1% CP. Based on theoretical computations, Dijkstra et al. (2013a) and van Vuuren and Chilibroste (2013) estimated that maximal N efficiency in dairy production may exceed 40%.

Ruminal Metabolites

Effects of experimental diets on ruminal pH and mean metabolite concentrations are in Table 5. We found diet and quadratic effects on ruminal pH, with lower pH values observed at intermediate dietary CP concentrations. We noted a trend for a quadratic effect of increasing CP content on total VFA concentration. Other major effects observed were diet and linear effects on ruminal concentrations of ammonia, total AA, and total AA plus peptides, as well as on molar proportions of isovalerate + 2-methylbutyrate and total

Table 5. Ruminal metabolite concentrations in lactating cows fed diets with increasing CP content but with constant estimated Lys:Met ratio of 3.1

Item	Dietary CP, % of DM				SEM ¹	Probability ²		
	11	13	15	17		Diet	Linear	Quadratic
pH	6.33 ^a	6.13 ^b	6.13 ^b	6.25 ^{ab}	0.076	0.03	0.12	0.01
Ammonia-N, mg/dL	1.9 ^c	2.9 ^{bc}	4.0 ^b	6.1 ^a	0.46	<0.01	<0.01	0.16
TAA, mM	1.6	2.1	1.9	2.0	0.15	0.06	0.02	0.42
TAA + peptides, ³ mM	3.1 ^b	3.9 ^a	3.8 ^a	3.8 ^a	0.23	0.03	0.01	0.28
Total VFA, mM	85.5	91.8	93.3	90.2	3.07	0.22	0.22	0.10
Molar proportions, mol/100 mol								
Acetate	58.8	58.4	59.2	59.7	2.15	0.70	0.34	0.61
Propionate	25.8	26.1	25.3	23.6	2.36	0.52	0.21	0.48
Acetate:propionate ratio	2.37	2.30	2.41	2.60	0.319	0.43	0.19	0.36
Butyrate	12.3	12.6	12.5	13.1	0.43	0.61	0.27	0.78
Isobutyrate	0.89	0.94	0.89	0.99	0.045	0.40	0.26	0.65
Isovalerate + 2-methyl butyrate	2.23 ^b	1.98 ^b	2.13 ^b	2.62 ^a	0.114	0.02	0.02	0.01
BCVFA ⁴	3.13 ^b	2.93 ^b	3.03 ^b	3.61 ^a	0.121	0.02	0.02	0.01
Valerate	2.20	2.47	2.52	2.29	0.685	0.85	0.79	0.43

^{a-c}Least squares means with different superscripts differ ($P < 0.05$).

¹Standard error of the LSM.

²Probability of significant effects of dietary treatment (Diet) and linear and quadratic effects of dietary CP concentration.

³Total AA plus small oligopeptides determined using *o*-phthaldialdehyde-absorbance assay (see text for details).

⁴Branched-chain VFA (isobutyrate plus isovalerate + 2-methyl butyrate).

branched-chain VFA (**BCVFA**), which are formed from microbial catabolism of the branched-chain AA (El-Shazly, 1952). Moreover, quadratic effects were observed for molar proportions of isovalerate + 2-methylbutyrate and BCVFA. All of these compounds are produced as a result of ruminal protein degradation; their elevation in the rumen represents accumulation in excess of microbial uptake for protein synthesis and increasing concentrations with increasing dietary CP was anticipated.

Quadratic Responses

Regression coefficients as well as estimated maximal or minimal dietary CP concentrations for the variables showing quadratic responses ($P \leq 0.10$) to dietary CP are in Table 6. We observed 8 responses that had maxima ranging from 14.0 to 17.5%, with a mean of 15.6%. The maxima for DMI and ECM yield were both 16.2% dietary CP. Maxima for yield of milk and true protein were both above 17% CP, which was greater than the highest level of CP fed in the trial. This suggested secretion of milk and protein was not yet maximal on the diet with 16.8% CP and Lys:Met ratio of 3.1. These results were similar to those of Colmenero and Broderick (2006b), who observed maxima for milk and true protein yield at, respectively, 16.7 and 17.1% CP on diets composed of similar ingredients but without added RPM. All of the responses related to energy utilization (BW change, OM and ADF digestibility, and ruminal total VFA

concentration) had similar maxima, ranging from 14.0 to 14.8% CP. Ruminal pH, which would be related to ruminal OM digestibility, was minimal at 13.7% dietary CP. Molar proportions of ruminal BCVFA would be expected to be the resultant of release from protein degradation and uptake for microbial protein synthesis (Van Soest, 1994); a minimum for BCVFA was detected at 13.2% CP. Of particular interest was the quadratic response of ADF digestibility. Fiber digestion occurs primarily in the rumen (Huhtanen et al., 2010), and results from several studies have shown depressed fiber digestion on low-CP diets, possibly because of inadequate supply of RDP. Broderick (2003) reported that milk and protein yield was the same at 16.5 and 18.4% CP, but that NDF and ADF digestibility was lower at 16.5% CP. Colmenero and Broderick (2006b) observed quadratic responses to dietary CP concentration and a maximum for both NDF and ADF digestibility was found at 16.7% CP. Lee et al. (2012) were able maintain milk and protein yield on a 13.6% CP supplemented with 3 rumen-protected AA (Met, Lys, and His), but observed depressed NDF and ADF digestibility compared with the positive control containing 15.7% CP. Two other traits related to N efficiency, MUN concentration and urinary urea excretion, had minima of, respectively, 8.0 and 6.6% CP. That both of these estimates were below the range of CP concentration fed in this trial reflected the general inverse relationship between dietary CP and N utilization often observed in feeding studies with lactating dairy cows (Huhtanen and Hristov, 2009).

Table 6. Regression coefficients and optimal dietary CP concentrations for traits showing quadratic responses ($P \leq 0.10$)

Item	Intercept	SE	Linear		Quadratic		Dietary CP, % of DM		<i>P</i> -value ¹
			Coefficient	SE	Coefficient	SE	Maximum	Minimum	
DMI, kg/d	7.7	7.08	2.16	1.013	−0.07	0.036	16.2		0.07
BW change, kg/d	−11.9	5.32	1.74	0.776	−0.06	0.028	14.8		0.04
Milk yield, kg/d	−5.9	14.17	4.42	2.027	−0.13	0.072	17.1		0.08
ECM yield, kg/d	−23.5	21.56	6.67	3.110	−0.21	0.111	16.2		0.08
True protein yield, kg/d	−0.4	0.57	0.17	0.082	−0.005	0.0029	17.5		0.10
OM digestibility, %	42.6	11.40	3.25	1.660	−0.12	0.059	14.0		0.05
ADF digestibility, %	−38.2	22.01	10.31	3.202	−0.35	0.114	14.7		<0.01
Ruminal total VFA, mM	−33.2	62.57	17.28	9.113	−0.59	0.326	14.6		0.10
MUN, mg/dL	12.6	9.36	−2.01	1.355	0.13	0.048		8.0	0.01
Urinary urea, g/d	48.3	151.9	−21.9	21.846	1.66	0.778		6.6	0.03
Ruminal pH	7.7	1.45	−0.23	0.211	0.009	0.0076		13.7	0.01
Ruminal BCVFA, ² mol/100 mol	11.6	2.53	−1.29	0.364	0.049	0.0130		13.2	0.01

¹Probability of quadratic effect of dietary CP content.²Branched-chain VFA (isobutyrate plus isovalerate + 2-methyl butyrate).

CONCLUSIONS

A lactation trial was conducted to determine the optimal CP content of diets based on corn silage as the main forage and supplemented with SBM and RPM added to maintain a Lys:Met ratio of 3.1 in MP. Feeding the 11% CP diet, in which MP was estimated to contain 2.1% Met and 6.4% Lys, depressed production. Diets containing 13 to 17% CP were estimated to contain 2.2% Met and 6.8% Lys in MP. Intake of DM and yield of milk, ECM, fat, lactose, SNF, and milk/DMI did not significantly increase above 13% CP. However, yield of true protein was lower on 13 versus 17% CP, but not different between 15 and 17% CP. Compared with 17% CP, feeding 15% CP reduced MUN and N excretion and improved N efficiency without altering apparent total-tract DM, OM, and fiber digestibility. Overall results from this trial indicated that, with added RPM, 15% CP was adequate for lactating dairy cows producing about 40 kg of milk/d when fed corn silage-based diets supplemented with SBM.

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