J. Dairy Sci. 91:1092–1102
doi:10.3168/jds.2007-0769

Effect of Supplementing Rumen-Protected Methionine on Production and Nitrogen Excretion in Lactating Dairy Cows[1]

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ABSTRACT

Two 4 × 4 Latin square trials (4-wk periods; 16 wk total) were conducted to see whether supplementing rumen-protected Met (RPM; fed as Mepron) would allow feeding less crude protein (CP), thereby reducing urinary N excretion, but without losing production. In trial 1, 24 Holsteins were fed 4 diets as total mixed rations containing [dry matter (DM) basis]: 18.6% CP and 0 g of RPM/d; 17.3% CP and 5 g of RPM/d; 16.1% CP and 10 g of RPM/d; or 14.8% CP and 15 g of RPM/d. Dietary CP was reduced by replacing soybean meal with high-moisture shelled corn. All diets contained 21% alfalfa silage, 28% corn silage, 4.5% roasted soybeans, 5.8% soyhulls, 0.6% sodium bicarbonate, 0.5% vitamins and minerals, and 27% neutral detergent fiber. There was no effect of diet on intake, weight gain, or yields of protein, lactose, and solids-not-fat. However, production was greater at 17.3% CP plus RPM and 16.1% CP plus RPM than on the other 2 diets. Apparent N efficiency (milk N:N intake) was greatest on the lowest CP diet containing the most RPM. Linear reductions in milk urea N and urinary N excretion were observed with lower dietary CP. In trial 2, 32 Holsteins were fed 4 diets as total mixed rations, formulated from ingredients used in trial 1 and containing 16.1 or 17.3% CP with 0 or 10 g of RPM/d. On average, cows were calculated to be in negative N balance on all diets because of lower than expected DM intake. There was no effect of RPM supplementation on any production trait. However, higher CP gave small increases in yields of milk, protein, and solids-not-fat and tended to increase DM intake and lactose yield. Apparent N efficiency was greater, and milk urea nitrogen was lower, on 16.1% CP. In trial 1, feeding lower CP diets supplemented with RPM resulted in improved N efficiency and reduced urinary N excretion. However, in trial 2, reducing dietary CP from 17.3 to 16.1% reduced milk secretion, an effect that was not reversed by RPM supplementation at low DM intakes when cows were apparently mobilizing body protein.
Key words: rumen-protected methionine, dietary crude protein, milk yield, nitrogen efficiency

INTRODUCTION

A substantial body of literature indicates that Met is the essential AA most limiting for production in dairy cows fed diets based on legume forages, corn silage, corn grain, and soybean meal (Schwab et al., 1976; Pisulewski et al., 1997; NRC, 2001). Several studies have shown that supplementing these diets with rumen-protected Met (**RPM**) increases milk yield (Schmidt et al., 1999) as well as milk protein content (Armentano et al., 1997; Berthiaume et al., 2006) and yield (Armentano et al., 1997; Rulquin and Delaby, 1997). Thus, supplementing RPM may allow the feeding of diets with lower CP content without losing milk and protein yields. Blood and milk urea concentrations and urinary urea excretion are directly related to dietary CP (Broderick and Clayton, 1997; Nousiainen et al., 2004). Recent research has shown that decreasing dietary CP may actually result in greater yields of both milk and milk components (Olmos Colmenero and Broderick, 2006b). Reducing dietary CP would decrease both feed costs and urinary urea excretion (Broderick, 2003); much of the urinary urea can be volatilized as ammonia after urea contacts the high microbial urease activity present in feces (Muck, 1982).

Leonardi et al. (2003) reported that supplementing diets with RPM increased milk protein concentration

Received October 10, 2007.
Accepted December 5, 2007.
[1]Mention of any trademark or proprietary product in this paper does not constitute a guarantee or warranty of the product by the USDA or the Agricultural Research Service and does not imply its approval to the exclusion of other products that also may be suitable.
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at both 16.1 and 18.8% dietary CP, with no interactions. This would be unexpected if there were a linear response to the first-limiting AA until its requirement was met. However, Lapierre et al. (2005) suggested that rather than the "broken-stick" response, in which the AA requirement for a specific function (such as milk secretion) remains constant until the need for that AA is met, a curvilinear response may be more appropriate to describe the effects of supplementation of the limiting essential AA.

Two trials were conducted to study the effects on production and nutrient utilization of supplementing diets with RPM as Mepron (Degussa Corp., Kennesaw, GA). In trial 1, dietary CP was reduced in a stepwise manner, and supplemental RPM was increased incrementally with each decrease in CP. In trial 2, the intermediate levels of dietary CP used in trial 1 were fed both with and without supplementation of an equal amount of RPM.

MATERIALS AND METHODS

Trial 1

Sixteen multiparous (mean parity 3.1, SD 1.7) and 8 primiparous Holstein cows, averaging overall (mean ± SD) 45 ± 6 kg of milk/d, 100 ± 42 DIM, and 598 ± 73 kg of BW at the beginning of the trial, were blocked into 6 squares by parity and DIM and, within squares, were randomly assigned to treatment sequences in 6 replicated 4 × 4 Latin squares. One additional square of 4 ruminally cannulated multiparous Holstein cows, averaging 33 kg of milk/d, 255 DIM, and 655 kg of BW, was used in this trial for ruminal sampling; however, production data from these cows were not analyzed. Treatment sequences within each Latin square were organized to balance the effects of carryover such that each treatment followed every other treatment one time within each square. Experimental periods lasted 28 d and consisted of 14 d for diet adaptation and 14 d for data and sample collection. All cows were injected with bST (500 mg of Posilac, Monsanto, St. Louis, MO) beginning on d 1 of the trial and at 14-d intervals thereafter until completion of the study. Cows were housed in tie stalls and had free access to water throughout the experiment. Care and handling of the animals were conducted as outlined by the guidelines of the University of Wisconsin institutional animal care and use committee.

Diets were fed as TMR and were formulated principally from alfalfa silage, rolled corn silage, rolled high-moisture shelled corn, solvent-extracted soybean meal, roasted soybeans, and soyhulls, plus minerals and vitamins. Dietary CP was lowered from 18.6% of DM in steps of approximately 1.3 percentage units by replacing the soybean meal with high-moisture shelled corn. With each step down in CP, increments of 5 g/d of RPM were added to the TMR as Mepron (Degussa Corp.), assuming an intake of 24 kg/d of DM and an equivalence of 0.6 g of metabolizable Met/g of Mepron (Berthiaume et al., 2001). The TMR were prepared by blending individual feed ingredients. A top-loading balance with readability to 0.1 g was used to measure the Mepron blended into the TMR. Diets were offered once daily at 1000 h after orts were collected daily at 0900 h. Amounts of feed offered to the cows were adjusted daily to allow refusals equal to 5 to 10% of intake.

Daily samples of approximately 0.5 kg of silages, high-moisture shelled corn, each TMR, and orts were collected, stored at –20°C, and used to make weekly composites. Weekly samples of soybean meal, roasted soybeans, and soyhulls also were taken.

Dry matter contents of samples of weekly composites and individual feed samples were determined by drying at 60°C (forced-air oven) for 48 h. The 60°C DM contents of dietary ingredients were used weekly to adjust as-fed compositions of the TMR. Dry matter intake was computed based on the 60°C DM contents of the TMR and orts. These samples were ground to pass a 1-mm Wiley mill screen (Arthur H. Thomas, Philadelphia, PA) and analyzed later for total N (Leco FP-2000 Nitrogen Analyzer, Leco Instruments Inc., St. Joseph, MI), absolute DM, ash, and OM (AOAC, 1980), sequentially (Van Soest et al., 1991) for NDF, ADF, and ADIN by using heat-stable α-amylase and Na_2SO_3 (Hintz et al., 1995), and also for neutral detergent insoluble N without the use of Na_2SO_3 (Licitra et al., 1996). For AA determination, samples of feed were predigested with performic acid to stabilize Met and Cys, treated with hydrobromic acid to destroy the performic acid, and then acid-hydrolyzed with 6 *N* HCl (method 994-12; AOAC, 1997). A separate acid hydrolysis (6 *N* HCl) digestion procedure was conducted for Phe, Tyr, and His, because those AA are destroyed during the oxidation process and by reaction with bromine. Concentrations of AA were quantified by ion-exchange chromatography (Beckmann 6300, Beckman Instruments, Palo Alto, CA). Silage extracts were prepared in distilled water from weekly composites according to Muck (1987), pH was determined immediately, and extracts were analyzed for ammonia and total free AA (Broderick et al., 2004) by flow injection (Lachat Quik-Chem 8000 FIA, Lachat Instruments, Milwaukee, WI), and for NPN (Muck, 1987; VarioMax CN, Elementar Analysensystem GmbH, Hanau, Germany). Weekly TMR composites were analyzed for indigestible ADF (ADF remaining after 12 d of in situ incubation; Huhtanen et al., 1994). The TMR composites also were analyzed for total fat (method 920.39; AOAC, 1997; Dairyland Laboratories,

Table 1. Chemical composition of silages and principal concentrate ingredients[1]

Item	AS	CS	HMSC	SSBM	RSB	Soyhulls
DM (%)	39.2	38.7	75.7	90.6	97.1	91.9
CP (% of DM)	25.4	6.8	8.2	55.3	42.9	12.2
Ash (% of DM)	11.0	4.33	1.66	7.45	5.63	5.36
NDF (% of DM)	33.7	40.8	8.7	7.8	23.9	64.0
ADF (% of DM)	24.6	22.3	2.1	4.2	4.4	44.8
Hemicellulose (% of DM)	9.1	18.5	6.6	3.6	19.5	19.2
Neutral detergent insoluble N (% of total N)	8.17	20.7	14.6	3.43	17.2	19.6
ADIN (% of total N)	3.02	7.20	4.86	0.69	1.03	5.60
NFC[2] (% of DM)	29.7	46.3	78.3	30.2	15.9	18.1
Essential AA (g/100 g of CP)						
Arg	2.36	2.16	4.09	7.16	7.06	5.03
His	1.45	1.29	2.54	2.57	2.50	2.47
Ile	4.30	2.73	3.40	4.44	4.44	3.63
Leu	7.00	7.05	11.57	7.50	7.39	6.18
Lys	3.27	2.30	2.68	5.92	5.24	6.10
Met	1.45	1.44	2.03	1.37	1.39	1.15
Met + Cys	2.03	2.45	3.88	2.72	2.69	2.72
Phe	4.14	3.17	4.64	4.98	4.86	3.71
Thr	3.48	2.73	3.54	3.94	3.90	3.63
Val	5.22	3.74	4.74	4.67	4.68	4.29
Ammonia (% of total N)	4.89	7.11	—	—	—	—
Total free AA N[3] (% of total N)	27.0	28.9	—	—	—	—
NPN (% of total N)	44.2	60.5	—	—	—	—
pH	4.72	3.94	—	—	—	—

[1]AS = alfalfa silage; CS = corn silage; HMSC = high-moisture shelled corn; SSBM = solvent-extracted soybean meal; RSB = roasted soybeans.

[2]NFC = 100 – (%NDF – NDIN × 6.25) – %CP – %Fat – %Ash, using tabular values for fat content of these ingredients (NRC, 2001).

[3]Total free AA N = total free AA, mmol × (40.3 mg of N/mmol of total free AA) (Broderick, 1987).

Arcadia, WI), starch (Hall et al., 1999; T. K. M. Webster, West Virginia Univ., Morgantown), and neutral detergent insoluble N (Van Soest et al., 1991; Hintz et al., 1995) to compute NFC. Compositions of the major feed ingredients are shown in Table 1, and compositions of the TMR are given in Table 2.

Cows were milked twice daily, and milk yield was recorded at each milking in all experimental periods. Milk samples from the a.m. and p.m. milkings were collected on d 17 and 24 of each period and analyzed for fat, true protein, lactose, and SNF by infrared analysis (AgSource, Verona, WI) with a Foss FT6000 (Foss North America Inc., Eden Prairie, MN) by using AOAC (1990) method 972.16. For MUN determination, 5-mL quantities of milk samples from both milkings were treated with 5 mL of 25% (wt/vol) TCA. Samples were vortexed and allowed to stand for 30 min at room temperature before filtering through Whatman no. 1 filter paper. Filtrates were stored at –20°C until MUN analysis by an automated colorimetric assay (Broderick and Clayton, 1997) adapted to flow injection (Lachat Quik-Chem 8000 FIA). Concentrations and yields of fat, true protein, lactose, and SNF, as well as MUN concentration all were computed as the weighted means from a.m. and p.m. milk yields on each test day. Efficiency of conversion of feed DM was computed for each cow over the last 2 wk of each period by dividing mean milk yield by mean DMI; similarly, apparent efficiency of utilization of feed N (assuming no retention or mobilization of body N) was calculated for each cow by dividing mean milk N output (milk true protein/6.38) by mean N intake. For computation of BW change, BW was measured on 3 consecutive days at the beginning of the experiment and at the end of each period.

Fecal grab samples were collected from the 16 multiparous cows at approximately 6 h prefeeding (a.m. sampling) and 6 h postfeeding (p.m. sampling) on d 26 or 27, transferred to aluminum pans, and held at 60°C in a forced-air oven until completely dried. Fecal samples were then ground to pass a 1-mm Wiley mill screen, and a single composite was prepared for each cow in each period by mixing equal DM from both samples. Fecal samples were analyzed for DM, OM, NDF, ADF, total N, and indigestible ADF as described earlier. Indigestible ADF was used as an internal marker to estimate apparent nutrient digestibility and fecal N output (Cochran et al., 1986). When fecal sampling was done, spot urine samples also were obtained from the same 16 cows by mechanical stimulation of the vulva. After collection, 15 mL of urine was pipeted into specimen containers holding 60 mL of 0.072 N H_2SO_4 and stored at –20°C until analysis. After thawing at room temperature, urine samples were analyzed for creatinine by using a picric acid assay (Oser, 1965) adapted to flow-

Table 2. Composition of diets[1] in trial 1 (18.6, 17.3, 16.1, or 14.8% dietary CP and 0, 5, 10, or 15 g/d of RPM[2]) and trial 2 (17.3% dietary CP and 0 or 10 g/d of RPM, or 16.1% dietary CP and 0 or 10 g/d of RPM)

	Trial 1: dietary CP (%), RPM (g/d)				Trial 2: dietary CP (%), RPM (g/d)			
Item	18.6, 0	17.3, 5	16.1, 10	14.8, 15	17.3, 0	17.3, 10	16.1, 0	16.1, 10
	% of DM							
Alfalfa silage	20.9	20.9	20.9	20.9	20.9	20.9	20.9	20.9
Corn silage	28.1	28.1	28.1	28.1	28.1	28.1	28.1	28.1
Rolled HMSC	28.0	30.7	33.3	36.0	30.7	30.7	33.3	33.3
SSBM	11.7	8.9	6.2	3.5	8.9	8.9	6.2	6.2
Roasted soybeans	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5
Soyhulls	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8
Mepron[3]	0	0.035	0.07	0.105	0	0.07	0	0.07
Sodium bicarbonate	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Dicalcium phosphate	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Salt	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Vitamins and trace minerals[4]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Chemical composition								
DM (%)	51.4	51.0	51.5	51.3	49.3	49.3	49.1	49.5
CP (% DM)	18.6	17.3	16.1	14.8	17.3	17.3	16.1	16.1
Ash (% of DM)	6.42	6.26	6.11	5.95	6.26	6.26	6.11	6.11
NDF (% of DM)	26.6	26.6	26.6	26.7	26.6	26.6	26.7	26.6
ADF (% of DM)	15.3	15.2	15.2	15.1	15.2	15.2	15.2	15.2
Neutral detergent insoluble N (% of DM)	0.41	0.40	0.40	0.37	0.38	0.41	0.39	0.39
ADIN (% of DM)	0.07	0.08	0.06	0.06	0.05	0.07	0.07	0.07
Fat (% of DM)	3.3	3.0	3.4	3.5	3.3	2.7	2.7	3.4
NFC,[5] % of DM	47.6	49.3	50.3	51.4	49.0	49.7	50.9	50.3
Starch (% of DM)	23.0	24.5	26.1	27.9	24.8	24.6	26.3	26.5
RDP[6] (% of DM)	12.3	11.5	10.8	10.0	11.7	11.6	11.0	11.0
RUP[6] (% of DM)	6.3	5.8	5.3	4.8	5.6	5.7	5.1	5.1
Digestible Lys[6] (g/d)	172	165	162	154	155	155	148	144
Digestible Met[6] (g/d)	47	51	56	59	43	53	42	50
Lys:Met ratio[6]	3.7	3.2	2.9	2.6	3.6	2.9	3.5	2.9
NE_L[7] (Mcal/kg of DM)	1.64	1.63	1.61	1.61	1.66	1.66	1.66	1.67

[1]HMSC = high-moisture shelled corn; RPM = rumen-protected Met; SSBM = solvent-extracted soybean meal.

[2]Rumen-protected Met product from Degussa Corp. (Kennesaw, GA).

[3]Computed assuming 60% metabolizability of RPM in Mepron (Berthiaume et al., 2001).

[4]Provided (per kg 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.

[5]NFC = 100 – (%NDF – NDIN × 6.25) – %CP – %Fat – %Ash.

[6]Estimated using the NRC (2001) model.

[7]Computed by discounting dietary energy content based on observed DMI (NRC, 2001).

injection analysis (Lachat Quik-Chem 8000 FIA), for total N (Leco FP-2000 Nitrogen Analyzer), and for urea with the colorimetric method used for MUN. Daily urine volume and excretion of urea N and total N were estimated from urinary creatinine concentration, assuming a creatinine excretion rate of 29 mg/kg of BW (Valadares et al., 1999).

Samples of whole ruminal contents (approximately 200 mL) were taken from the ventral sac of the rumen of the 4 ruminally cannulated cows on d 27 to 28 of each period at 0 (prefeeding), 1, 2, 3, 6, 9, 12, and 18 h postfeeding, strained through 2 layers of cheesecloth, and followed immediately by pH measurement. Two 10-mL samples were then preserved in scintillation vials by addition of 0.2 mL of 50% H_2SO_4 and stored at –20°C until analysis. Samples were thawed at room temperature, centrifuged (15,000 × *g*, 15 min, 4°C), and the supernatants analyzed for ammonia and total free AA (Broderick et al., 2004) by flow injection (Lachat Quik-Chem 8000 FIA) and for VFA by gas chromatography (Brotz and Schaefer, 1987).

Trial 2

Twenty multiparous Holstein cows (mean parity 3.1, SD 1.6), including 4 with ruminal cannulas, plus 12 primiparous Holstein cows, averaging overall (mean ± SD) 44 ± 5 kg of milk/d, 116 ± 29 DIM, and 604 ± 46 kg of BW at the beginning of the trial, were blocked into squares by parity and DIM and, within squares, were randomly assigned to treatment sequences in 8 replicated 4 × 4 Latin squares. A 2 × 2 arrangement of treatments was used in this trial: 17.3 or 16.1% dietary CP with either 0 or 10 g/d of RPM fed as Mepron. The 4 diets were formulated from the same ingredients fed in trial 1; dietary compositions are shown in Table 2.

Except for obtaining production data on 2 additional squares of cows (the 4 ruminally cannulated cows plus 4 more primiparous cows), feeding protocol, feed sampling and analysis, milk sampling and analysis, injection with bST, animal housing, BW measurements, and sampling and analysis of urine, feces, and ruminal contents were as described for trial 1.

Statistical Analysis

Average intake and milk production data from each cow over the last 14 d of each period, and individual data obtained by using spot fecal and urine sampling from the 16 multiparous cows, were analyzed in each trial as replicated 4 × 4 Latin squares with the mixed procedures of SAS (SAS Institute, 1999–2000). For both trials, model sums of squares were separated into overall mean, cow (within square), square, period, diet, and square × diet and period × diet interactions. For trial 1, linear and quadratic effects of dietary CP content (ignoring RPM supplementation) on MUN, apparent N efficiency, and traits related to N excretion also were tested by using single degree of freedom contrasts in the model. For trial 2, single degree of freedom orthogonal contrasts were used to test for effects of CP level (17.3 or 16.1% dietary CP), RPM supplement (0 or 10 g/d), and CP × RPM interaction. All variables were considered fixed except cow (within square) and overall error, which were considered random; interaction terms were removed from the model when $P \geq 0.25$. For ruminal traits, model sums of squares for data collected at different times after feeding were separated into overall mean, cow, period, diet, square × diet interaction, whole-plot error, hours postfeeding (repeated measures), hours postfeeding × diet interaction, and subplot error. Repeated measures analyses were performed by using the SP(POW) structure of SAS (SAS Institute, 1999–2000). All variables were considered fixed except cow, whole-plot error, and subplot error, which were considered random. The interaction term square × diet was removed from the model when $P \geq 0.25$. For all statistical analyses, significance was declared at $P \leq 0.05$ and trends at $P \leq 0.10$. Mean separation was done by using the PDIFF statement in SAS and is reported only when $\alpha \leq 0.05$.

RESULTS AND DISCUSSION

Trial 1

Composition of the major feed ingredients fed in this study is shown in Table 1. Corn silage contained 41% NDF, 22% ADF, and approximately 7% CP, values that were only slightly lower than those reported for "normal" corn silage by the NRC (2001). However, chemical composition of the alfalfa silage—more than 25% CP and less than 34% NDF and 24% ADF—indicated it was of much higher quality than the alfalfa silage (21% CP and 42% NDF) being fed from the same silo immediately before the trial began. Both silages had typical proportions of NPN and low levels of ammonia N and ADIN, indicating that they were well fermented and that N utilization would be expected to be normal. Dietary NDF and NE_L contents, averaging 26.6% and 1.62 Mcal/kg (Table 2), were indicative of the high-energy diets that are appropriate for feeding high-producing dairy cows. Crude protein contents of the major feed ingredients were determined weekly throughout the trial, and as a result, the target of a 1.3% decrease in CP with each reduction in soybean meal, and increment of high-moisture corn plus RPM, was achieved in the trial. However, NDF and ADF analyses were not conducted until the study was completed, and dietary fiber concentrations were lower than the original formulations of 28% NDF and 20% ADF. As expected, levels of NFC increased when dietary CP was stepped down because high-moisture corn replaced solvent-extracted soybean meal. Levels of NFC ranged from approximately 48 to 51% of DM (Table 2), and although a dietary NFC content greater than 45% may be considered excessive (M. B. Hall, US Dairy Forage Research Center, Madison, WI; personal communication), substantial portions of the NFC fraction likely came from organic acids in silages and high-moisture corn plus pectin and other soluble-fiber compounds in alfalfa silage (Hall, 2003). Lanzas et al. (2007; Table 2) reported mean concentrations of total organic acids (VFA plus lactic acid) of 6.4, 7.2, and 2.3% of DM in, respectively, alfalfa silage, corn silage, and high-moisture corn. If the same levels of organic acids were present in these feedstuffs in the current trial, then organic acid-adjusted NFC contents of the diets would have ranged from 44 to 47% of DM. Moreover, alfalfa silage contains approximately 20% soluble fiber (Lanzas et al., 2007). Furthermore, TMR starch levels ranged from 24 to 28% of DM, indicative of high, but not excessive, starch concentrations (Oba and Allen, 2003).

Dry matter intake, weight gain, and yields of protein, lactose, and SNF were unaffected by diet (Table 3), despite an overall reduction in dietary CP of 3.8 percentage units. However, there were significant effects ($P \leq 0.05$) on production of milk and 3.5% FCM and trends ($P \leq 0.09$) for the effects on milk:DMI and fat yield (Table 3). Yields of milk and FCM actually were greater at 17.3% CP plus 5 g/d of RPM and 16.1% CP plus 10 g/d of RPM than on the other 2 diets. Previously, we observed a quadratic response to CP content on diets formulated from similar feedstuffs, with maximal milk and protein yields predicted at 16.7 and 17.1% CP

Table 3. Effect of reducing CP by replacing dietary soybean meal with high-moisture corn plus rumen-protected Met (RPM) on production, excretion, and apparent digestibility of lactating dairy cows (trial 1)

	Dietary CP (%), RPM[1] (g/d)					
Item	18.6, 0	17.3, 5	16.1, 10	14.8, 15	SE[2]	$P > F$[3]
DMI (kg/d)	23.4	23.4	23.8	23.7	0.6	0.85
BW gain (kg/d)	0.14	0.42	0.55	0.42	0.16	0.35
Milk yield (kg/d)	39.7^{b}	41.6^{a}	41.6^{a}	39.7^{b}	1.2	0.05
3.5% FCM (kg/d)	38.9^{b}	42.0^{a}	41.2^{ab}	38.6^{b}	1.7	0.05
Milk:DMI	1.72^{ab}	1.80^{a}	1.77^{ab}	1.69^{b}	0.05	0.07
Milk fat (%)	3.55	3.58	3.40	3.31	0.12	0.19
Milk fat (kg/d)	1.37^{ab}	1.49^{a}	1.43^{ab}	1.32^{b}	0.07	0.09
Milk protein (%)	3.02	2.98	2.97	3.04	0.05	0.42
Milk protein (kg/d)	1.15^{b}	1.23^{a}	1.23^{a}	1.20^{ab}	0.04	0.16
Milk lactose (%)	4.81	4.82	4.78	4.81	0.05	0.81
Milk lactose (kg/d)	1.90	2.00	1.99	1.92	0.07	0.17
Milk SNF (%)	8.74	8.72	8.68	8.71	0.06	0.45
Milk SNF (kg/d)	3.43	3.61	3.61	3.46	0.12	0.14
MUN (mg/dL)	14.5^{a}	11.8^{b}	9.5^{c}	7.9^{d}	0.4	<0.01
Milk N:N intake (%)	26.2^{c}	29.9^{b}	31.7^{b}	34.0^{a}	0.9	<0.01
Excretion[4]						
Urine volume (L/d)	27.9^{ab}	29.4^{a}	24.8^{bc}	24.2^{c}	1.9	0.01
Urinary urea N (g/d)	205^{a}	148^{b}	115^{c}	80^{d}	6	<0.01
Total urinary N (g/d)	260^{a}	207^{b}	188^{c}	150^{d}	8	<0.01
Urea N/total urinary N (%)	78.5^{a}	72.0^{b}	61.7^{c}	52.9^{d}	1.2	<0.01
Fecal DM (kg/d)	7.3^{c}	7.4^{bc}	8.2^{a}	7.9^{ab}	0.3	<0.01
Fecal N (g/d)	250	246	259	237	9	0.20
Total manure N (g/d)	510^{a}	453^{b}	447^{b}	387^{c}	13	<0.01
Estimated N balance[5] (g/d)	28	25	14	−7	9	0.01
Apparent digestibility[6]						
DM digestibility (%)	70.6^{a}	69.5^{ab}	68.5^{bc}	67.7^{c}	0.7	<0.01
OM digestibility (%)	71.5^{a}	70.6^{a}	69.1^{b}	68.7^{b}	0.7	<0.01
NDF digestibility (%)	58.5^{a}	56.2^{b}	54.2^{c}	52.2^{d}	1.1	<0.01
ADF digestibility (%)	54.0^{a}	53.1^{a}	51.0^{b}	47.7^{c}	1.2	<0.01
N digestibility (%)	66.0^{a}	63.6^{a}	61.3^{bc}	59.0^{c}	0.9	<0.01

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

[1]Computed assuming 60% metabolizability of RPM in Mepron (Degussa Corp., Kennesaw, GA; Berthiaume et al., 2001).

[2]SE = SE of the LSM difference.

[3]Probability of a significant effect of diet.

[4]Urinary excretion estimated using creatinine as a volume marker and fecal excretion estimated by using indigestible ADF as an internal marker in 16 of the 24 cows.

[5]Nitrogen balance computed assuming milk protein contains 6.38% N.

[6]Apparent digestibilities estimated from spot fecal sampling, using indigestible ADF as an internal marker.

(Olmos Colmenero and Broderick, 2006b). Reduced ($P < 0.01$) MUN (Table 3), as is typically found with decreased dietary CP, also occurred in this trial and was paralleled by increased apparent N efficiency (milk N:N intake). A significant ($P < 0.01$) negative linear relationship between dietary CP content and MUN and N efficiency was observed. Apparent N efficiency improved by nearly 8 percentage units from the highest to lowest CP, and was greatest ($P < 0.01$) on the diet containing the least CP and most RPM. However, the highest N efficiency on 14.8% CP occurred along with lost yields of milk and milk components relative to the 2 intermediate diets. The greatest N efficiency, accompanied by production and feed efficiency similar or equal to the highest observed in this trial, occurred on the RPM-supplemented diet containing 16.1% CP.

The NRC (2001) model predicted an NE_L-limited milk yield of 38 kg/d on all 4 diets but MP-limited milk yields of 39, 37, 35, and 32 kg/d on the diets containing, respectively, 18.6, 17.3, 16.1, and 14.8% CP. Responses in yields of milk and FCM indicated that MP and AA supplies were much better than predicted on the lower CP, RPM-supplemented diets. Assuming that Met limited production, the predicted supply of digestible Met was substantially greater on the 3 lower CP diets (Table 2); this probably accounted for the milk production observed in this trial. If it is assumed that the optimal ratio of absorbed Lys to Met is 3.0 (NRC, 2001) and that Lys is the second-limiting essential AA (Schwab et al., 1992), then the 2 median diets with Lys:Met ratios of 3.2 and 2.9 had the most favorable AA patterns and supplies in the current trial. On the basis of the

NRC (2001) model, digestible Lys became limiting when the estimated supply fell from 162 to 154 g/d (Table 2), resulting in a drop in milk yield from 41.6 to 39.7 kg/d between the diets containing 16.1 and 14.8% CP (Table 3).

A number of studies have shown that supplementing lactating dairy cows with RPM has improved milk protein synthesis. Feeding RPM increased milk concentrations of total protein (Armentano et al., 1997; Berthiaume et al., 2006), true protein (Berthiaume et al., 2006), and casein N (Overton et al., 1998), and yields of milk (Schmidt et al., 1999), total protein (Armentano et al., 1997), and true protein (Rulquin and Delaby, 1997). Blum et al. (1999) found that an elevated blood Met concentration was accompanied by a lower MUN concentration when RPM was fed as Mepron, indicating improved N utilization. Berthiaume et al. (2001) measured a 55% net appearance in the portal vein of D,L-Met fed to lactating dairy cows as Mepron. Although not affected overall by diet ($P = 0.16$), PDIFF-mean separations indicated that protein yield on the 2 median diets with added RPM actually was greater ($P < 0.05$) than on the diet with 18.6% CP. As discussed above, the improved milk production observed in the present trial probably occurred because of stimulation of milk protein synthesis when the limiting AA Met was supplied by feeding Mepron.

The stepwise improvement in apparent N efficiency that occurred when dietary soybean meal was replaced with high-moisture corn plus RPM resulted from lower ($P < 0.01$) urinary excretion of urea N and total N (Table 3). There was a significant ($P < 0.01$) linear relationship between dietary CP content and urinary urea N and total N. Reducing dietary CP concentration also resulted in highly significant ($P \leq 0.01$) reductions in estimated urine volume and proportion of urea N in total urinary N, which fell from 78 to 53%. As dietary CP was decreased from 18.6 to 14.8%, urea N and total N excreted in the urine, as estimated by spot urine sampling, declined by, respectively, 125 and 110 g/d; this suggested that the decline in urinary N excretion was accounted for by reduced output of urea N. We have observed a similar correspondence between the reductions in urea N and total N in the urine when using the same methodology in earlier trials (Broderick, 2003; Olmos Colmenero and Broderick, 2006b). Apparent N balance, computed from observed N intake and milk N secretion (milk N = milk protein/6.38), and estimated manure N excretion also showed a significant diet effect ($P \leq 0.01$); only cows fed the RPM-supplemented diet with 14.8% CP were in apparent negative N balance (Table 3). If N utilization was considered optimal on the diet with 16.1% CP plus RPM, then the 72 g/d reduction in total urinary N vs. that on the 18.6% CP would correspond to approximately 22 kg/cow of N over a 300-d lactation. In this trial, fecal N excretion was unaffected by diet.

As commonly observed, lower dietary CP concentration decreased apparent N digestibility because of a reduced dilution of metabolic fecal N that was proportional to DMI (Schneider and Flatt, 1975). However, decreasing dietary CP also lowered ($P < 0.01$) apparent digestibility of DM, OM, NDF, and ADF, and increased fecal DM output, as estimated by using spot fecal sampling and indigestible ADF as the fecal output marker (Table 3). Fecal DM output, which is important because it influences the manure volumes that must be handled by producers, was higher on 16.1% CP vs. 18.6 and 17.3% CP. There also was lower ruminal ammonia with decreased CP feeding, and reduced digestibility was paralleled by lower concentrations of acetate, propionate, and total VFA, as well as a trend for higher pH, in the rumen (Table 4). Digestibilities of DM and NDF fell by, respectively, 2.9 and 6.3 percentage units. Because NDF was 27% of consumed DM, lower NDF digestibility accounted for 58% of the reduction in DM digestibility as dietary CP was decreased in this trial (Table 3). Soybean meal and high-moisture corn had similar NDF contents (Table 1) and have similar NDF digestibilities (NRC, 2001), suggesting that an inadequate RDP supply depressed NDF digestion as CP was lowered. Stokes et al. (1991) found that decreasing RDP from 11.8 to 9% of dietary DM depressed ruminal OM digestion from 65 to 56%; diets with 16.1 and 14.8% CP in the present study were estimated to contain 10.8 and 10.0% RDP (Table 2). There was no effect of diet on ruminal total AA, and mean daily ammonia N concentration exceeded 5 mg/dL (Satter and Slyter, 1974), even on 14.8% CP; Stokes et al. (1991) also found that ruminal ammonia N ranged from 6 to 12 mg/dL on the 9% RDP diet with lowered ruminal OM digestion. A quadratic effect of dietary CP content on DM, OM, and fiber digestion was observed in another experiment in which SBM replaced high-moisture corn to elevate CP (Olmos Colmenero and Broderick, 2006b). However, increasing CP from 15.6 to 17.6% on diets formulated from similar ingredients did not alter total tract DM and NDF digestibility in a third study (Olmos Colmenero and Broderick, 2006a). An additional consideration is that dietary NFC and starch were elevated as dietary CP was decreased (Table 2). Ruminal pH was actually greater (Table 4) on the diet with the lowest CP and highest starch, suggesting that depressed DM or NDF digestibility was not due to excessive feeding of readily fermentable carbohydrate.

Results from this experiment indicated that RPM could be used to replace part of the CP that is normally fed as solvent-extracted soybean meal. By supplemen-

Table 4. Effect of varying CP by altering dietary soybean meal and high-moisture corn and supplementing with rumen-protected Met (RPM) on concentrations of ruminal metabolites in lactating dairy cows

	Trial 1: dietary CP (%), RPM[1] (g/d)					
Item	18.6, 0	17.3, 5	16.1, 10	14.8, 15	SE[2]	$P > F^3$
pH	6.47[b]	6.47[b]	6.49[b]	6.64[a]	0.05	0.07
Ammonia N (mg/dL)	9.75[a]	7.88[b]	6.10[bc]	5.86[c]	0.86	< 0.01
Total free AA (m*M*)	6.78	5.56	4.95	5.26	0.70	0.26
Acetate (m*M*)	60.3[a]	56.1[ab]	53.9[b]	53.3[b]	2.1	0.07
Propionate (m*M*)	20.4[a]	20.0[ab]	17.8[c]	18.3[bc]	0.7	0.02
Butyrate (m*M*)	11.4	10.2	9.9	10.2	0.8	0.24
BCVFA[4] (m*M*)	2.42	2.26	2.46	2.30	0.14	0.35
Valerate (m*M*)	1.97	1.77	1.86	2.03	0.17	0.19
Total VFA (m*M*)	98.8[a]	92.4[ab]	89.0[b]	87.9[b]	3.2	0.08
Acetate:propionate ratio	2.96	2.84	3.04	2.93	0.16	0.82
	Trial 2: dietary CP (%), RPM (g/d)					
	17.3, 0	17.3, 10	16.1, 0	16.1, 10		
pH	6.51	6.59	6.57	6.61	0.07	0.65
Ammonia-N (mg/dL)	8.96	7.48	8.99	7.13	0.79	0.22
Total free AA (m*M*)	7.75[ab]	5.30[bc]	8.06[a]	5.10[c]	0.94	0.05
Acetate (m*M*)	57.6	59.6	58.3	56.5	3.3	0.93
Propionate (m*M*)	18.7	20.1	18.3	19.2	1.0	0.63
Butyrate (m*M*)	11.7	10.6	10.9	10.4	0.6	0.38
BCVFA[4] (m*M*)	2.27	2.49	2.23	2.26	0.10	0.27
Valerate (m*M*)	1.80	1.73	1.69	1.71	0.08	0.84
Total VFA (m*M*)	92.0	94.5	91.4	90.1	5.0	0.94
Acetate:propionate ratio	3.12	2.97	3.20	2.94	0.07	0.20

[a–c]Means within a row with different superscripts differ ($P < 0.05$).

[1]Computed assuming 60% metabolizability of RPM in Mepron (Degussa Corp., Kennesaw, GA; Berthiaume et al., 2001).

[2]SE = SE of the LSM difference.

[3]Probability of a significant effect of diet.

[4]Total branched-chain VFA (sum of isobutyrate, isovalerate, and 2-methyl butyrate).

tation with RPM, it was possible to reduce dietary CP from 18.6 to as little as 16.1% CP without losing production of milk and milk components. This reduction also decreased excretion of urinary N, potentially the most polluting form of manure N. When feeding RPM, N efficiency was optimized at 16.1% CP. Reducing dietary CP to 14.8% could not be compensated for by RPM supplementation; that diet depressed milk production and resulted in mobilization of body protein.

Trial 2

Diets were formulated from the same feed ingredients as were fed in trial 1, and the targets of 17.3 and 16.1% CP, with or without approximately 10 g/d of RPM, also were achieved in this second study (Table 2). Surprisingly, no significant effects ($P \geq 0.27$) of RPM supplementation were detected for any production trait measured in this trial (Table 5). However, higher dietary CP resulted in slightly, but significantly, greater ($P \leq 0.05$) yields of milk, protein, and SNF, as well as trends ($P \leq 0.09$) for greater DMI and increased lactose secretion (Table 5). As expected, MUN was higher and apparent N efficiency (milk N:N intake) was lower on 17.3% CP, indicating better ($P < 0.01$) N utilization on the lower CP diet (Table 5).

Results from this study differed from trial 1 and the report of Leonardi et al. (2003) in that there was no response to RPM, regardless of protein level. Also unlike the findings of Leonardi et al. (2003), trends ($P \leq 0.10$) were detected for the interaction of dietary CP and RPM for protein and SNF yield. Cows in the Leonardi study were calculated to be in positive energy balance. However, because of lower than expected DMI in the present experiment, cows were calculated to be in negative energy balance, and mean N balance, estimated from milk protein yield and spot fecal and urine sampling, was negative on all 4 diets (Table 5). This occurred despite apparent BW gains, which averaged 0.35 kg/d. At the start of the experiment, cows in this study were at levels of production and stages of lactation (means 44 kg/d and 116 DIM) similar to those in trial 1 (means 45 kg/d and 100 DIM). Milk yields predicted from intake of NE_L and MP (NRC, 2001) were, respectively, 33 to 35 and 30 to 34 kg/d, compared with observed yields of 39 to 40 kg/d. The high mean feed

Table 5. Effect of supplementing rumen-protected Met (RPM) at 2 levels of dietary CP on production and excretion of lactating dairy cows (trial 2)

	Dietary CP (%), RPM[1] (g/d)					Contrasts (*P*-value)[3]		
Item	17.3, 0	17.3, 10	16.1, 0	16.1, 10	SE[2]	CP	RPM	CP × RPM
DMI (kg/d)	21.7	21.8	21.6	20.9	0.6	0.08	0.40	0.18
BW gain (kg/d)	0.49	0.24	0.33	0.36	0.10	0.89	0.27	0.16
Milk yield (kg/d)	39.8	40.1	39.2	38.7	1.0	0.04	0.80	0.54
3.5% FCM (kg/d)	39.7	40.7	39.9	39.1	1.2	0.28	0.91	0.33
Milk:DMI	1.87	1.87	1.84	1.89	0.04	0.97	0.33	0.34
Fat (%)	3.59	3.59	3.65	3.62	0.12	0.59	0.86	0.86
Fat (kg/d)	1.41	1.43	1.42	1.38	0.06	0.60	0.95	0.42
Protein (%)	3.07	3.09	3.07	3.06	0.03	0.42	0.95	0.65
Protein (kg/d)	1.21	1.23	1.19	1.17	0.03	0.04	0.88	0.19
Lactose (%)	4.84	4.84	4.84	4.83	0.03	0.89	0.62	0.99
Lactose (kg/d)	1.91	1.96	1.89	1.86	0.06	0.09	0.87	0.23
SNF (%)	8.85	8.85	8.83	8.83	0.04	0.25	0.83	0.97
SNF (kg/d)	3.48	3.57	3.44	3.38	0.09	0.05	0.78	0.19
MUN (mg/dL)	12.4	12.1	10.2	10.2	0.3	<0.01	0.43	0.51
Milk N:N intake (%)	31.9	32.4	34.3	34.7	0.7	<0.01	0.40	0.94
Excretion[4]								
Urine volume (L/d)	26.5	25.0	23.3	24.1	1.2	0.09	0.76	0.34
Urinary urea N (g/d)	133	148	122	121	5	<0.01	0.15	0.13
Total urinary N (g/d)	188	204	176	180	6	<0.01	0.08	0.24
Urea N:total urinary N (%)	69.8	71.2	68.3	68.7	1.6	0.18	0.56	0.71
Fecal DM (kg/d)	8.0	7.7	7.5	7.5	0.4	0.09	0.59	0.50
Fecal N (g/d)	270	262	242	242	12	0.01	0.60	0.65
Total manure N (g/d)	458	466	419	422	14	<0.01	0.58	0.79
Estimated N balance[5] (g/d)	−18	−22	−13	−23	15	0.87	0.55	0.77
Apparent digestibility[6]								
DM digestibility (%)	65.1	66.4	67.5	67.0	1.0	0.09	0.67	0.33
OM digestibility (%)	66.3	67.7	68.4	68.2	1.0	0.13	0.48	0.35
NDF digestibility (%)	50.4	52.6	55.4	55.7	1.1	0.01	0.17	0.28
ADF digestibility (%)	46.1	51.4	54.4	54.2	1.2	<0.01	0.10	0.06
N digestibility	62.4	62.2	62.1	61.8	1.2	0.78	0.81	0.95

[1]Computed assuming 60% metabolizability of RPM in Mepron (Degussa Corp., Kennesaw, GA; Berthiaume et al., 2001).

[2]SE of the LSM.

[3]Orthogonal contrasts: CP = 17.3% CP vs. 16.1% CP; RPM = 0 vs. 10 g/d of RPM; CP × RPM = interaction of CP level and RPM.

[4]Urinary excretion estimated using creatinine as a volume marker and fecal excretion estimated using indigestible ADF as an internal marker in 16 of the 32 cows.

[5]Nitrogen balance computed assuming milk protein contains 6.38% N.

[6]Apparent digestibilities estimated from spot fecal sampling, using indigestible ADF as an internal marker.

efficiency (milk yield:DMI) of 1.87 reflected the considerable mobilization of tissue AA and fat to support milk synthesis. Production likely was limited by energy supply, and the small effects of CP may have been driven by greater DMI, which trended higher on 17.3% CP (Table 5). The positive response to RPM supplementation in trial 1 apparently was related to the mean 2.1 kg/d greater DMI and the fact that cows were in positive N balance on the 3 highest CP diets (Table 3). The lack of response to RPM in trial 2 may also be related to AA balance. As discussed for trial 1, milk yield declined when digestible Lys fell below 160 g/d. Predicted supply of digestible Lys (Table 2) was below this value for all 4 diets in trial 2; thus, Lys limitation may have inhibited the RPM response.

Proportion of total urinary N excreted as urea also differed between studies. In trial 1, urea N accounted for 72 and 62% of total urinary N on, respectively, 17.3 and 16.1% CP (Table 3). In trial 2, these proportions were not different on 17.3 and 16.1% CP, averaging 69% across diets (Table 5), possibly reflecting similar catabolism of AA for energy. Schei et al. (2007) speculated that inadequate supplies of glucogenic substrates limit the response to AA when cows are in negative energy balance. Greater amounts of RUP on the 17.3% CP diets may have increased the supply of glucogenic AA to the liver. Schwab et al. (1992) reported that milk protein content and yield were greater with duodenal infusion of Lys rather than Met during early and peak lactation, when cows are more likely to be mobilizing body protein, whereas the responses were similar in midlactation. Furthermore, Socha et al. (2005) observed a significant effect on milk protein yield when feeding rumen-protected Met plus Lys, but not RPM alone, in

early-lactation cows fed diets that were similar to those in the present trial except for 1.4% added blood meal. Socha et al. (2005) also detected greater milk protein yield with rumen-protected Met plus Lys at 18.5% vs. 16.0% dietary CP.

Apparent NDF and ADF digestion was slightly lower ($P \leq 0.01$), and there was a trend ($P = 0.09$) for greater fecal DM output, on 17.3% compared with 16.1% CP (Table 5); this was opposite what was observed in trial 1. This apparently anomalous effect was not related to ruminal pH and concentrations of ammonia, which were similar across diets, or to total free AA, which averaged 6.5 and 6.6 m*M* on, respectively, 17.3 and 16.1% dietary CP (Table 4). Ruminal total free AA were lower ($P = 0.05$) with RPM supplementation regardless of CP level. We have no explanation for this surprising result, and further experimentation may be required to confirm its validity and importance.

Under the conditions of this trial, in which DMI was more than 2 kg/d lower than in the earlier study, decreasing dietary CP from 17.3 to 16.1% caused small decreases in milk and protein yields, reductions that were not reversed by RPM supplementation. Despite an apparent BW gain, estimated N retention was negative on all 4 diets. The findings from this trial suggest that RPM might be ineffective when low feed intake results in substantial mobilization of body protein.

CONCLUSIONS

Two feeding studies were conducted to assess the effects of supplementing cows with RPM at approximately 100 to 200 DIM and feeding TMR based on alfalfa and corn silages, high-moisture corn, roasted soybeans, and soyhulls, with CP varied by addition of solvent-extracted soybean meal. In trial 1, dietary CP was decreased from 18.6 to 17.3, 16.1, and 14.8% CP and supplemented with, respectively, 0, 5, 10, and 15 g/d of RPM (fed as 0, 8, 17, and 25 g/d of Mepron). Milk:DMI and yields of milk, 3.5% FCM, and fat were greater at 17.3% CP plus RPM and 16.1% CP plus RPM than on higher or lower CP concentrations. Apparent N efficiency (milk N:N intake) was greatest ($P < 0.01$) on the lowest CP diet containing the most RPM; MUN and urinary N excretion decreased in parallel with dietary CP. Cows were in apparent positive N balance on all diets except the diet containing 14.8% CP. Feeding lower CP diets supplemented with RPM improved N efficiency and reduced urinary N excretion; the best compromise between milk production and lowered N excretion occurred on 16.1% CP plus 10 g/d of RPM (17 g/d of Mepron). In trial 2, cows were fed a TMR formulated from the same ingredients to contain 16.1 or 17.3% CP, with or without 10 g/d of RPM. On average, cows consumed 2.1 kg/d less DM than in trial 1 and were calculated to be in negative N balance. There was no effect of RPM supplementation on production, but there were small positive effects of dietary CP on yields of milk, protein, and SNF, and a trend for greater DMI. Apparent N efficiency was greater, and MUN lower, on 16.1% CP. Mobilization of body protein at intakes too low to maintain milk yield may have prevented a positive response to RPM supplementation.

ACKNOWLEDGMENTS

The authors thank Rick Walgenbach and his farm crew for harvesting and storing the feedstuffs used in these trials; Jill Davidson and her barn crew for feeding and animal care at the US Dairy Forage Center Research Farm (Prairie du Sac, WI); Wendy Radloff, Mary Becker, and Steven Diederich for assisting with sampling and laboratory analyses; and Peter Crump for his help with statistical analyses. Partial financial support for this research from Degussa Co. is also gratefully acknowledged.

REFERENCES

AOAC. 1980. Official Methods of Analysis. 13th ed. Assoc. Off. Anal. Chem., Washington, DC.

AOAC. 1990. Official Methods of Analysis. 15th ed. Assoc. Off. Anal. Chem., Arlington, VA.

AOAC. 1997. Official Methods of Analysis. 16th ed. Assoc. Off. Anal. Chem., Arlington, VA.

Armentano, L. E., S. J. Bertics, and G. A. Ducharme. 1997. Response of lactating cows to methionine or methionine plus lysine added to high protein diets based on alfalfa and heated soybeans. J. Dairy Sci. 80:1194–1199.

Berthiaume, R., P. Dubreuil, M. Stevenson, B. W. McBride, and H. Lapierre. 2001. Intestinal disappearance and mesenteric and portal appearance of amino acids in dairy cows fed ruminally protected methionine. J. Dairy Sci. 84:194–203.

Berthiaume, R., M. C. Thivierge, R. A. Patton, P. Dubreuil, M. Stevenson, B. W. McBride, and H. Lapierre. 2006. Effect of ruminally protected methionine on splanchnic metabolism of amino acids in lactating dairy cows. J. Dairy Sci. 89:1621–1634.

Blum, J. W., R. M. Bruckmaier, and F. Jans. 1999. Rumen-protected methionine fed to dairy cows: Bioavailability and effects on plasma amino acid pattern and plasma metabolite and insulin concentrations. J. Dairy Sci. 82:1991–1998.

Broderick, G. A. 1987. Determination of protein degradation rates using a rumen in-vitro system containing inhibitors of microbial nitrogen metabolism. Br. J. Nutr. 58:463–476.

Broderick, G. A. 2003. Effects of varying dietary protein and energy levels on the production of lactating dairy cows. J. Dairy Sci. 86:1370–1381.

Broderick, G. A., and M. K. Clayton. 1997. A statistical evaluation of animal and nutritional factors influencing concentrations of milk urea nitrogen. J. Dairy Sci. 80:2964–2971.

Broderick, G. A., P. Udén, M. L. Murphy, and A. Lapins. 2004. Sources of variation in rates of in vitro ruminal protein degradation. J. Dairy Sci. 87:1345–1359.

Brotz, P. G., and D. M. Schaefer. 1987. Simultaneous determination of lactic acid and volatile fatty acids in microbial fermentation extracts by gas-liquid chromatography. J. Microbiol. Methods 6:139–144.

Cochran, R. C., D. C. Adams, J. D. Wallace, and M. L. Galyean. 1986. Predicting digestibility of different diets with internal markers: Evaluation of four potential markers. J. Anim. Sci. 63:1476–1487.
Hall, M. B. 2003. Challenges with nonfiber carbohydrate methods. J. Anim. Sci. 81:3226–3232.
Hall, M. B., W. H. Hoover, J. P. Jennings, and T. K. M. Webster. 1999. A method for partitioning neutral detergent-soluble carbohydrates. J. Sci. Food Agric. 79:2079–2086.
Hintz, R. W., D. R. Mertens, and K. A. Albrecht. 1995. Effects of sodium sulfite on recovery and composition of detergent fiber and lignin. J. AOAC. 78:16–22.
Huhtanen, P., K. Kaustell, and S. Jaakkola. 1994. The use of internal markers to predict total digestibility and duodenal flow of nutrients in cattle given six different diets. Anim. Feed Sci. Technol. 48:211–227.
Lanzas, C., C. J. Sniffen, S. Seo, L. O. Tedeschi, and D. G. Fox. 2007. A revised CNCPS feed carbohydrate fractionation scheme for formulating rations for ruminants. Anim. Feed Sci. Technol. 136:167–190.
Lapierre, H., R. Berthiaume, G. Raggio, M. C. Thivierge, L. Doepel, D. Pacheco, P. Dubreuil, and G. E. Lobley. 2005. The route of absorbed nitrogen into milk protein. Anim. Sci. 80:10–22.
Leonardi, C., M. Stevenson, and L. E. Armentano. 2003. Effect of two levels of crude protein and methionine supplementation on performance of dairy cows. J. Dairy Sci. 86:4033–4042.
Licitra, G., T. M. Hernandez, and P. J. Van Soest. 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim. Feed. Sci. Technol. 57:347–358.
Muck, R. E. 1982. Urease activity in bovine feces. J. Dairy Sci. 65:2157–2163.
Muck, R. E. 1987. Dry matter level effects on alfalfa silage quality. 1. Nitrogen transformations. Trans. ASAE 30:7–14.
Nousiainen, J., K. J. Shingfield, and P. Huhtanen. 2004. Evaluation of milk urea nitrogen as a diagnostic of protein feeding. J. Dairy Sci. 87:386–398.
NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Sci., Washington, DC.
Oba, M., and M. S. Allen. 2003. Effects of diet fermentability on efficiency of microbial nitrogen production in lactating dairy cows. J. Dairy Sci. 86:195–207.
Olmos Colmenero, J. J., and G. A. Broderick. 2006a. Effect of amount and ruminal degradability of soybean meal protein on performance of lactating dairy cows. J. Dairy Sci. 89:1635–1643.
Olmos Colmenero, J. J., and G. A. Broderick. 2006b. Effect of dietary crude protein concentration on milk production and nitrogen utilization in lactating dairy cows. J. Dairy Sci. 89:1704–1712.
Oser, B. L. 1965. Hawk's Physiological Chemistry. 14 ed. McGraw-Hill, New York, NY.
Overton, T. R., L. S. Emmert, and J. H. Clark. 1998. Effects of source of carbohydrate and protein and rumen-protected methionine on performance of cows. J. Dairy Sci. 81:221–228.
Pisulewski, P. M., H. Rulquin, J. L. Peyraud, and R. Verite. 1997. Lactational and systemic responses of dairy cows to postruminal infusions of increasing amounts of methionine. J. Dairy Sci. 79:1781–1791.
Rulquin, H., and L. Delaby. 1997. Effects of the energy balance of dairy cows on lactational responses to rumen-protected methionine. J. Dairy Sci. 80:2513–2522.
SAS Institute. 1999–2000. SAS/STAT User's Guide: Release 8.1. SAS Inst. Inc., Cary, NC.
Satter, L. D., and L. L. Slyter. 1974. Effect of ammonia concentration on rumen microbial protein production in-vitro. Br. J. Nutr. 32:199–208.
Schei, I., A. Danfær, I. A. Boman, and H. Volden. 2007. Post-ruminal or intravenous infusions of carbohydrates or amino acids to dairy cows. 1. Early lactation. Animal 1:501–514.
Schmidt, J., P. Sipocz, E. Cenkvari, and J. Sipocz. 1999. Use of protected methionine (Mepron M 85) in cattle. Acta Vet. Hung. 47:409–418.
Schneider, B. H., and W. P. Flatt. 1975. The Evaluation of Feeds Through Digestibility Experiments. Univ. Georgia Press, Athens.
Schwab, C. G., C. K. Bozak, N. L. Whitehouse, and M. M. A. Messbah. 1992. Amino acid limitation and flow to duodenum at four stages of lactation. 1. Sequences of lysine and methionine limitation. J. Dairy Sci. 75:3486–3502.
Schwab, C. G., L. D. Satter, and A. B. Clay. 1976. Responses of lactating dairy cows to abomasal infusions of amino acids. J. Dairy Sci. 59:1254–1270.
Socha, M. T., D. E. Putnam, B. D. Garthwaite, N. L. Whitehouse, N. A. Kierstead, C. G. Schwab, G. A. Ducharme, and J. C. Robert. 2005. Improving intestinal amino acid supply of pre- and postpartum dairy cows with rumen-protected methionine and lysine. J. Dairy Sci. 88:1113–1126.
Stokes, S. R., W. H. Hoover, T. K. Miller, and R. Blauweikel. 1991. Ruminal digestion and microbial utilization of diets varying in type of carbohydrate and protein. J. Dairy Sci. 74:871–881.
Valadares, R. F. D., G. A. Broderick, S. C. Valadares Filho, and M. K. Clayton. 1999. Effect of replacing alfalfa silage with high moisture corn on ruminal protein synthesis estimated from excretion of total purine derivatives. J. Dairy Sci. 82:2686–2696.
Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583–3597.