Replacing dietary soybean meal with canola meal improves production and efficiency of lactating dairy cows¹

Glen A. Broderick,^{*2,3} Antonio P. Faciola,[†] and Louis E. Armentano[‡]

^{*}Agricultural Research Service, USDA, US Dairy Forage Research Center, 1925 Linden Drive, Madison, WI 53706

[†]Department of Agriculture, Nutrition, and Veterinary Sciences, 1664 North Virginia Street, University of Nevada, Reno 89557

[‡]Department of Dairy Science, 1675 Observatory Drive, University of Wisconsin, Madison 53706

ABSTRACT

Previous research suggested that crude protein (CP) from canola meal (CM) was used more efficiently than CP from solvent soybean meal (SBM) by lactating dairy cows. We tested whether dietary CP content influenced relative effectiveness of equal supplemental CP from either CM or SBM. Fifty lactating Holstein cows were blocked by parity and days in milk into 10 squares (2 squares with ruminal cannulas) in a replicated 5 × 5 Latin square trial. Five squares were fed: (1) low (14.5–14.8%) CP with SBM, (2) low CP with CM, (3) low CP with SBM plus CM, (4) high (16.4–16.7%) CP with SBM, and (5) high CP with CM; the other 5 squares were fed the same diets except with rumen-protected Met plus Lys (RPML) added as Mepron (Degussa Corp., Kennesaw, GA) and AminoShure-L (Balchem Corp., New Hampton, NY), which were assumed to provide 8 g/d of absorbed DL-Met and 12 g/d of absorbed L-Lys. Diets contained [dry matter (DM) basis] 40% corn silage, 26% alfalfa silage, 14 to 23% corn grain, 2.4% mineral-vitamin premixes, and 29 to 33% neutral detergent fiber. Periods were 3 wk (total 15 wk), and data from the last week of each period were analyzed using the Mixed procedures of SAS (SAS Institute Inc., Cary, NC). The only effects of RPML were increased DM intake and milk urea N (MUN) and urinary N excretion and trends for decreased milk lactose and solids-not-fat concentrations and milk-N:N intake; no significant RPML × protein source interactions were detected. Higher dietary CP increased milk fat yield and tended to increase milk yield but also elevated MUN, urine volume, urinary N excretion, ru-

minal concentrations of ammonia and branched-chain volatile fatty acids (VFA), lowered milk lactose concentration and milk-N:N intake, and had no effect on milk true protein yield. Feeding CM instead of SBM increased feed intake, yields of milk, energy-corrected milk, and true protein, and milk-N:N intake, tended to increase fat and lactose yields, and reduced MUN, urine volume, and urinary N excretion. At low CP, MUN was lower and intake tended to be greater on SBM plus CM versus SBM alone, but MUN and N excretion were not reduced to the same degree as on CM alone. Interactions of parity × protein source and parity × CP concentration indicated that primiparous cows were more responsive than multiparous cows to improved supply of metabolizable protein. Replacing SBM with CM reduced ruminal ammonia and branched-chain VFA concentrations, indicating lower ruminal degradation of CM protein. Replacing SBM with CM improved milk and protein yield and N-utilization in lactating cows fed both low- and high-CP diets.

Key words: canola meal, soybean meal, milk production, nitrogen efficiency

INTRODUCTION

In recent years, increasing production of canola has given rise to greater availability of canola meal (CM) as a protein supplement for livestock feeding (Harker et al., 2012). Greater access to CM has made it a viable alternative to soybean meal (SBM) for lactating dairy cows (Hickling, 2008). We observed a numeric increase in milk and protein yields when CM replaced equal supplemental protein from solvent-extracted SBM in 16.5% CP diets fed to dairy cows (Brito and Broderick, 2007). Recent meta-analyses of results published in peer-reviewed journals showed greater DMI and yield of milk and milk components when CM substituted for several commonly fed proteins (Martineau et al., 2013). These meta-analyses reported that replacing SBM with CM significantly increased milk protein yield (Martineau et al., 2013) and increased intake and yield of milk and milk components (Huhtanen et al., 2011).

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²Retired; present address: Broderick Nutrition & Research LLC, 221 Glen Hollow Road, Madison, WI 53705.

³Corresponding author: gbroderi@wisc.edu

The NRC (2001) model indicates ruminal degradation rates of 4.5%/h for SBM protein and 10.4%/h for CM protein, although larger insoluble fractions B and C in CM tend to equalize the predicted RUP and MP value of these proteins. Brito et al. (2007) found that the proportion of RUP in CM was numerically greater than that in SBM. Huhtanen et al. (2011) also concluded that CM contributed amounts of RUP and MP that were at least equal to those from SBM. Lactating dairy cows fed SBM-supplemented diets often respond to rumen-protected Met (e.g., Broderick et al., 2009), and the concentration of Met is greater in CM protein than in SBM (NRC, 2001). This suggests that (1) lactating cows would be less responsive to rumen-protected Met when fed CM than SBM, and (2) the AA pattern in MP from a blend of CM plus SBM might be complementary. Furthermore, recent research has indicated that dietary CP content can be lowered substantially without reducing milk and protein yields in lactating cows (Broderick, 2003; Kalscheur et al., 2006). If MP from CM contributes more absorbed Met than SBM, then CM might prove more effective in low-CP diets.

Therefore, the objectives of this experiment were to (1) determine the relative effectiveness of CM and SBM as supplemental protein sources in both low- and high-CP diets; (2) compare effectiveness of feeding a blend of SBM plus CM versus SBM or CM alone; and (3) evaluate whether lactation performance on diets based on SBM or CM diets would be improved by supplementation with rumen-protected Met plus Lys. Moreover, the design of this study allowed evaluation of relative response to protein source and CP content of primiparous versus multiparous cows.

MATERIALS AND METHODS

Experimental Design

Thirty multiparous Holstein cows, including 10 fitted with permanent 10-cm ruminal cannulas (Bar Diamond Inc., Parma, ID), with mean (SD) 2.5 (0.6) parity, 81 (29) DIM, 47.8 (5.1) kg of milk/d, 620 (50) kg of BW, and $SCC = 41 (62) \times 10^3$ cells/mL, plus 20 primiparous cows with mean (SD) 100 (39) DIM, 36.5 (4.4) kg milk/day, 543 (31) kg of BW, and $SCC = 26 (20) \times 10^3$ cells/mL, were used in the trial. All cows were in good health and, on average, gaining BW at the start of the trial. Cows were grouped into ten 5×5 Latin squares to give 6 squares (2 squares ruminally cannulated) of multiparous cows and 4 squares of primiparous cows, all blocked into squares by DIM. Cows were randomly assigned to dietary treatment sequences

within 5 pairs of 5×5 Latin squares (3 square-pairs of multiparous and 2 square-pairs of primiparous cows) that were balanced such that each square-pair had equal numbers of change-overs among the 5 basic diets. The 5 basic diets were fed as TMR composed of alfalfa silage, corn silage, and high-moisture shelled corn (ground to approximately 1.4-mm mean particle size; Ekinci and Broderick, 1997), plus minerals and vitamins. Dietary protein supplements were solvent-extracted SBM, solvent-extracted CM, or both, fed in the following arrangement: (1) low CP (SBM), (2) low CP (CM), (3) low CP (blend of SBM plus CM), (4) high CP (SBM), and (5) high CP (CM). Rumen-protected Met plus Lys (**RPML**) was fed to 1 square in each square-pair, alternating the supplement such that RPML was fed to the square at later DIM for the first square-pair, and then to the square at earlier DIM for the second square-pair, and so on. Mean (SD) DIM for cows fed without and with RPML supplement were, respectively, 80 (28) and 96 (29) at the start of the trial. To improve distribution of RPML in the TMR, 2 premixes were prepared containing ground shelled corn plus molasses only or ground shelled corn, molasses, plus RPML added as Mepron (Evonik Corp., Kenosaw, GA) and AminoShure-L containing 38% L-Lys (Balchem Corp., New Hampton, NY). Depending on batch size, between 2.2 and 2.4 kg of either the control or RPML premix was added to the TMR batch fed to cows in the square in each square-pair receiving that specific treatment. The RPML supplement provided 15 g/d of chemical DL-Met and 19 g/d of chemical L-Lys; assuming, respectively, 72 and 64% bioavailability (Lee et al., 2012b), this corresponded to 8 g/d of absorbed Met and 12 g/d of absorbed Lys. Mean composition of the major feed ingredients fed during the trial is in Table 1. Compositions of the experimental diets actually fed during the trial (based on daily mean as-fed weights and weekly mean DM contents of each ingredient mixed into the TMR) are in Table 2.

All cows were injected every other week with bST (500 mg of Posilac; Elanco Animal Health, Greenfield, IN) beginning about 60 DIM; injections were synchronized such that animals received a full dose on d 1 of period 1 and continuing at 14-d intervals throughout the trial. Therefore, cows received bST twice during periods 1, 3, and 5 (on d 1 and 15) and once during periods 2 and 4 (on d 8). Because the design was a balanced 5×5 Latin square, an equal number of observations were made for each dietary treatment during periods in which bST was injected on both d 1 and d 15 and only once on d 8. This arrangement proved satisfactory in a previous trial (Valadares Filho et al., 2000). Cows were housed in tiestalls and had free access to water.

Table 1. Composition of principal dietary ingredients¹

Component	Alfalfa silage		Corn silage		HMSC		SBM		CM	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
DM, %	40.1	2.1	41.9	2.0	73.7	0.1	89.0	0.3	89.6	0.4
CP, % of DM	21.5	0.5	6.8	0.1	7.7	0.1	53.6	0.5	40.6	0.2
OM, % of DM	87.6	1.5	96.0	0.4	98.4	0.3	92.4	0.1	91.0	0.1
NDF, % of DM	39.5	1.0	41.7	3.3	5.4	0.3	7.0	0.3	29.9	0.3
ADF, % of DM	32.1	1.0	25.4	2.1	1.5	0.1	4.2	0.2	18.2	0.2
NDIN, % of total N	12.3	0.2	2.3	0.2	2.1	0.2	7.3	0.3	26.9	0.9
ADIN, % of total N	6.7	0.6	0.7	0.1	0.1	0.1	1.6	0.2	6.2	0.1
B3, ² % of total N	5.7	0.7	1.6	0.1	2.0	0.2	5.8	0.2	20.7	0.8
Ether extract, % of DM	—	—	—	—	—	—	1.7	0.1	3.0	0.1
NPN, % of total N	49.5	4.7	69.1	8.0	—	—	—	—	—	—
NH ₃ , % of total N	1.9	0.2	4.4	0.8	—	—	—	—	—	—
Total AA-N, ³ % of total N	22.0	3.3	18.4	5.4	—	—	—	—	—	—
pH	4.48	0.04	4.26	0.55	—	—	—	—	—	—

¹CM = canola meal, HMSC = high-moisture shelled corn, SBM = solvent soybean meal.

²B3 = NDIN – ADIN.

³Computed assuming 40.3 μ mol of total free AA/mg of N in alfalfa and corn silages (Broderick, 1987).

The Animal Care and Use Committee of the College of Agricultural and Life Sciences of the University of Wisconsin-Madison approved all procedures involving animals.

Each of the 5 experimental periods lasted 21 d and consisted of 14 d for adaptation after diet reversal and 7 d for collection of intake and production data. Diets were offered once daily at 1000 h; orts were collected and weights recorded at 0900 h. Feeding rate was adjusted daily to yield refusals equivalent to about 5 to 10% of intake. The mean refusal rate observed over the trial was 8.6% of feed DM offered. Weekly composites of alfalfa silage, corn silage, high-moisture shelled corn, and the 10 different TMR and orts were obtained from daily subsamples of about 0.5 kg of each material that were stored at -20°C . Weekly samples also were collected of SBM, CM, and control and RPML premixes and stored at room temperature. Dry matter was determined in weekly composites of corn silage, alfalfa silage, high-moisture shelled corn, and orts by drying at 60°C for 48 h and in weekly samples of SBM, CM, and RPML premixes by drying for 24 h at 105°C (method 967.03; AOAC, 1990). These DM contents were used to adjust DM composition of TMR every week over 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, CM, and the 2 premixes from wk 3 of each period (5 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 (method 967.03;

AOAC, 1990), 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 15 TMR samples and 5 samples each of SBM and CM from wk 3 of each period were analyzed for total lipid (ether extract) according to AOAC International (1997; method 920.39; Dairyland Laboratories, Arcadia, WI). The 15 TMR samples were also analyzed for starch using a modification of the method of Knudsen (1997; Dairyland laboratories, Arcadia, WI). Frozen composites of alfalfa and corn silages were thawed and analyzed for NPN (Muck, 1987; Leco FP-2000 N Analyzer).

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 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 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 (Krause and Combs, 2003) were also computed. Efficiency of conversion of feed DM was calculated for each cow over the last week of each period by dividing mean yields 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 was measured on

Table 2. Composition of diets (g/kg of DM, unless otherwise noted) containing either 15 or 17% CP with combinations of soybean meal (SBM) and canola meal (CM) and with or without rumen-protected Met and Lys (RPM_L; +, -)

Item	15% CP						17% CP					
	SBM			CM			SBM+CM			SBM		
	-	+	-	-	+	+	-	+	+	-	+	+
Ingredient	26.2	26.2	26.2	26.2	26.2	26.2	26.2	26.2	26.2	26.2	26.1	26.1
Alfalfa silage	40.0	40.0	40.0	40.0	40.0	40.1	40.1	40.1	40.1	40.0	39.9	39.9
Corn silage	21.3	21.3	18.6	18.5	18.5	20.0	20.0	20.0	17.2	17.2	13.1	13.1
High-moisture shelled corn	1.38	1.11	1.38	1.11	1.11	1.38	1.38	1.11	1.38	1.11	1.38	1.11
Ground shelled corn	8.7	8.7	0	0	0	4.3	4.3	4.3	12.7	12.8	0.0	0.0
Solvent soybean meal	0	0	11.4	11.5	11.5	5.6	5.6	5.6	0	0	17.1	17.1
Canola meal	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Molasses	0	0.06	0	0.06	0.06	0	0	0.06	0	0.06	0	0.06
Mepron ¹	0	0.21	0	0.21	0.21	0	0	0.21	0	0.21	0	0.21
AminoShure-L ²	1.36	1.36	1.36	1.36	1.36	1.36	1.36	1.36	1.36	1.36	1.36	1.36
Calcium sulfate	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22
Monocalcium phosphate	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Sodium chloride	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Magnesium oxide/sulfate	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13
Vitamins-trace minerals ³	14.7	14.8	14.5	14.6	14.6	14.6	14.6	14.7	16.6	16.7	16.4	16.5
CP	31	31	0	32	32	16	16	16	41	41	0	0
SBM CP, % of total CP	0	0	32	32	32	16	16	16	0	0	42	42
CM CP, % of total CP	5.9	5.9	6.2	6.2	6.2	6.0	6.0	6.0	6.1	6.1	6.6	6.6
Ash	28.9	28.9	31.6	31.6	31.6	30.3	30.3	30.2	29.0	29.0	32.9	32.9
NDF	19.2	19.2	20.9	20.9	20.9	20.1	20.1	20.1	19.4	19.4	21.8	21.8
ADF	14.0	14.0	20.5	20.7	20.7	17.0	17.0	16.0	11.4	12.1	20.7	20.2
NDIN, % of total N	8.6	7.2	7.4	9.1	9.1	8.5	8.5	7.0	5.3	4.0	5.8	6.9
ADIN, % of total N	5.4	6.8	13.2	11.6	11.6	8.5	8.5	8.9	6.1	8.1	14.9	13.2
B3, % of total N	2.5	2.8	2.8	2.9	2.9	2.6	2.6	3.2	2.5	2.4	2.7	2.9
Ether extract	50.1	49.7	47.8	47.7	47.7	49.0	49.0	48.3	47.7	47.8	44.8	44.5
NFC ⁵	28.8	30.8	27.3	28.7	28.7	29.1	29.1	28.4	28.4	25.8	23.1	22.0
Starch	0.89	0.89	0.94	0.94	0.94	0.91	0.91	0.91	0.90	0.90	0.98	0.98
Calcium, % of DM	0.36	0.36	0.42	0.42	0.42	0.39	0.39	0.39	0.38	0.38	0.47	0.47
Phosphorus, % of DM	1.51	1.51	1.50	1.51	1.51	1.51	1.51	1.51	1.51	1.51	1.51	1.51
NE _L , Mcal/kg of DM	38	38	38	38	38	38	38	38	38	38	38	38
NE _L -allowable milk, ⁶ kg/d	30	30	30	30	30	30	30	30	32	32	33	33
MP-allowable milk, ⁶ kg/d	2,216	2,216	2,249	2,249	2,249	2,224	2,224	2,224	2,299	2,299	2,367	2,367
MP, ⁶ g/d	3.7	3.3	3.3	3.0	3.0	3.5	3.5	3.2	3.8	3.4	3.4	3.1
Lys:Met ratio in MP ⁶												

¹Rumen-protected Met product from Degussa Corp. (Kennesaw, GA).²Rumen-protected Lys product from Balchem Corp. (New Hampton, NY).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, 16 IU of vitamin E, and 12 mg of monensin.⁴B3 = NDIN - ADIN.⁵NFC = 100 - % NDF - [% CP × (100 - % NDIN)/100] - % fat - % ash.⁶Computed according to NRC (2001) model using mean observed DMI (25.2 kg/d) and ingredient composition from Table 1 and NRC tables; Lys:Met ratios were estimated assuming 72% bioavailability of Met and 64% bioavailability of Lys in supplements of rumen-protected AA.

3 consecutive days at the beginning of the experiment and at the end of each period.

Spot urine samples were collected on d 20 of each period at 6 h before and 6 h after feeding. Urine was immediately diluted by mixing 15 mL of each sample with 60 mL of 0.072 *N* H₂SO₄ and storing it at -20°C until analysis. 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 Quik-Chem 8000 FIA, Lachat Instruments, Milwaukee, WI), and for creatinine (Valadares et al., 1999). Daily urine volume and excretion of urea N and total N were estimated from mean urinary concentrations in each period, assuming a creatinine excretion rate of 29 mg/kg of BW (Valadares et al., 1999).

On d 20 to 21 of each period, about 100 mL of fluid digesta was collected from 4 locations under the ruminal mat in the ventral rumen at 0 (just before feeding), 1, 2, 4, 6, 8, 12, 18, and 24 h after feeding from the 10 lactating Holstein cows fitted with ruminal cannulas using the probe described by Olmos Colmenero and Broderick (2006a). At each sampling, mixed fluid digesta was strained through 2 layers of cheesecloth and pH measured immediately in strained fluid using a glass electrode. Two 10-mL aliquots of ruminal fluid were then preserved 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), and total AA using a fluorimetric procedure based on the reaction with *o*-phthaldialdehyde (Roth, 1971). Leucine was the standard for this assay and total AA values are reported in Leu equivalents. Samples were thawed and centrifuged (28,000 × *g* for 30 min at 4°C) before VFA determination using a modification of the GLC method for free fatty acids described in Supelco Bulletin 855B (Supelco Inc., Supelco Park, Bellefonte, PA) with flame-ionization detection. Standards or supernatants (0.5 or 1 µL) were injected onto a ZB-FFAP capillary column (30 m × 0.53 mm × 1.0 µm; no. 7HK-G009-22; Phenomenex Inc., Torrance, CA) with helium carrier gas at 100 kPa and a flow rate of 20 mL/min. Column oven temperature was 100°C at injection; after 2 min, the temperature was increased to 130°C at 10°C/min. Injector and detector temperatures were 230°C and 250°C. This method did not resolve isovalerate and 2-methyl butyrate, which are reported as isovalerate plus 2-methylbutyrate.

Statistical Analysis

The basic design of the lactation trial was a 5 × 5 Latin square, replicated 10 times. Half of the squares were supplemented with RPML and half were not; thus, there were 10 different diets (Table 2). Results from the lactation trial were analyzed using the Mixed procedures of SAS (2003, SAS Institute Inc., Cary, NC) for replicated Latin squares applying 3 different statistical models. The following model (model 1) was used to compute least squares means for production and excretion in cows fed each of the 10 diets:

$$Y_{ijk} = \mu + P_i + T_j + P \times T_{ij} + C_k + E_{ijk}, \quad [1]$$

where Y_{ijk} = dependent variable, μ = overall mean, P_i = effect of period *i* (*i* = 1 to 5), T_j = effect of dietary treatment *j* (*j* = 1 to 10), $P \times T_{ij}$ = interaction of period *i* and treatment *j*, C_k = effect of cow *k* (*k* = 1 to 50), and E_{ijk} = residual error. This model used 151 denominator degrees of freedom. All terms were considered fixed, except for C_k and E_{ijk} , which were considered random. Results from this analysis are in Table 3.

The following model (model 2) was used to fit production and excretion data to assess effects of RPML, protein source, CP concentration, parity, and their interactions:

$$\begin{aligned} Y_{ijklmn} = & \mu + P_i + \text{RPML}_j + \text{CPPS}_k + \text{Par}_l \\ & + \text{PSQ}(\text{Par})_{lm} + \text{RPML} \times \text{CPPS}_{jk} + \text{RPML} \times \text{Par}_{jl} \\ & + \text{CPPS} \times \text{Par}_{kl} + C_{n(j)} + E_{ijklmn}, \end{aligned} \quad [2]$$

where Y_{ijklmn} = dependent variable, μ = overall mean, P_i = effect of period *i* (*i* = 1 to 5), RPML_j = effect of RPML supplement *j* (*j* = 1 to 2), CPPS_k = effect of CP level-protein source *k* (*k* = 1 to 5), Par_l = effect of parity *l* (primiparous or multiparous), $\text{PSQ}(\text{Par})_{lm}$ = effect of square-pair *m* (*m* = 1 to 5) within parity *l*, $\text{RPML} \times \text{CPPS}_{jk}$ = interaction of RPML supplement *j* and CP level-protein source *k*, $\text{RPML} \times \text{Par}_{jl}$ = interaction of RPML supplement *j* and parity *l*, $\text{CPPS} \times \text{Par}_{kl}$ = interaction of CP level-protein source *k* and parity *l*, $C_{n(j)}$ = effect of cow *n* (*n* = 1 to 25) within RPML supplement *j*, and E_{ijklmn} = residual error. All terms were considered fixed, except for $C_{n(j)}$ and E_{ijklmn} , which were considered random. This model used 183 denominator degrees of freedom. Contrasts were used to evaluate the effects of RPML, protein source, CP concentration, parity, and the interactions, without the SBM plus CM treatment; least squares means and

SEM were estimated using LSM-estimate statements. Results from analysis of the effects of RPML, protein source, and dietary CP concentration and their interactions are in Table 4. Contrasts were used to evaluate the effects of SBM versus SBM plus CM, and CM versus SBM plus CM, only at low dietary CP; results from this analysis are in Table 5. Results from analysis of the effects of parity are in Table 6 and interactions of parity with protein source and with CP concentration are in Table 7.

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: Time-weighted mean = $[2 \times \text{avg}(0-2 \text{ h}) + 2 \times \text{avg}(2-4 \text{ h}) + 2 \times \text{avg}(4-6 \text{ h}) + 2 \times \text{avg}(6-8 \text{ h}) + 4 \times \text{avg}(8-12 \text{ h}) + 6 \times \text{avg}(12-18 \text{ h}) + 6 \times \text{avg}(18-24 \text{ h})]/24$, where $\text{avg}(0-2 \text{ h}) \dots \text{avg}(18-24 \text{ h})$ represents the mean value for each ruminal trait observed at 0 and 2 h ... 18 and 24 h after feeding. Time-weighted means were fit to the following model (model 3):

$$Y_{ijkl} = \mu + P_i + T_j + C_{k(l)} + E_{ijkl}, \quad [3]$$

where Y_{ijkl} = dependent variable, μ = overall mean, P_i = effect of period i ($i = 1$ to 5), T_j = effect of dietary treatment j ($j = 1$ to 10), $C_{k(l)}$ = effect of cow k ($k = 1$ to 4) within square l ($l = 1$ to 2), and E_{ijkl} = residual error. All terms were considered fixed, except for $C_{k(l)}$ and E_{ijkl} , which were considered random. This model used 28 denominator degrees of freedom. Contrasts were used to evaluate the effects of RPML, protein source, and CP concentration, without the SBM plus CM treatment; least squares means and SEM were estimated using LSM-estimate statements. Contrasts were also used to evaluate the effects of SBM versus SBM plus CM, and CM versus SBM plus CM, only at low dietary CP. Results from this analysis are in Table 8. For all models, significance was declared at $P \leq 0.05$ and trends were declared at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Feed Quality and Diet Composition

Composition data on the major feed ingredients used in preparation of 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 50% of total N; NPN normally accounts for 45 to 55% of total N in alfalfa silage (Broderick, 1995b). Very low contents of ammonia-N in both alfalfa and corn silages indicated

that these feeds were well preserved (McDonald et al., 1991).

The chemical composition of both SBM and CM (Table 1) differed somewhat from NRC (2001) tabular values. Although CP, NDF, and ADF contents were similar, SBM fed in the current trial was substantially higher in NDIN and protein fraction B3, which were reported as, respectively, 1.3 and 0.6% of total N in Table 15-1 (NRC, 2001). The principal differences from NRC (2001) values for CM were in CP, lipid, NDIN, and protein fraction B3, which were tabulated as, respectively, 37.8 and 5.3% of DM and 16.7 and 10.3% of total N (NRC, 2001). The NRC (2001) refers to CM as "mechanically extracted"; mechanically extracted CM has lower CP and substantially higher fat contents than solvent-extracted CM (Newkirk, 2009). Solvent-extracted CM was fed in the current trial; solvent CM was reported to contain (DM basis) 40.9% CP and 4.0% crude fat (Newkirk, 2009). Higher NDIN contents explain the greater protein fraction B3 observed for both SBM and CM relative to NRC (2001) values. The NDIN values reported here derived from using a method in which both amylase and Na_2SO_3 are omitted from the neutral detergent extraction, which increases observed NDIN values (Licitra et al., 1996).

Composition of the 10 diets fed in this trial is given in Table 2. Diets ranged from (DM basis) 29 to 33% NDF, 1.50 to 1.51 estimated Mcal of NE_L/kg (computed using NRC, 2001 software), 22 to 31% starch, and 45 to 50% NFC (Table 2). As judged from milk fat content (3.92 to 4.09%; Table 3) and mean rumen pH (6.39 to 6.45; Table 8), the relatively high starch and NFC contents appeared to have no negative effects on ruminal environment or animal performance. Dietary CP was slightly lower than formulated, ranging from 14.5 to 14.8% (low protein) and from 16.4 to 16.7% (high protein); diets containing SBM had 0.2 percentage units more CP than those with CM (Table 2). Supplemental SBM and CM supplied between 31 and 32% (low protein) and 41 to 42% (high protein) of total dietary CP. Based on NRC (2001) computations at the overall mean DMI (25.2 kg/d), predicted MP-allowable milk was 30 kg/d (low protein) and 32 to 33 kg/d (high protein); MP-allowable milk was 5 to 8 kg/d less than NE_L -allowable milk. The large magnitude of these differences may have occurred because the NRC (2001) model underestimates MP-allowable milk yield at low MP supply (Lee et al., 2012a). However, these predictions suggest that milk and protein yield were limited by MP supply in the current trial. Estimates made with the NRC (2001) model also indicated that the CM diets supplied 14 to 39 g/d more RUP and 4 g/d more absorbable Met (low protein) and 73 g/d more RUP

Table 3. Least squares means for production and urinary excretion of lactating dairy cows fed the 10 individual dietary treatments containing either 15 or 17% CP with different supplemental sources of CP and with or without rumen-protected Met and Lys (RPML; +, -)^{1,2}

Trait	15% CP						17% CP				SEM ³
	SBM		CM		SBM+CM		SBM		CM		
	–	+	–	+	–	+	–	+	–	+	
Production											
DMI, kg/d	23.7	25.9	24.3	26.3	24.2	26.5	24.5	25.8	24.5	26.4	0.62
BW gain, kg/d	0.36	0.20	0.56	0.53	0.46	0.46	0.53	0.47	0.41	0.41	0.130
Milk, kg/d	38.4	40.6	39.3	41.1	39.1	41.3	39.4	40.5	40.5	41.7	1.32
Milk:DMI	1.62	1.57	1.62	1.57	1.62	1.56	1.60	1.57	1.65	1.58	0.037
ECM, kg/d	37.9	39.1	38.1	40.2	37.5	40.6	38.7	39.6	39.9	40.5	1.33
ECM:DMI	1.59	1.51	1.56	1.53	1.55	1.53	1.57	1.54	1.62	1.54	0.035
Fat, %	3.99	3.97	3.95	4.03	3.92	4.02	3.97	4.06	4.09	3.98	0.099
Fat, kg/d	1.53	1.59	1.54	1.64	1.52	1.65	1.57	1.63	1.64	1.66	0.062
True protein, %	3.06	3.02	3.06	3.04	2.98	3.08	3.05	3.03	3.10	3.00	0.054
True protein, kg/d	1.17	1.21	1.19	1.24	1.16	1.26	1.20	1.21	1.23	1.25	0.038
Lactose, %	4.98	4.82	4.92	4.86	4.83	4.91	4.89	4.81	4.89	4.73	0.058
Lactose, kg/d	1.91	1.95	1.91	1.99	1.88	2.02	1.93	1.94	1.95	1.99	0.067
SNF, %	8.94	8.72	8.86	8.78	8.69	8.87	8.84	8.72	8.88	8.59	0.101
SNF, kg/d	3.43	3.51	3.44	3.59	3.38	3.64	3.48	3.50	3.54	3.60	0.113
SCC, ×10 ³ cells/mL	276	189	307	331	247	159	291	354	447	444	135.7
MUN, mg/dL	9.9	9.9	8.4	9.1	9.2	9.9	12.7	13.6	11.8	12.2	0.26
Milk-N:N intake, %	32.8	30.9	32.9	31.7	32.1	31.8	28.8	27.6	30.0	28.4	0.64
Urinary excretion ⁴											
Urine volume, L/d	23.9	27.7	24.3	23.8	26.2	25.8	29.2	30.9	28.1	29.7	1.35
Urea-N, g/d	105.2	109.2	80.7	94.0	91.3	111.6	171.8	179.0	142.1	166.6	6.67
Total urinary-N, g/d	184.2	202.4	164.0	179.4	187.1	200.9	265.9	280.0	233.3	259.3	8.20
Urea-N:total-N, %	57.9	52.9	49.0	51.9	48.5	54.6	63.8	66.8	61.4	64.1	2.15

¹Sources of supplemental CP: CM = canola meal, SBM = solvent soybean meal, SBM+CM = blend with equal CP from solvent soybean meal and canola meal.²Minus (-) indicates no RPML supplement; plus (+) indicates supplementation of 8 g/d of absorbed Met and 12 g/d of absorbed Lys (assuming 72% bioavailability of Met and 64% bioavailability of Lys from rumen-protected AA and 25.2 kg/d of DMI).³Standard error of the least squares means determined using statistical model 1.⁴Estimated from urinary excretion of creatinine according to Valadares et al. (1999).

and 5 g/d more absorbable Met (high protein), and improved mean Lys:Met ratio (averaged across those 8 treatments) from 3.55 to 3.2.

Production Responses

Least squares means from the 10 individual treatments are reported in Table 3. Lactation performance was relatively high in this experiment, with DMI and yields of milk, fat, and protein averaging, respectively, 25.2, 40.2, 1.60, and 1.21 kg/d. However, our main objective was to compare production responses to SBM and CM at low and high dietary protein, to determine whether these were influenced by RPML supplementation and whether responses differed according to parity. Evaluation of the effects of blending CM and SBM CP was a secondary objective; thus, we omitted both SBM + CM treatments (with and without RPML) from the statistical analyses presented in Table 4. Although milk SCC were relatively high, SCC was not associated with any main effect or interaction, indicating that subclinical mastitis did not influence differences in production among treatments. Replacing SBM with CM increased ($P \leq 0.05$) DMI (0.4 kg/d) and yields of milk (0.9 kg/d), ECM (0.4 kg/d), and true protein (0.03 kg/d), and resulted in trends for greater fat and SNF yields. Moreover, the CM diets improved ($P \leq 0.01$) N-utilization as indicated by increased milk-N:N intake (from 30.0 to 30.8%) and reduced MUN and urinary excretion of urea-N, total-N, and urea-N:total-N. Urine volumes, which were estimated based on creatinine concentration and ranged from 24 to 31 L/d, were lower than that found by Valadares et al. (1999), who reported a mean volume of 40 L of urine/d using the method applied in the present study. Urine volume is influenced by mineral intake, particularly potassium consumption. Eriksson and Rustas (2014) measured (by total collection) excretions of 14, 27, and 40 L of urine/d when dairy cows consumed 20.2 kg of DM/d of diets containing, respectively, potassium at 1.19, 2.31, and 3.22% of DM. Dietary potassium in the present trial, computed from NRC (2001) tables, ranged from 1.51 to 1.65% of DM. Urine volume tended to be reduced when CM replaced SBM in the diet (Table 4). In an earlier comparison of CM with SBM (Brito and Broderick, 2007), we observed numeric improvements in these same traits but likely had insufficient power to detect statistical significance. In their meta-analysis, Huhtanen et al. (2011) reported generally positive responses when CM replaced other dietary proteins but noted that these effects were smaller when CM replaced SBM. Those researchers attributed increased DMI on CM to a “pull effect,” whereby a better AA pattern in MP improved yield of milk and milk components, which

in turn stimulated greater feed consumption to support the elevated yield. However, Martineau et al. (2013), in their meta-analysis, observed improved protein yield but not improved yield of milk and ECM or milk-N:N intake, when CM was substituted for SBM.

Greater dietary CP content was associated with increased ($P = 0.05$) fat yield with a trend for increased milk yield (Table 4). However, greater dietary CP also elevated MUN, urinary volume, and N excretion and depressed apparent N-efficiency. Kalscheur et al. (1999) found that increasing dietary CP increased the yields of milk, fat, and protein from wk 4 through 14 of lactation, but not after wk 19 of lactation, in high-producing dairy cows. On average, cows were about 13 wk into lactation at the start of the current trial. Our findings generally differed from earlier observations of greater production of milk and milk components, on diets formulated from similar feedstuffs with SBM as supplemental protein, when CP was increased from 15.4 to 16.7% but not from 16.7 to 18.4% CP (Broderick, 2003), and from 15.7 to 16.7% but not from 16.7 to 17.7% CP (Olmos Colmenero and Broderick, 2006b). Several other studies have shown that reducing dietary CP substantially improved apparent N efficiency (Wu and Satter, 2000; Olmos Colmenero and Broderick, 2006a), but maximal N efficiency in another trial came at the expense of depressed yields of milk and milk components (Broderick et al., 2008). An interesting observation was reduced milk lactose content ($P = 0.03$), but not yield, when dietary CP was increased.

Although RPML supplementation increased DMI by 1.8 kg/d ($P = 0.01$), it had no significant effect on yields of milk, ECM, or milk components (Table 4). It is unclear why RPML stimulated such a large feed intake effect, and contradictory responses have been reported in the literature. A recent meta-analysis (Zanton et al., 2014) indicated small DMI increases with abomasally infused DL-Met (0.12 kg/d) and with feeding 2-hydroxy-4-methylthio-butyrate (0.15 kg/d) and Smartamine-M (Adisseo Corp., Alpharetta, GA; 0.31 kg/d), but a negative effect (-0.25 kg/d) of feeding Mepron, which was also fed in present study. Ariola Apelo et al. (2014) found no effect on DMI when feeding rumen-protected Met, Lys, and Leu, either singly or in combination. Supplementing diets with small amounts of “low-solubles” fish meal, a protein source that is high in both RUP and Met, had differential effects depending on whether it was fed with diets based on alfalfa silage or alfalfa hay. Addition of fish meal to the higher CP alfalfa silage diet increased DMI by 1.2 kg/d but had no effect on the alfalfa hay diet, on which cows were already consuming 2.6 kg/d more DM and secreting 0.10 kg/d more milk protein (Broderick, 1995a). Supplemental RPML in the present

Table 4. Effects of source of dietary protein, CP concentration, and supplemental rumen-protected Met plus Lys (RPML) on least squares means for production and urinary excretion in lactating dairy cows¹

Trait	Protein source		Formulated [CP]		SEM ²	RPML ³			Probability ⁴					
	SBM	CM	15%	17%		–	+	SEM ²	Source	[CP]	RPML	Source × [CP]	Source × RPML	[CP] × RPML
Production														
DMI, kg/d	24.8	25.2	24.9	25.0	0.39	24.1	25.9	0.53	0.05	0.47	0.01	0.91	0.51	0.17
BW gain, kg/d	0.38	0.47	0.41	0.45	0.067	0.46	0.39	0.067	0.32	0.67	0.43	0.07	0.61	0.73
Milk, kg/d	39.3	40.3	39.5	40.1	0.84	38.9	40.7	1.16	<0.01	0.07	0.26	0.33	0.87	0.21
Milk:DMI	1.59	1.60	1.59	1.60	0.026	1.61	1.58	0.035	0.11	0.14	0.44	0.17	0.43	0.88
ECM, kg/d	38.5	39.5	38.6	39.3	0.82	38.2	39.7	1.11	0.04	0.11	0.34	0.39	0.74	0.36
ECM:DMI	1.55	1.57	1.55	1.57	0.023	1.58	1.54	0.030	0.21	0.16	0.27	0.14	0.96	0.98
Fat, %	3.99	4.02	3.99	4.02	0.062	4.00	4.01	0.081	0.49	0.50	0.95	0.51	0.58	0.59
Fat, kg/d	1.56	1.61	1.56	1.61	0.039	1.55	1.63	0.052	0.06	0.05	0.32	0.34	0.94	0.44
True protein, %	3.04	3.06	3.05	3.05	0.033	3.08	3.03	0.044	0.51	0.80	0.43	0.99	0.57	0.50
True protein, kg/d	1.19	1.22	1.19	1.22	0.021	1.19	1.22	0.029	0.02	0.14	0.34	0.47	0.58	0.30
Lactose, %	4.88	4.87	4.91	4.84	0.032	4.93	4.83	0.039	0.71	0.03	0.06	0.76	0.89	0.91
Lactose, kg/d	1.92	1.95	1.93	1.94	0.043	1.90	1.96	0.059	0.13	0.72	0.47	0.62	0.50	0.39
SNF, %	8.81	8.81	8.85	8.77	0.056	8.90	8.72	0.066	1.00	0.18	0.06	0.85	0.88	0.61
SNF, kg/d	3.45	3.53	3.47	3.51	0.069	3.44	3.54	0.093	0.07	0.39	0.42	0.54	0.51	0.30
SCC, ×10 ³ cells/mL	277	384	274	388	73.7	336	325	84.4	0.22	0.19	0.93	0.83	0.90	0.72
MUN, mg/dL	11.5	10.3	9.3	12.5	0.17	10.7	11.2	0.22	<0.01	<0.01	0.11	0.63	0.56	0.40
Milk-N:N intake, %	30.0	30.8	32.1	28.8	0.38	31.1	29.8	0.51	<0.01	<0.01	0.07	0.32	0.78	0.80
Urinary excretion ⁵														
Urine volume, L/d	27.7	26.3	24.8	29.2	0.75	26.1	27.9	0.91	0.07	<0.01	0.15	0.70	0.21	0.81
Urea-N, g/d	138	119	96	161	3.4	122	135	4.0	<0.01	<0.01	0.02	0.95	0.10	0.25
Total-N, g/d	229	206	180	254	4.4	208	226	5.6	<0.01	<0.01	0.02	0.67	0.47	0.67
Urea-N:total-N, %	60.4	56.7	52.7	64.4	1.07	58.0	59.1	1.11	0.01	<0.01	0.49	0.49	0.21	0.16

¹Sources of supplemental CP: CM = canola meal, SBM = solvent soybean meal; RPML = rumen-protected Met plus Lys.²Standard error of the least squares means determined using statistical model 2; SEM for protein source and [CP] were identical.³Minus (–) indicates no RPML supplement; plus (+) indicates supplementation with RPML.⁴Probability of dietary treatment effects: Source = SBM versus CM; [CP] = 15% versus 17% CP; RPML = no RPML versus plus RPML; Source × [CP] = interaction of protein source and CP concentration; Source × RPML = interaction of protein source and RPML supplement; [CP] × RPML = interaction of CP concentration and RPML supplement.⁵Estimated from urinary excretion of creatinine according to Valadares et al. (1999).

study elevated ($P \leq 0.05$) MUN and urinary urea-N and total-N excretion, and tended to reduce milk-N:N intake. This likely occurred because elevated N intake, due to the substantial increase in DMI, was not accompanied by significantly increased secretion of milk or protein. Thus, the extra CP consumed contributed to the urea pool and was excreted in the urine. Note that supplemental RPML supplied an additional 11 g/d to N intake; urinary urea-N increased by 13 g/d on the RPML treatment. As was detected with elevated CP, dietary RPML tended to reduce milk lactose content, which was related to a trend for reduced milk SNF content. Yields of lactose and SNF were not, however, altered. In an earlier trial (Broderick, 1992), reduced milk lactose concentration was observed when cows were supplemented with small amounts of low-solubles fish meal, the high-Met, high-RUP source mentioned above. Depressed milk lactose content may derive from increased supply of absorbable Met, acting through some unknown mechanism. However, lactose yield was not different and reduced lactose concentration was likely due to dilution from elevated milk yield.

Aside from a trend for an interaction of protein source and dietary CP concentration on DMI and a trend for an interaction of protein source and RPML on urinary excretion of urea-N, no other interaction of protein source $\times$ CP or protein source $\times$ RPML ($P \geq 0.14$) was detected in this study (Table 4). This indicated that replacing SBM with CM was just as effective at high CP as at low CP and that there were no differential effects of adding RPML, regardless of whether diets contained SBM or CM. Previously, we had observed production responses with addition of rumen-protected Met alone to diets supplemented with protein as SBM plus roasted soybeans or solvent plus expeller SBM: increased milk and protein yield and N-efficiency occurred when rumen-protected Met was fed as Mepron (Broderick and Muck, 2009; Broderick et al., 2009), and increased milk protein content and fat yield occurred when rumen-protected Met was fed as Smart-amine-M or Meta-Smart (Adisseo Corp.; Chen et al., 2011). A large body of literature documents that feeding rumen-protected Met 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, rumen-protected Met supplementation has elevated yields of milk (Schmidt et al., 1999), total protein (Armentano et al., 1997), and true protein (Rulquin and Delaby, 1997). The greater Met concentration in CM and the predicted greater supply of absorbable Met (Table 2) implied that cows would be less responsive to RPML on CM diets. Lee et al. (2012b) found that adding RPML as Mepron plus AminoShure-L to a 13.6% CP diet

(with supplemental protein from roasted soybeans and CM) resulted in milk and protein yields that were equal to that on a 15.7% CP diet, in which the additional CP came from expeller SBM. Polan et al. (1991) reported that rumen-protected Lys improved milk yield in cows fed diets containing corn gluten meal, a low Lys protein, as the principal CP supplement. It is noted that SBM, CM, and rumen microbial protein all have relatively high Lys concentrations (NRC, 2001) and would be expected to contribute large amounts of absorbable Lys. However, Socha et al. (2005) observed that rumen-protected Met (without Lys) and an RPML treatment both improved ECM:DMI and N-efficiency in early-lactation cows fed a soy-protein based diet containing 18.6% CP; these researchers also reported that their RPML treatment was substantially more effective than rumen-protected Met alone for increasing milk yield.

We were interested in whether dilution of 50% of the dietary CM protein with SBM protein would give production responses similar to CM alone; this was done only at the low CP concentration. The only responses detected were lower ($P = 0.03$) MUN and a trend for lower proportion of urea-N in total urinary-N (Table 5). Although DMI was the same on CM alone as on SBM + CM, this numeric difference was not different from that on SBM alone ($P = 0.11$). Intake and production were similar ($P \geq 0.24$) on the CM and SBM + CM treatments except that traits related to N-efficiency (MUN, urine volume, and urinary excretion of urea-N and total-N) were poorer on the SBM + CM diet. These results suggest that, although yield of milk and milk components would be comparable on CM and SBM + CM, this occurred without the improved protein utilization occurring on CM alone.

As expected, multiparous cows had greater ($P \leq 0.03$) DMI and yields of milk, true protein, lactose, and SNF, with trends for greater fat and ECM yield compared with primiparous cows (Table 6). Reduced ($P \leq 0.02$) lactose and SNF contents in milk secreted by multiparous cows was also observed; reduced lactose accounted for most of the reduction in SNF concentration. Reduced lactose and SNF concentrations were only partly due to dilution because milk yield was 8.8% greater in multiparous cows, whereas lactose and SNF yields were, respectively, 5.9 and 6.8% greater in multiparous versus primiparous cows. Miglior et al. (2007) reported that milk lactose concentration declined from 4.71 to 4.53% between parity 1 and parity 3 in a survey of Canadian Holsteins. Urine volume ($P = 0.04$) and excretion of urea-N and total-N ($P < 0.01$) were also higher in multiparous cows. However, greater N excretion was probably driven by greater CP intake because MUN, milk-N:N intake, and urinary urea-N as a proportion of total-N were not different by parity (P

Table 5. Effects of source and concentration of dietary supplemental protein on least squares means for production and urinary excretion in lactating dairy cows¹

Trait	Protein source (15% CP)			SEM ²	Contrast ³	
	SBM	SBM+CM	CM		S vs. S+C	C vs. S+C
Production						
DMI, kg/d	24.7	25.1	25.1	0.41	0.11	0.90
BW gain, kg/d	0.27	0.46	0.54	0.096	0.17	0.53
Milk, kg/d	39.2	39.9	39.8	0.87	0.15	0.95
Milk:DMI	1.59	1.59	1.59	0.027	0.84	0.97
ECM, kg/d	38.3	38.6	38.9	0.90	0.59	0.77
ECM:DMI	1.55	1.54	1.55	0.025	0.56	0.68
Fat, %	3.99	3.96	3.99	0.070	0.67	0.65
Fat, kg/d	1.55	1.57	1.58	0.043	0.66	0.82
True protein, %	3.05	3.03	3.06	0.038	0.70	0.40
True protein, kg/d	1.18	1.20	1.21	0.024	0.48	0.69
Lactose, %	4.91	4.88	4.91	0.040	0.41	0.44
Lactose, kg/d	1.92	1.93	1.94	0.046	0.66	0.78
SNF, %	8.85	8.79	8.86	0.070	0.48	0.40
SNF, kg/d	3.45	3.48	3.50	0.075	0.59	0.74
SCC, $\times 10^3$ cells/mL	211	192	337	96.5	0.88	0.24
MUN, mg/dL	9.9	9.5	8.7	0.19	0.03	<0.01
Milk-N:N intake, %	31.8	31.9	32.3	0.43	0.78	0.26
Urinary excretion ⁴						
Urine volume, L/d	25.7	25.8	24.0	0.92	0.89	0.09
Urea-N, g/d	105	99	86	4.3	0.29	0.02
Total-N, g/d	191	191	170	5.2	0.99	<0.01
Urea-N:total-N, %	55.1	51.3	50.4	1.48	0.07	0.64

¹Sources of supplemental protein: CM = canola meal, SBM = solvent soybean meal, SBM+CM = blend with equal CP from solvent soybean meal and canola meal.

²Standard error of the least squares means determined using statistical model 2.

³Probability of contrasts: S vs. S+C = SBM versus SBM+CM; C versus S+C = CM vs. SBM+CM.

⁴Estimated from urinary excretion of creatinine according to Valadares et al. (1999).

Table 6. Effect of parity on least squares means for production and urinary excretion in lactating dairy cows

Trait	Primiparous	SEM ¹	Multiparous	SEM ¹	Probability ²
Production					
DMI, kg/d	23.9	0.58	26.0	0.47	0.01
BW gain, kg/d	0.40	0.074	0.46	0.060	0.53
Milk, kg/d	37.9	1.27	41.7	1.04	0.03
Milk:DMI	1.59	0.039	1.60	0.032	0.81
ECM, kg/d	37.5	1.22	40.4	0.99	0.06
ECM:DMI	1.57	0.033	1.55	0.027	0.70
Fat, %	4.02	0.089	4.00	0.073	0.86
Fat, kg/d	1.52	0.057	1.65	0.047	0.07
True protein, %	3.07	0.048	3.03	0.039	0.55
True protein, kg/d	1.16	0.031	1.25	0.026	0.03
Lactose, %	4.96	0.043	4.80	0.035	<0.01
Lactose, kg/d	1.88	0.065	1.99	0.053	0.18
SNF, %	8.92	0.073	8.71	0.059	0.02
SNF, kg/d	3.37	0.102	3.60	0.084	0.09
SCC, $\times 10^3$ cells/mL	334	92.5	327	75.5	0.95
MUN, mg/dL	10.7	0.24	11.1	0.20	0.16
Milk-N:N intake, %	30.6	0.56	30.3	0.45	0.69
Urinary excretion ³					
Urine volume, L/d	25.7	0.99	28.4	0.81	0.04
Urea-N, g/d	116	4.3	141	3.5	<0.01
Total-N, g/d	198	6.1	236	5.0	<0.01
Urea-N:total-N, %	58.8	1.22	58.3	0.99	0.74

¹Standard error of the least squares means determined using statistical model 2.

²Probability of parity effects.

³Estimated from urinary excretion of creatinine according to Valadares et al. (1999).

≤ 0.16), indicating similar efficiency of MP utilization between primiparous and multiparous cows.

Interactions of parity with protein source and with CP concentration for production and urinary excretion traits are given in Table 7. No significant RPML $\times$ parity interactions were observed ($P \geq 0.18$) and these have been omitted from Table 7. However, parity $\times$ protein source interactions were detected ($P \leq 0.04$) for ECM:DMI, milk lactose, and SNF contents, and milk-N/N intake; trends also were detected for ECM yield and milk fat and true protein contents. The general pattern of these effects was that CM increased these traits in primiparous cows, whereas little change or even slight declines occurred in multiparous cows; components increased in concentration in milk from primiparous cows and tended to decrease in milk from multiparous cows. For example, ECM yield, milk fat content, true protein content, and milk-N:N intake increased from, respectively, 36.6 to 38.4 kg/d, 3.96 to 4.07%, 3.04 to 3.10%, and 29.9 to 31.3% when CM replaced SBM in diets fed to primiparous cows; however, ECM yield, milk fat content, true protein content, and milk-N:N intake changed from, respectively, 40.3 to 40.5 kg/d, 4.02 to 3.97%, 3.05 to 3.02%, and 30.1 to 30.4% when CM replaced SBM in diets fed to multiparous cows. This suggested that production and N-efficiency in primiparous cows were somewhat more responsive to improved amount and quality of MP supply. Nevertheless, replacement of SBM with CM resulted in greater reduction ($P \leq 0.05$) in urinary excretion of urea-N and total-N in multiparous than in primiparous cows. Evaluating the interactions of parity with dietary CP concentration revealed that increased dietary CP content reduced DMI in primiparous cows but elevated DMI in multiparous cows ($P < 0.01$; Table 7); however, the opposite trend was observed for milk:DMI ($P = 0.02$). Interactions for total urinary N excretion ($P = 0.01$) and trends for milk-N:N intake, urinary urea-N, and urea-N:total-N, reflected greater effects on all 4 traits in multiparous than primiparous cows. Results in the present trial appear to contradict the assumption that primiparous dairy cows are less responsive than multiparous cows to increasing nutrient supply. Additional requirements for growth as well as lactation (NRC, 2001) may make primiparous animals more responsive to improved amount and EAA pattern of MP. There are few literature reports of parity $\times$ nutrient interactions related to N efficiency. Drackley et al. (2003) observed no parity $\times$ diet interactions when varying dietary NE_L content from 1.53 to 1.62 Mcal/kg of DM by adding starch or fat. Flis and Wattiaux (2005) detected parity $\times$ RUP interactions for metabolism of absorbed N: although similar proportions of N intake were secreted as milk N

in both parities, urinary N excretion was greater in primiparous cows whereas apparent tissue retention was greater in multiparous cows, a result at variance with our findings. Although they observed parity effects on milk and component yield when increasing dietary CP from 14 to 16%, Hymøller et al. (2014) reported no parity $\times$ diet interactions.

Ruminal Metabolites

Ruminal pH and metabolites are given in Table 8. Ruminal pH and concentrations of total VFA, the major VFA (acetate, propionate, and butyrate), and acetate:propionate ratio were not affected by diet in this trial ($P \geq 0.15$). The RPML supplement also did not alter traits related to protein breakdown in the rumen. As expected, increasing dietary CP concentration increased ($P \leq 0.01$) ruminal concentrations of ammonia, isobutyrate, and total branched-chain VFA (BCVFA), and gave rise to a trend for increased isovalerate concentration. These compounds all derive from AA catabolism by ruminal microbes. The BCVFA are formed from microbial deamination and decarboxylation of branched-chain AA, and reduced BCVFA levels reflect reduced ruminal protein degradation (Van Soest, 1994). Moreover, we detected a trend for increased valerate concentration on 17% versus 15% dietary CP. El-Shazly (1952) reported that *n*-valeric acid was formed from ruminal catabolism of the protein AA Arg, Pro, and Lys, likely explaining the elevated ruminal valerate. Ruminal concentrations of total free AA were not influenced by diet ($P \geq 0.18$) and notably were not elevated with the greater dietary protein diet. The α -phthalaldehyde fluorescence assay used in this trial detects free AA only but is insensitive to oligopeptides, which are the major metabolites formed in protein degradation (Colombini et al., 2011). Earlier, we observed no relationship between protein degradation rate, measured in the in vivo rumen, and total AA concentrations measured using α -phthalaldehyde fluorescence (Reynal and Broderick, 2003). We conclude that ruminal total free AA concentrations alone are an unreliable index of ruminal protein degradation. Replacing SBM with CM in the diet clearly reduced ($P \leq 0.01$) concentrations in the rumen of ammonia and individual and total BCVFA. Relative to the CM diet, isobutyrate and ammonia were also elevated ($P \leq 0.03$), with a trend for elevated BCVFA, on the SBM + CM diet, indicating that ruminal metabolites related to protein degradation on this diet were more similar to the SBM diet than to the CM diet. These results strongly suggest lower ruminal degradation of, and greater RUP contribution from, CM than SBM.

Table 7. Effects of parity, source of dietary protein and CP concentration on least squares means for production and urinary excretion in lactating dairy cows¹

Trait	Protein source			Protein source			Prob. ³	Formulated [CP]				Prob. ³
	Primiparous		SEM ²	Multiparous		SEM ²		Primiparous		Multiparous		
	SBM	CM		SBM	CM			15%	17%	15%	17%	
Production							Par × source					Par × [CP]
DMI, kg/d	23.7	24.1	0.60	25.8	26.2	0.49	0.88	24.2	23.7	25.6	26.4	<0.01
BW gain, kg/d	0.33	0.46	0.104	0.43	0.48	0.085	0.67	0.38	0.41	0.43	0.48	0.92
Milk, kg/d	37.4	38.5	1.30	41.3	42.0	1.06	0.50	37.8	38.1	41.2	42.1	0.32
Milk:DMI	1.57	1.60	0.040	1.60	1.60	0.032	0.21	1.57	1.61	1.61	1.60	0.02
ECM, kg/d	36.6	38.4	1.28	40.3	40.5	1.04	0.09	37.3	37.7	39.8	41.0	0.42
ECM:DMI	1.54	1.60	0.035	1.56	1.54	0.029	0.03	1.55	1.59	1.55	1.55	0.16
Fat, %	3.96	4.07	0.096	4.02	3.97	0.078	0.08	4.02	4.01	3.96	4.03	0.39
Fat, kg/d	1.48	1.56	0.060	1.65	1.66	0.049	0.11	1.51	1.54	1.62	1.69	0.45
True protein, %	3.04	3.10	0.052	3.05	3.02	0.042	0.09	3.08	3.06	3.03	3.04	0.42
True protein, kg/d	1.13	1.19	0.033	1.24	1.25	0.027	0.13	1.15	1.17	1.23	1.26	0.51
Lactose, %	4.93	4.99	0.050	4.84	4.75	0.041	0.03	5.02	4.90	4.81	4.78	0.20
Lactose, kg/d	1.84	1.91	0.067	1.99	1.99	0.055	0.13	1.88	1.87	1.98	2.00	0.43
SNF, %	8.86	8.98	0.086	8.77	8.64	0.070	0.03	8.99	8.85	8.72	8.69	0.32
SNF, kg/d	3.31	3.44	0.107	3.60	3.61	0.087	0.11	3.37	3.38	3.57	3.63	0.49
SCC, ×10 ³ cells/mL	274	395	114.3	280	374	93.3	0.88	264	405	283	370	0.76
MUN, mg/dL	11.2	10.2	0.26	11.8	10.5	0.21	0.58	9.1	12.3	9.5	12.8	0.53
Milk-N:N intake, %	29.9	31.3	0.60	30.1	30.4	0.49	0.04	31.9	29.2	32.2	28.4	0.06
Urinary excretion ⁴												
Urine volume, L/d	25.8	25.5	1.15	29.6	27.1	0.94	0.16	23.5	27.8	26.2	30.6	0.97
Urea-N, g/d	121	111	5.2	154	128	4.3	0.04	86	145	105	177	0.09
Total-N, g/d	206	191	6.8	252	221	5.6	0.05	167	230	193	279	0.01
Urea-N:total-N, %	60.5	57.2	1.65	60.4	56.2	1.35	0.77	51.8	65.9	53.7	62.9	0.10

¹Sources of supplemental CP: CM = canola meal, SBM = solvent soybean meal.²Standard error of the least squares means determined using statistical model 2; SEM for protein source and CP concentration within primiparous and multiparous cows were identical.³Par $\times$ source = probability (Prob.) of interaction of parity and protein source; Par $\times$ [CP] = probability of interaction of parity and CP concentration.⁴Estimated from urinary excretion of creatinine according to Valadares et al. (1999).

Table 8. Effects of source and concentration of dietary supplemental protein on ruminal pH and metabolite concentrations¹

Trait	15% CP			17% CP			Prob. ³	Contrasts ⁴		
	SBM	CM	SBM+CM	SBM	CM	SEM ²		[CP]	SBM vs. CM	S vs. S+C
pH	6.51	6.53	6.58	6.53	6.51	0.052	0.97	0.96	0.93	0.09
Ammonia-N, mg/dL	4.34	4.14	4.85	7.22	6.25	0.349	0.83	<0.01	0.01	0.11
Total AA, mM	2.05	2.12	1.95	2.11	1.92	0.148	0.74	0.43	0.49	0.44
Total VFA, mM	87.6	91.0	88.9	91.9	88.0	3.21	0.77	0.78	0.91	0.68
Acetate, mM	54.1	56.5	55.6	56.8	55.5	1.90	0.96	0.51	0.67	0.42
Propionate, mM	20.6	21.6	20.4	20.6	19.6	1.20	0.54	0.25	0.99	0.90
Acetate:propionate ratio	2.83	2.77	2.84	2.86	2.94	0.115	0.34	0.15	0.98	0.99
Butyrate, mM	9.3	9.3	9.2	10.2	9.4	0.46	0.59	0.18	0.20	0.82
Isovalerate, mM	0.83	0.76	0.85	0.99	0.84	0.032	0.51	<0.01	<0.01	0.59
Isovalerate + 2-methyl butyrate, mM	1.58	1.47	1.59	1.80	1.43	0.103	0.82	0.09	<0.01	0.93
BCVFA, ⁵ mM	2.41	2.23	2.43	2.78	2.27	0.131	0.97	0.01	<0.01	0.83
Valerate, mM	1.23	1.31	1.27	1.43	1.30	0.057	0.17	0.08	0.64	0.61

¹Sources of supplemental CP: CM = canola meal, SBM = solvent soybean meal, SBM+CM = blend with equal CP from solvent soybean meal and canola meal.²Standard error of the least squares means determined using statistical model 3.³Probability (Prob.) of no rumen-protected Met plus Lys versus supplementation with rumen-protected Met plus Lys (RPML).⁴Probability of contrasts for ruminal traits: [CP] = 15% versus 17% CP (excluding the SBM+CM treatment); SBM vs. CM = SBM versus CM at both 15 and 17% CP (excluding the SBM+CM treatment); S vs. S+C = SBM versus SBM+CM at 15% CP; C vs. S+C = CM versus SBM+CM at 15% CP.⁵Branched-chain VFA (isobutyrate plus isovalerate + 2-methyl butyrate).

CONCLUSIONS

Replacing equal SBM CP with CP from CM in diets formulated from corn silage, alfalfa silage, and high-moisture corn and averaging 14.7 or 16.5% CP increased intake and yields of milk and true protein, improved milk-N:N intake, and depressed MUN, urine volume, and urinary N excretion. Responses to CM feeding were greater in primiparous than in multiparous cows. Higher dietary CP gave rise to small increases in fat yield but substantially elevated MUN and urinary N excretion. We detected no protein source $\times$ CP concentration interaction, indicating that replacing SBM with CM was effective at both low and high dietary CP. Reduced ruminal concentrations of ammonia and BCVFA indicated lower degradation of CM protein. Rumen-protected Met plus Lys increased intake without affecting production in this trial. Blending equal CP from SBM and CM at low CP decreased MUN and urinary N excretion compared with SBM alone but was not as effective as CM alone in improving N-utilization. Replacing SBM with CM increased production of milk and milk protein, reduced urinary N-excretion, and improved N-efficiency in lactating dairy cows fed both low and high dietary CP.

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