J. Dairy Sci. 94:1978–1988
doi:10.3168/jds.2010-3578

Effect of feeding different sources of rumen-protected methionine on milk production and N-utilization in lactating dairy cows[1]

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ABSTRACT

Objectives of this study were to quantify production responses of lactating dairy cows to supplying absorbable Met as isopropyl-2-hydroxy-4-(methylthio)-butanoic acid (HMBi), or rumen-protected Met (RPM, Smartamine M; Adisseo, Alpharetta, GA) fed with or without 2-hydroxy-4-(methylthio)-butanoic acid (HMB), and to determine whether Met supplementation will allow the feeding of reduced dietary crude protein (CP). Seventy cows were blocked by parity and days in milk into 14 blocks and randomly assigned within blocks to 1 of the 5 dietary treatments based on alfalfa and corn silages plus high-moisture corn: 1 diet with 15.6% CP and no Met source (negative control); 3 diets with 15.6% CP plus 0.17% HMBi, 0.06% RPM + 0.10% HMB, or 0.06% RPM alone; and 1 diet with 16.8% CP and no Met supplement (positive control). Assuming that 50% of ingested HMBi was absorbed from the gastrointestinal tract and 80% of the Met in RPM was absorbed at intestine, the HMBi and RPM supplements increased metabolizable Met supply by 9 g/d and improved the Lys:Met ratio from 3.6 to 3.0. After a 2-wk covariate period during which all cows received the same diet, cows were fed test diets continuously for 12 wk. Diet did not affect dry matter intake (mean ± SD, 25.0 ± 0.3 kg/d), body weight gain (0.59 ± 0.2 kg/d), or milk yield (41.7 ± 0.6 kg/d). However, feeding HMBi increased yield of energy-corrected milk and milk content of protein and solids-not-fat. Moreover, trends were observed for increased milk fat content and yield of fat and true protein on all 3 diets containing supplemental Met. Apparent N efficiency (milk N/N intake) was highest on the RPM treatment. Feeding 16.8% CP without a Met source elevated milk urea N and urinary excretion of urea N and total N and reduced apparent N efficiency from 34.5 to 30.2%, without improving production. Overall results suggested that feeding HMBi or RPM would give similar improvements in milk production and N utilization.

Key words: rumen-protected methionine, isopropyl-2-hydroxy-4-(methylthio)-butanoic acid, nitrogen efficiency

INTRODUCTION

Overfeeding of CP results in the extra CP above requirement being excreted largely as urinary N, the most environmentally labile form of excretory N. Reducing dietary CP results in an overall reduction in N excretion, but may also depress yields of milk and milk protein (Broderick, 2003). Previous research has shown that milk production was not increased when diets contained more than 17.0% CP in early lactation (Wu and Satter, 2000) and about 16 to 16.5% CP after the peak of the lactation curve (Wu and Satter, 2000; Broderick, 2003). Methionine and Lys are considered the most limiting essential AA for the synthesis of milk and milk protein in the high-producing dairy cow (Schwab et al., 1992; Rulquin et al., 1993). Methionine often limits milk secretion in high-producing dairy cows when diets are formulated from typical North American ingredients, particularly when supplemental protein comes principally from soybean meal (**SBM**). Hence, lactating cows often respond to postruminal Met supplementation with increased milk protein synthesis and secretion. Balancing rations with Met supplementation to improve the profile of essential AA in MP is fundamental to allowing the feeding of lower levels of dietary CP and RUP, maximizing lactation performance and minimizing N excretion.

An effective approach to increasing postruminal Met supply is to feed rumen-protected Met (**RPM**). Tech-

Received June 30, 2010.
Accepted December 16, 2010.
[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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nologies that have been used include physically coating a core of Met with a matrix of lipid/pH-sensitive polymer or with several thin layers of ethylcellulose and stearic acid (Schwab, 1995). Studies have shown that these forms of RPM were effective for increasing both milk and component yield (Armentano et al., 1997; Rulquin and Delaby, 1997; Leonardi et al., 2003; Broderick et al., 2008). Another approach for Met protection from rumen degradation is to supply derivatives and analogs of Met that resist microbial breakdown. Some experiments have shown the benefits of adding different types and forms of Met analogs to the diet to improve milk and milk component production. Evidence also exists that supplementing with the hydroxylated analog of Met [2-hydroxy-4-(methylthio)-butanoic acid, **HMB**] stimulates microbial metabolism in the rumen (e.g., Gil et al., 1973). The most consistent result of feeding HMB has been an increase in milk fat content (Chandler et al., 1976; Huber et al., 1984; Lundquist et al., 1985). However, several studies have found no effect of HMB on milk protein concentration (Stokes et al., 1981; Hansen et al., 1991; St-Pierre and Sylvester, 2005).

Esterification of HMB with isopropanol [isopropyl-2-hydroxy-4-(methylthio)-butanoic acid, **HMBi**] results in reduction of the extent of its ruminal breakdown (Robert et al., 2001). Recent evidence showed that as much as 50% of orally fed HMBi was absorbed from the gastrointestinal tract, with the balance being microbially metabolized in the rumen (Graulet et al., 2005). Thus, HMBi offers the potential to both stimulate ruminal microbial activity and supply metabolizable Met. Because HMBi does not contain N, supplementation with HMBi may result in lower excretion of urinary N than supplementation with an equivalent amount of DL-Met (Römer and Abel, 1999). Feeding HMBi increased milk and protein production and protein concentration while reducing the amount of N excreted (Sylvester et al., 2003; Noftsger et al., 2005) and positively affected N efficiency (St-Pierre and Sylvester, 2005). Therefore, feeding HMBi could benefit milk production while reducing dietary CP and urinary N excretion and improving feed efficiency.

The hypotheses tested in this trial were (1) feeding diets containing HMBi, HMB+RPM, or RPM alone in amounts that result in a 3.0:1 ratio of Lys:Met in MP would increase secretion of milk components and enhance milk composition; (2) HMBi would have the same effect on milk protein and milk fat production as a physically protected form of RPM; and (3) HMBi, RPM, or HMB+RPM supplementation would allow the feeding of decreased dietary CP and give rise to increased N efficiency.

MATERIALS AND METHODS

Experimental Design

The trial was conducted as a randomized complete block design, and 70 lactating cows (50 multiparous and 20 primiparous) were selected for the study. Multiparous cows had mean (±SD) 2.7 (±1.3) parity, 143 (±55) DIM, 44 (±6) kg/d of milk, and 632 (±63) kg of BW; primiparous cows had mean (±SD) 158 (±47) DIM, 39 (±3) kg/d of milk, and 535 (±42) kg of BW. Cows were grouped into 14 blocks of 5 cows by parity and DIM. Cows were fed the same diet for a 2-wk covariate period. Cows were then randomly assigned within blocks to 1 of the 5 experimental diets and fed assigned diets throughout the remaining 12 weeks of the trial. Experimental diets were based on alfalfa silage, corn silage, and high-moisture corn; Table 1 lists the chemical composition of silages and principal concentrate ingredients. Four diets were formulated to contain 15.5% CP and had (1) no added Met (negative control, **NC**) or were supplemented with (2) HMBi (MetaSmart, Adisseo, Alpharetta, GA), (3) RPM (Smartamine M, Adisseo) and HMB (AT-88, Adisseo; HMB+RPM), or (4) RPM only; 1 diet was formulated to contain 17.0% CP (positive control, **PC**) with added solvent and expeller SBM plus distillers dried grains plus solubles (**DDGS**) but without a supplemental Met source. As fed in the trial, the first 4 diets contained 15.6% CP and diet PC contained 16.8% CP (Table 2). Methionine supplements were added to the diets as premixes that also contained ground corn and molasses; premixes were fed at 2.0% of DM. Levels of Met supplementation (Table 2) were adjusted to yield predicted concentration ratios of Lys:Met in MP of 3.0:1 as computed according to NRC (2001) using molecular weights of 192.3 and 149.2 for HMBi and Met, respectively, and assuming that 50% of ingested HMBi (Graulet et al., 2005) and 80% of ingested Met in the RPM source (Schwab, 1995) were absorbed from the gastrointestinal tract.

Cows were housed in a tie-stall barn and had free access to water during the trial. All cows were injected with rbST (Posilac, Monsanto, St. Louis, MO) beginning on d 1 of the trial and at 14-d intervals throughout. Cows were individually fed their respective TMR once a day at about 1100 h. Orts were collected and recorded at about 1000 h so that DMI could be calculated daily. The feeding rate was adjusted daily to yield orts of about 5 to 10% of offered feed to obtain ad libitum intake. Adjustments to the TMR were made weekly based on DM content of dietary components. Cows were milked twice a day at 0500 and 1600 h. Data on milk yield were collected daily.

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

Item	AS	CS	HMSC	SSBM	ESBM	DDGS	Dried molasses	GSC
DM (%)	34.5	32.3	76.9	89.2	90.4	92.0	97.9	88.6
CP (% of DM)	23.9	7.72	8.07	54.6	48.2	29.6	8.11	12.2
Ash (% of DM)	10.9	4.71	1.34	7.68	6.83	5.04	9.37	1.53
NDF (% of DM)	38.1	39.4	8.62	7.83	20.3	28.1	27.6	9.12
ADF (% of DM)	27.5	20.6	2.31	4.08	6.69	6.62	18.7	1.84
Hemicellulose (% of DM)	10.6	16.4	6.34	3.73	13.6	21.5	9.9	7.31
NDIN (% of total N)	9.87	8.32	9.06	4.14	17.2	6.34	28.8	8.09
ADIN (% of total N)	5.22	2.21	1.59	0.34	0.81	1.03	8.35	1.52
Fraction B_3[2] (% of total N)	4.74	6.13	7.46	3.81	16.4	5.31	20.4	6.60
NPN (% of total N)	40.8	47.8	7.34					
Ammonia (% of total N)	6.76	6.07	1.62					
Total free AA N[3] (% of total N)	24.2	21.0	2.73					
pH	4.45	4.00	6.03					

[1]AS = alfalfa silage; CS = corn silage; ESBM = expeller soybean meal; DDGS = distillers dried grains plus solubles; GSC = ground shelled corn; HMSC = high-moisture shelled corn; SSBM = solvent-extracted soybean meal.

[2]Fraction B_3 = NDIN (% of total N) − ADIN (% of total N) (Sniffen et al., 1992).

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

Daily samples of about 0.5 kg of corn silage, alfalfa silage, high-moisture shelled corn, TMR, and orts were collected to give weekly composites that were used to determine the composition of the rations actually consumed throughout the trial. The dry ingredients (the SBM, dried molasses, DDGS, fat supplement, ground shelled corn, and the premixes containing the Met sources) were sampled weekly. Forage composite samples were divided into 2 parts: one subsample was used to prepare silage extracts and the second subsample was dried at 60°C and retained for further analyses. After drying, ingredients and TMR were ground through a 1-mm screen (Wiley mill, Arthur H. Thomas, Philadelphia, PA). Intake of DM was computed based on the 60° DM values for TMR and orts. Dried weekly samples of feed ingredients were analyzed for total N by combustion assay (Leco 2000, Leco Instruments Inc., St. Joseph, MI) and for NDF and ADF using heat stable α-amylase (Van Soest et al., 1991) and Na_2SO_3 (Hintz et al., 1996). Silage extracts were prepared in distilled water (Muck, 1987) from weekly composites, pH was determined and extracts were analyzed for NPN (VarioMax CN, Elementar Analysensystem GmbH, Hanau, Germany), and for ammonia and total free AA using flow injection (Broderick et al., 2004; Lachat Quik-Chem 8000 FIA, Hach Company, Loveland, CO). Proportions of each ration ingredient on an as-fed basis were adjusted weekly based on DM determined by drying weekly composites at 60°C (48 h). Data on composition of individual ration ingredients are in Table 1.

Milk samples were collected midweek at both daily milkings during every week of the trial (in both the 2-wk covariate and the 12-wk experimental periods) and analyzed for fat, true protein, lactose and SNF by infrared techniques (AgSource Inc., Verona, WI) with a Foss FT6000 (Foss North America Inc., Eden Prairie, MN) using AOAC (1990; method no. 972.16). For determination of MUN, separate 5-mL samples of a.m. and p.m. milk were each mixed with 5 mL of 25% (wt/vol) TCA and then vortexed and held for 30 min at room temperature before being filtered through Whatman #1 filter paper. Filtrates were stored at −20°C until MUN analysis was conducted by an automated colorimetric assay (Broderick and Clayton, 1997) adapted to flow injection (Lachat QuickChem 8000 FIA). Concentrations and yields of fat, true protein, lactose, SNF, and MUN were computed as the weighted means from p.m. and a.m. milk yields on each test day. Yields of 3.5% FCM (Sklan et al., 1992) and ECM (Charbonneau et al., 2006) were also computed. Efficiency of conversion of feed DM to milk, FCM, and ECM was computed weekly for each cow by dividing mean milk yield by mean DMI. Similarly, efficiency of utilization of feed N was computed for each cow by dividing mean milk N output (milk true protein/6.38) by mean N intake. Cows were weighed on 3 consecutive days at the start and at the end of wk 4, 8, and 12 of the experimental phase of the trial to compute BW change. Efficiency of MP utilization was estimated as milk protein yield plus MP required for maintenance plus protein retained in the tissues (computed from BW change), divided by MP supplied in the diet, as calculated using the NRC (2001) model. Blood samples were collected from the coccygeal vein or artery into heparinized tubes at approximately 4 h postfeeding from each cow at the end of wk 4, 8, and 12 of the trial and placed on ice for transport to the laboratory. Blood was centrifuged (700 × *g*, 15 min, 4°C) and the resulting plasma was deproteinized by mixing 1 volume of 25% TCA with 4 volumes of plasma and centrifuging (24,700 × *g*, 15

min, 4°C). The supernatant was stored at –20°C until analyzed for plasma urea nitrogen (**PUN**) with the flow injection system used for MUN.

Urine and fecal grab samples were collected at the end of wk 4, 8, and 12 at about 6 and 18 h after feeding. Fecal samples were transferred to aluminum pans, held at 60°C in a forced-air oven until completely dry, and then ground through a 1-mm screen (Wiley mill). Equal weight of DM from the 2 fecal subsamples was mixed to obtain a single composite for each cow in each sample week. All fecal samples were analyzed for DM, ash, OM, NDF, ADF, total N, and indigestible ADF using the assays described above. Indigestible ADF was used as an internal marker to estimate apparent nutrient digestibility and fecal output (Cochran et al., 1986). At the time of fecal sampling, spot urine samples were also collected from all cows by stimulation of the vulva. Fresh urine samples were acidified by diluting 1 volume of urine with 4 volumes of 0.072 N H_2SO_4 and stored at –20°C until analyzed. At the end of the trial,

Table 2. Composition of diets

	Diet[1]				
Item	NC	HMBi	HMB +RPM	RPM	PC
Ingredients (% of DM)					
Alfalfa silage	25.2	25.2	25.2	25.2	25.2
Corn silage	34.4	34.4	34.4	34.4	34.4
High-moisture shelled corn	21.3	21.3	21.3	21.3	14.8
48% Solvent soybean meal	8.62	8.62	8.62	8.62	3.66
Expeller soybean meal (SoyPlus[2])	0	0	0	0	3.96
Distillers dried grains plus solubles	0	0	0	0	7.53
Dried molasses	2.00	2.00	2.00	2.00	2.00
Fat supplement (EnergyBooster[3])	2.00	2.00	2.00	2.00	2.00
Ground shelled corn	4.16	3.99	4.00	4.10	4.16
Liquid molasses	0.05	0.05	0.05	0.05	0.05
HMBi product (57% HMBi)	0	0.17	0	0	0
HMB product (88% HMB)	0	0	0.10	0	0
Rumen-protected Met product (75%)	0	0	0.06	0.06	0
Vitamin-mineral concentrate[4]	2.39	2.39	2.39	2.39	2.39
Chemical composition					
CP (% of DM)	15.6	15.6	15.6	15.6	16.8
DM (%)	44.8	45.3	45.2	45.1	45.0
Ash (% of DM)	6.07	6.07	6.07	6.07	6.37
NDF (% of DM)	26.7	26.6	26.6	26.7	28.7
ADF (% of DM)	15.3	15.3	15.3	15.3	15.7
NFC (% of DM)	46.8	47.0	47.0	46.8	43.6
NDIN (% of total N)	7.18	7.18	7.18	7.18	8.9
ADIN (% of total N)	2.71	2.71	2.71	2.71	2.68
Fraction B_3 (% of CP)	4.51	4.51	4.51	4.51	6.20
NE_L[5] (Mcal/kg of DM)	1.61	1.61	1.61	1.61	1.64
Rumen-protected Met[6] (g/d)	0	9	9	9	0
Absorbed Met[5] (g/d)	45	45	45	45	48
Total absorbed Met (g/d)	45	54	54	54	48
Absorbed Lys[5] (g/d)	161	161	161	161	160
Metabolizable Lys (% of MP)	6.59	6.59	6.59	6.59	6.17
Metabolizable Met (% of MP)	1.84	2.21	2.21	2.21	1.85
Lys:Met ratio	3.58	2.98	2.98	2.98	3.33

[1]NC = negative control diet (15.5% CP, no added Met); HMBi = diet with 15.5% CP, supplemented with isopropyl-2-hydroxy-4-(methylthio)-butanoic acid (MetaSmart, Adisseo, Alpharetta, GA); RPM = diet with 15.5% CP, supplemented with rumen-protected Met (Smartamine M, Adisseo); HMB+RPM = diet with 15.5% CP, supplemented with 2-hydroxy-4-(methylthio)-butanoic acid (HMB, AT-88, Adisseo) and Smartamine M (Adisseo); RPM = diet with 15.5% CP, supplemented with rumen-protected Met only; PC = positive control diet (16.8% CP, with added solvent and expeller soybean meal plus distillers dried grains plus solubles but without a supplemental Met source).

[2]West Central (Ralston, IA).

[3]MSC (Carpentersville, IL)

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

[5]Values for NE_L, absorbed Met, and absorbed Lys calculated from NRC (2001) model.

[6]Estimated supply of absorbed Met from HMBi (assuming 50% absorption) and RPM (assuming 80% absorption) at 24 kg/d of DM intake, and assuming no HMB was absorbed from the gastrointestinal tract.

urine samples were thawed at room temperature and filtered through Whatman #1 filter paper. Filtrates were analyzed for creatinine using a picric acid assay (Oser, 1965; Valadares et al., 1999) adapted to flow injection analysis (Lachat Quik-Chem 8000 FIA), total N (Leco FP-2000 Nitrogen Analyzer), urea with an automated colorimetric assay (Broderick and Clayton, 1997), allantoin by the method of Vogels and van der Grift (1970) adapted to a 96-well plate reader, and uric acid with a commercial kit (no. 683-100P, Sigma, St. Louis, MO). Daily urine volume and excretion of urea N, total N, and purine derivatives (allantoin plus uric acid) were estimated from urinary creatinine concentration, assuming a creatinine excretion rate of 29 mg/kg of BW (Valadares et al., 1999). Throughout the trial, care and handling of all experimental animals was conducted under protocols approved by the University of Wisconsin Institutional Animal Care and Use Committee.

Statistical Analysis

Statistical analyses of all data except BW change and excretion results were conducted using the MIXED procedure (SAS Institute, 1999–2000) with a repeated measures model using an SP(POW) structure that included the covariate mean for each trait for each cow, plus block, diet and week, and the interaction of week × diet. The same approach was used for data on BW change and excretion except that the model did not contain a covariate. All variables were considered fixed, except cow, whole-plot error, and subplot error, which were considered random. The interaction term was removed from the model when $P \geq 0.25$. Four contrasts were run to test different treatment effects. Overall treatment differences were examined using least squares means with the lowest standard error. Significance was declared at $P \leq 0.05$ and trends at $P \leq 0.10$ for production traits and at $P \leq 0.10$ for N excretion traits. Week and block were included in the final statistical model for all analyses. The PDIFF option of SAS (SAS Institute, 1999–2000) was used to test treatment differences among least squares means, and the SLICE option in MIXED was used to analyze treatment differences among weekly treatment means.

RESULTS AND DISCUSSION

Composition of major dietary ingredients is presented in Table 1. The chemical composition of the alfalfa silage was 23.9% CP, 38.1% NDF, and 27.5% ADF, indicating that it was of high quality; however, the corn silage contained 39.4% NDF, 20.6% ADF, and 7.7% CP, which was similar to values in NRC (2001) tables. Nonprotein N represented 40.8 and 47.8% of the CP in alfalfa silage and corn silage, respectively. Alfalfa silage typically has NPN contents that exceed 50% of total N (Broderick, 1995), so that fed in this trial was relatively low in NPN. Both forages contained similar amounts of ammonia-N, total free AA-N, and total NPN as proportions of total N. High-moisture shelled corn and solvent SBM fed in the experiment contained, respectively, 8.1 and 54.6% CP and 8.6 and 7.8% NDF; 9.1 and 4.1%, respectively, of total N was present as NDIN.

By design, the proportions of alfalfa silage and corn silage were similar across all treatments, making up about 60% of the dietary DM. The CP contents of the diets fed in this study were 15.6% (NC and the 3 diets supplemented with Met sources) and 16.8% (PC), which were close to the target values of 15.5 and 17.0% CP (Table 2). The NRC (2001) model suggests that the Lys and Met contents in MP for optimal milk protein production are 7.2 and 2.4%, respectively. These concentrations are difficult to achieve without feeding very high levels of protein. Therefore, it is recommended that the first step in balancing diets for Lys and Met is to maintain a Lys:Met ratio in MP of 3.0 with more practical levels of Lys and Met in MP being 6.6 and 2.2% (Schwab et al., 2003). In this study, supplementing with HMBi (assuming 50% absorption) and RPM (assuming 80% absorption of Met) substantially improved Met status, as indicated by the reduction of the predicted (NRC, 2001) Lys:Met ratio in MP from 3.6 to 3.0 (Table 2). A smaller improvement was observed in predicted Met status with supplementation of CP from DDGS plus expeller SBM; Lys:Met ratio was reduced to 3.3 on diet PC because of an estimated 3 g/d increase in metabolizable Met supply.

The least squares means for DMI and production of milk and milk components are reported in Table 3. Treatments had no significant effect on DMI and BW gain, which averaged 25.0 and 0.59 kg/d across the 5 diets. Treatment did not affect yields of milk or 3.5% FCM or milk lactose content and yield. However, yield of ECM and ECM/DMI as well as true protein and SNF concentrations in milk were influenced by diet ($P \leq 0.03$; Table 3). Compared with the PC diet, feeding the NC diet reduced yields of both FCM and fat ($P \leq 0.04$; Table 3).

Mean separation indicated that, relative to the NC diet, ECM yield was greater on HMBi and ECM/DMI was greater on the RPM-only treatment. Moreover, HMBi and HMB+RPM increased ($P = 0.01$) milk SNF concentration. Furthermore, trends ($P \leq 0.08$) were observed for significant effects on milk fat content and yield with HMBi and RPM supplementation compared

Table 3. Effect of diet on production of milk and milk components (LS means)

	Diet[1]							Contrast[3]			
Item	NC	HMBi	HMB +RPM	RPM	PC	SEM	$P > F$[2]	LPM	HPM	SCE	PRO
DM intake (kg/d)	24.9	25.7	25.1	24.6	24.7	0.44	0.44	0.27	0.36	0.08	0.65
BW gain (kg/d)	0.65	0.69	0.66	0.50	0.45	0.07	0.43	0.22	0.08	0.06	0.28
Milk (kg/d)	41.8	42.1	41.7	41.7	41.2	0.92	0.98	0.99	0.93	0.98	0.94
3.5% FCM (kg/d)	40.8	43.9	43.1	44.8	43.8	1.37	0.29	0.29	0.13	0.60	0.04
ECM (kg/d)	37.9[b]	41.0[a]	39.0[ab]	40.2[ab]	39.4[ab]	0.95	0.02	0.15	0.56	0.43	0.12
Milk/DMI	1.69	1.68	1.67	1.69	1.67	0.03	0.97	0.77	0.70	0.66	0.63
FCM/DMI	1.65	1.71	1.70	1.82	1.78	0.05	0.12	0.23	0.12	0.07	0.41
ECM/DMI	1.54[b]	1.59[ab]	1.57[ab]	1.63[a]	1.61[ab]	0.04	0.04	0.19	0.47	0.92	0.11
Fat (%)	3.52	3.93	3.66	3.77	3.85	0.11	0.08	0.08	0.35	0.44	0.06
Fat (kg/d)	1.42	1.60	1.54	1.62	1.61	0.06	0.07	0.07	0.07	0.85	0.01
True protein (%)	3.03[c]	3.19[a]	3.17[a]	3.15[ab]	3.05[bc]	0.04	0.01	0.03	0.03	0.48	0.84
True protein (kg/d)	1.24	1.30	1.33	1.33	1.25	0.03	0.09	0.16	0.11	0.42	0.72
Lactose (%)	4.79	4.83	4.87	4.79	4.81	0.05	0.81	0.64	0.73	0.63	0.42
Lactose (kg/d)	1.99	1.97	2.05	2.05	1.98	0.05	0.72	0.58	0.48	0.30	0.78
SNF (%)	8.73[b]	8.94[a]	8.92[a]	8.84[ab]	8.73[b]	0.05	0.01	0.06	0.02	0.13	0.81
SNF (kg/d)	3.59	3.65	3.77	3.76	3.61	0.08	0.32	0.35	0.31	0.40	0.85
MUN (mg/dL)	10.0[c]	10.2[c]	10.8[bc]	11.2[b]	13.2[a]	0.33	<0.01	0.02	<0.01	0.02	<0.01

[a–c]Values with different superscript letters in rows are different ($P < 0.05$).

[1]NC = negative control diet (15.5% CP, no added Met); HMBi = diet with 15.5% CP, supplemented with isopropyl-2-hydroxy-4-(methylthio)-butanoic acid (MetaSmart, Adisseo, Alpharetta, GA); RPM = diet with 15.5% CP, supplemented with rumen-protected Met (Smartamine M, Adisseo); HMB+RPM = diet with 15.5% CP, supplemented with 2-hydroxy-4-(methylthio)-butanoic acid (HMB, AT-88, Adisseo) and Smartamine M (Adisseo); RPM = diet with 15.5% CP, supplemented with rumen-protected Met only; PC = positive control diet (16.8% CP, with added solvent and expeller soybean meal plus distillers dried grains plus solubles but without a supplemental Met source).

[2]Probability of a significant effect of diet.

[3]Contrasts: LPM = NC vs. HMBi, HMB+RPM, RPM; HPM = PC vs. HMBi, HMB+RPM, RPM; SCE = HMBi vs. RPM; PRO = NC vs. PC.

with the NC diet (Table 3). Significant responses in milk fat content (Figure 1a) and yield (Figure 1b) were detected by wk 1 of supplementation with PC, HMBi, and RPM, but results were variable throughout the trial. Effects of HMBi, HMB+RPM, and RPM supplementation were more consistent for milk true protein content ($P = 0.01$; Table 3); responses were also rapid, and statistical significance for milk content of true protein was detected by wk 2 of the experimental period (Figure 2a and 2b). Supplementation with HMBi, HMB+RPM, and RPM resulted in a trend ($P = 0.09$; Table 3) for milk true protein yield to be increased by, respectively, 55, 85, and 85 g/d, compared with mean protein yield on the NC and PC diets. Except for lower MUN, the HMBi diet had similar effects on yields of milk and milk components as did the RPM diet ($P \geq 0.13$, Table 3). These results are consistent with previous reports of the positive effects of feeding the same RPM supplement (Rulquin and Delaby, 1997) and HMBi (Graulet et al., 2005) and confirm the effectiveness of both as sources of absorbed Met. Several 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 (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). The improved ECM production observed in the present trial may have occurred because of stimulation of milk protein synthesis when the limiting AA Met was supplemented by feeding HMBi or RPM.

The concentration of MUN was lower in cows fed the 4 diets containing 15.6% CP compared with that in cows fed the diet with 16.8% CP (Table 3). A consistently positive relationship of dietary CP content with MUN concentrations has been reported in many previous trials (Broderick and Clayton, 1997; Jonker et al., 1998; Nousiainen et al., 2004). Also, MUN was lower on the NC and HMBi diets compared with the PC treatment, which was consistent with research reported by St-Pierre and Sylvester (2005) showing that supplementation with HMBi decreased MUN significantly. However, the diet supplemented with RPM only was observed to result in greater MUN than the other diets containing 15.6% CP. Because the RPM diet gave lower N intake and higher milk true protein yield, one explanation for this result could be that cows on that treatment catabolized tissue protein to support milk production. Although all cows were apparently gaining weight, a trend ($P = 0.06$) was observed for lower gain

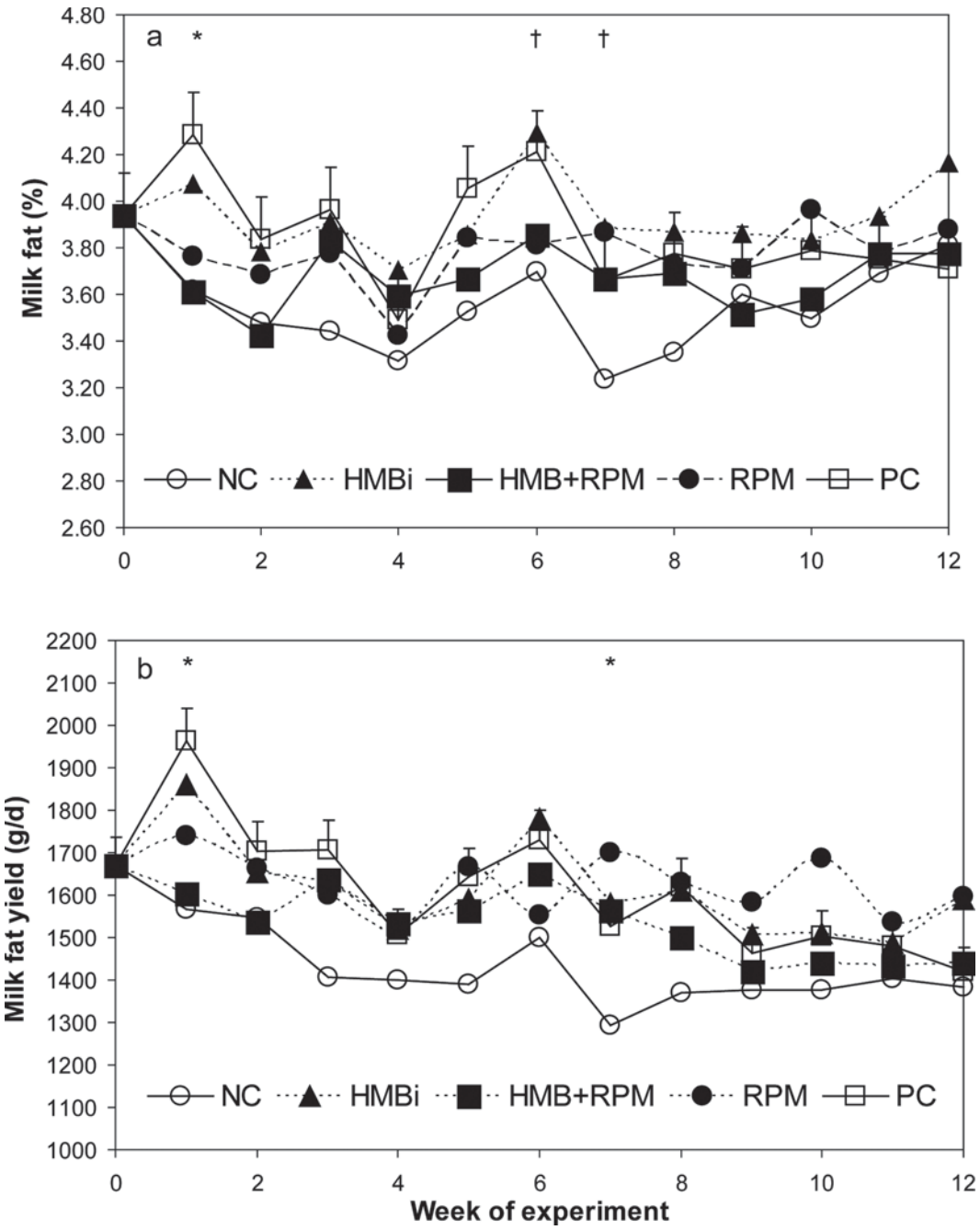

Figure 1. Effects of dietary treatment on (a) milk fat content by week and (b) milk fat yield by week. NC = negative control diet (15.5% CP, no added Met); HMBi = diet with 15.5% CP, supplemented with isopropyl-2-hydroxy-4-(methylthio)-butanoic acid (MetaSmart, Adisseo, Alpharetta, GA); RPM = diet with 15.5% CP, supplemented with rumen-protected Met (Smartamine M, Adisseo); HMB+RPM = diet with 15.5% CP, supplemented with 2-hydroxy-4-(methylthio)-butanoic acid (HMB, AT-88, Adisseo) and Smartamine M (Adisseo); RPM = diet with 15.5% CP, supplemented with rumen-protected Met only; PC = positive control diet (16.8% CP, with added solvent and expeller soybean meal plus distillers dried grains plus solubles but without a supplemental Met source).Significant effect of diet at $\dagger P < 0.10$ and $^*P < 0.05$.

on RPM versus HMBi. It is possible that cows on the RPM diet were, in fact, mobilizing body tissue, contributing to elevated MUN.

As expected, nitrogen intake was significantly higher ($P < 0.01$, Table 4) on the PC diet than on the other diets. As expected, fecal N excretion, estimated using indigestible ADF as internal marker, was not affected by treatment and averaged 216 g/d. Urinary excretion of urea-N and total N, estimated using creatinine as the volume marker, was significantly increased ($P =$

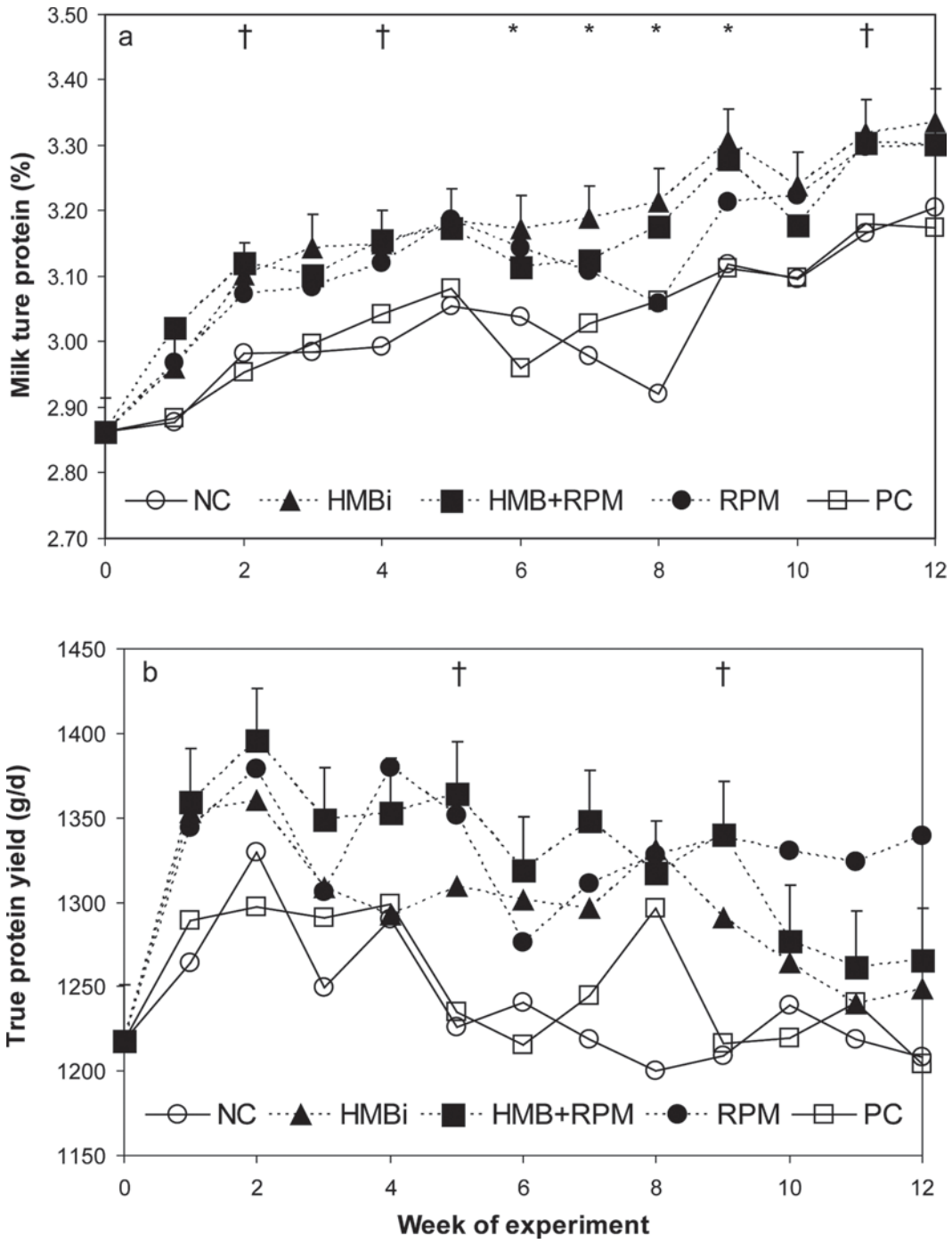

Figure 2. Effects of dietary treatment on (a) milk true protein content by week and (b) milk true protein yield by week. NC = negative control diet (15.5% CP, no added Met); HMBi = diet with 15.5% CP, supplemented with isopropyl-2-hydroxy-4-(methylthio)-butanoic acid (MetaSmart, Adisseo, Alpharetta, GA); RPM = diet with 15.5% CP, supplemented with rumen-protected Met (Smartamine M, Adisseo); HMB+RPM = diet with 15.5% CP, supplemented with 2-hydroxy-4-(methylthio)-butanoic acid (HMB, AT-88, Adisseo) and Smartamine M (Adisseo); RPM = diet with 15.5% CP, supplemented with rumen-protected Met only; PC = positive control diet (16.8% CP, with added solvent and expeller soybean meal plus distillers dried grains plus solubles but without a supplemental Met source).Significant effect of diet at †$P < 0.10$ and *$P < 0.05$.

0.04) by feeding 16.8% dietary CP and urinary urea N was 17 and 10 g/d lower, respectively, on the diets supplemented with HMBi and RPM compared with the PC diet (Table 4). Urea is the form of urinary N most rapidly degraded to ammonia and lost through volatilization, thereby contributing to environmental

Table 4. Effect of dietary treatment on nutrient digestibility and nitrogen metabolism (LSM)

	Diet[1]						
Item	NC	HMBi	HMB +RPM	RPM	PC	SEM	$P > F$[2]
N intake (g/d)	623[b]	645[ab]	630[b]	619[b]	670[a]	10.6	<0.01
Milk N	199[b]	208[ab]	213[a]	213[a]	201[ab]	4.60	0.09
Plasma urea N (mg/dL)	11.7[b]	12.2[b]	12.8[b]	12.8[b]	15.7[a]	0.53	<0.01
N excretion (g/d)							
Fecal-N	214	224	206	216	219	7.60	0.58
Urinary urea-N	95.2[b]	96.9[b]	97.0[b]	90.2[b]	107[a]	3.75	0.04
Urinary total-N	133[ab]	135[ab]	128[b]	121[b]	146[a]	6.07	0.06
Total-N	347	359	335	337	365	11.1	0.24
Excretion (% N intake)							
Fecal-N	35.0[a]	34.9[a]	32.9[bc]	34.4[ab]	32.4[c]	0.77	0.08
Urinary total-N	21.6	21.3	20.6	19.5	21.4	0.88	0.45
Total-N	56.6	56.2	53.5	54.0	53.8	1.23	0.26
Apparent digestibility (%)							
DM	66.6	66.7	68.2	67.8	67.5	0.75	0.46
NDF	48.6	48.2	50.9	51.7	51.6	1.43	0.26
N	65.0	65.1	66.8	65.7	67.6	0.84	0.15
N efficiency							
Milk N/N intake (%)	32.0[bc]	32.3[b]	33.6[ab]	34.5[a]	30.2[c]	0.69	<0.01
Predicted MP efficiency[3] (%)	79.6	91.0	86.1	86.0	75.5	4.64	0.16

[a–c]Values within rows with different superscript letters are different ($P < 0.10$).

[1]NC = negative control diet (15.5% CP, no added Met); HMBi = diet with 15.5% CP, supplemented with isopropyl-2-hydroxy-4-(methylthio)-butanoic acid (MetaSmart, Adisseo, Alpharetta, GA); RPM = diet with 15.5% CP, supplemented with rumen-protected Met (Smartamine M, Adisseo); HMB+RPM = diet with 15.5% CP, supplemented with 2-hydroxy-4-(methylthio)-butanoic acid (HMB, AT-88, Adisseo) and Smartamine M (Adisseo); RPM = diet with 15.5% CP, supplemented with rumen-protected Met only; PC = positive control diet (16.8% CP, with added solvent and expeller soybean meal plus distillers dried grains plus solubles but without a supplemental Met source).

[2]Probability of a significant effect of diet.

[3]MP efficiency computed as milk protein yield divided by estimated MP supplied by the diet minus MP required for maintenance and protein retained in the tissues (estimated from BW change), computed using the NRC (2001) model.

pollution (Van Horn et al., 1994). For cows fed the PC diet, the extra N was excreted mainly via the urine; thus, feeding a lower protein diet with Met supplementation may meet the need for maximum profitability with reduced environmental impact.

In this study, apparent digestibility of DM, NDF, and N was not significantly affected by diet (Table 4). Although some trials have reported improved digestibility of DM (Polan et al., 1970) and NDF (Noftsger et al., 2005) with Met supplementation, most have shown no effect on NDF and hemicellulose digestibility (Windschitl and Stern, 1988; Noftsger and St-Pierre, 2003). Previous research reported reduced DM, OM, and fiber digestibility when dietary CP was decreased from 17.1 to 15.8% (Broderick et al., 2009). No differences were detected in the present study comparing digestibilities estimated on the 4 diets containing 15.6% CP with that on the diet containing 16.8% CP.

Diet did not affect estimated MP efficiency ($P = 0.16$; Table 4); however, MP efficiency was numerically higher when comparing the 3 Met-supplemented diets with either the NC or the PC diet. Supplementation with HMBi, HMB+RPM, and RPM resulted in 2.1 to 4.3 percentage units greater ($P < 0.01$) apparent N efficiency compared with the PC diet. Moreover, the RPM diet achieved the highest efficiency, with a 14% improvement in apparent N utilization versus the PC diet. Furthermore, the NC and HMBi diets had lower N efficiency than the RPM diet. This could be the result of lower true protein yield on the NC diet and higher N intake on the HMBi diet. A significant increase ($P < 0.01$) in PUN concentration was observed when the PC diet was fed (Table 4). This was due to the higher N intake, which did not improve milk protein secretion on this diet, and supports the observation that PUN and MUN are in equilibrium (Gustafsson and Palmquist, 1993). Compared with that in cows on the NC diet, PUN concentrations were not affected by the source of Met supply (Table 4). Increased MUN and PUN ($P < 0.01$) and urinary excretion of urea N ($P = 0.04$) and total N ($P = 0.06$) reflected the decreased N efficiency, all indicating that N utilization on the PC diet was impaired. However, statistical contrasts indicated that milk protein yields on the 3 Met-supplemented diets were not greater ($P = 0.11$) than on the PC diet (Table 3).

CONCLUSIONS

Over a 12-wk feeding study, supplementation of lactating cows with HMBi or RPM (with or without

HMB) increased yield of ECM, ECM/DMI, and milk content of true protein and SNF, and resulted in trends for increased protein yield and milk fat content and yield. Overall results from this experiment suggested that HMBi was comparable in effectiveness to the RPM source, in which Met was protected with a physical coating, when fed to lactating dairy cows. These responses indicated that when either source of absorbed Met was added to a 15.6% CP diet, performance was equal to or better than that of cows fed a 16.8% CP diet, but with reduced N excretion and improved N utilization.

ACKNOWLEDGMENTS

The authors gratefully acknowledge Adisseo (Commentry, France) for partial financial support of this research; herd manager Jim Meronek and the barn crew for feeding and animal care at the US Dairy Forage Center Research Farm (Prairie du Sac, WI); Wendy Radloff and Mary Becker (US Dairy Forage Research Center, Madison, WI) and Wangshan Guo, and Hangshu Xin (University of Wisconsin, Madison) for assisting with sampling and laboratory analyses; and Peter Crump (University of Wisconsin, Madison) for assisting with statistical analyses.

REFERENCES

AOAC. 1990. Official Methods of Analysis. 15th 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., 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.

Broderick, G. A. 1987. Determination of protein degradation rates using a rumen in vitro system containing inhibitors of microbial nitrogen metabolism. Br. J. Nutr. 58:463–475.

Broderick, G. A. 1995. Desirable characteristics of forage legumes for improving protein utilization in ruminants. J. Anim. Sci. 73:2760–2773.

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., M. J. Stevenson, and R. A. Patton. 2009. Effect of dietary protein concentration and degradability on response to rumen-protected methionine in lactating dairy cows. J. Dairy Sci. 92:2719–2728.

Broderick, G. A., M. J. Stevenson, R. A. Patton, N. E. Lobos, and J. J. Olmos Colmenero. 2008. Effect of supplementing rumen-protected methionine on production and nitrogen excretion in lactating dairy cows. J. Dairy Sci. 91:1092–1102.

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

Chandler, P. T., C. A. Brown, R. P. Johnston Jr., G. K. Macleod, R. D. McCarthy, B. R. Moss, A. H. Rakes, and L. D. Satter. 1976. Protein and methionine hydroxy analog for lactating cows. J. Dairy Sci. 59:1897–1909.

Charbonneau, E., P. Y. Chouinard, G. Allard, H. Lapierre, and D. Pellerin. 2006. Milk from forage as affected by carbohydrate source and degradability with alfalfa silage-based diets. J. Dairy Sci. 89:283–293.

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.

Gil, L. A., R. L. Shirley, and J. E. Moore. 1973. Effect of methionine hydroxy analog on growth, amino acid content, and catabolic products of glucolytic rumen bacteria in vitro. J. Dairy Sci. 56:757–762.

Graulet, B., C. Richard, and J. C. Robert. 2005. Methionine availability in plasma of dairy cows supplemented with methionine hydroxy analog isopropyl ester. J. Dairy Sci. 88:3640–3649.

Gustafsson, A. H., and D. L. Palmquist. 1993. Diurnal variation of rumen ammonia, serum urea, and milk urea in dairy cows at high and low yields. J. Dairy Sci. 76:475–484.

Hansen, W. P., D. E. Otterby, J. G. Linn, and J. D. Donker. 1991. Influence of forage type, ratio of forage to concentrate, and methionine hydroxy analog on performance of dairy cows. J. Dairy Sci. 74:1361–1369.

Hintz, R. W., D. R. Mertens, and K. A. Albrecht. 1996. Effects of sodium sulfite on recovery and composition of detergent fiber and lignin. J. AOAC Int. 789:16–22.

Huber, J. T., R. S. Emery, W. G. Bergen, J. S. Liesman, L. Kung Jr., K. J. King, R. W. Gardner, and M. Checketts. 1984. Influences of methionine hydroxy analog on milk and milk fat production, blood serum lipids, and plasma amino acids. J. Dairy Sci. 67:2525–2531.

Jonker, J. S., R. A. Kohn, and R. A. Erdman. 1998. Using milk urea nitrogen to predict nitrogen excretion and utilization efficiency in lactating dairy cows. J. Dairy Sci. 81:2681–2692.

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.

Lundquist, R. G., D. E. Otterby, and G. G. Linn. 1985. Influence of three concentrations of DL-methionine or methionine hydroxyl analog on milk yield and composition. J. Dairy Sci. 68:3350–3354.

Muck, R. E. 1987. Dry matter level effects on alfalfa silage quality. 1. Nitrogen transformations. Trans. ASAE 30:7–14.

Noftsger, S., and N. R. St-Pierre. 2003. Supplementation of methionine and selection of highly digestible rumen undegradable protein to improve nitrogen efficiency for milk production. J. Dairy Sci. 86:958–969.

Noftsger, S., N. R. St-Pierre, and J. T. Sylvester. 2005. Determination of rumen degradability and ruminal effects of three sources of methionine in lactating cows. J. Dairy Sci. 88:223–237.

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.

Oser, B. L. 1965. Hawk's Physiological Chemistry. 14th 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.

Polan, C. E., P. T. Chandler, and C. N. Miller. 1970. Methionine analog effect on ruminant digestion. J. Dairy Sci. 31:251. (Abstr.)

Robert, J. C., C. Richard, T. d'Alfonso, N. Ballet, and E. Depres. 2001. Investigation of the site of absorption and metabolism of a novel source of metabolizable methionine: 2-Hydroxy-4-(methylthio) butanoic acid isopropyl ester (HMBi). J. Dairy Sci. 84(Suppl.1):35. (Abstr.)

Römer, A., and H. Abel. 1999. Effects of DL-methionine hydroxyanalogue (MHA) or DL-methionine (DL-Met) on N-retention in broiler chickens and pigs. Anim. Feed Sci. Technol. 81:193–203.

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.

Rulquin, H., P. M. Pisulewski, R. Verite, and J. Guinard. 1993. Milk production and composition as function of postruminal lysine and methionine supply: A nutrient-response approach. Livest. Prod. Sci. 37:69–90.

SAS Institute. 1999–2000. SAS/STAT User's Guide. Release 8.1. SAS Institute Inc., Cary, NC.

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.

Schwab, C. G. 1995. Protected proteins and amino acids for ruminants. Pages 115–141 in Biotechnology in Animal Feeds and Animal Feeding. R. J. Wallace and A. Chesson, ed. V. C. H. Press, Weinheim, Germany.

Schwab, C. G., C. K. Bozak, N. L. Whitehouse, and M. M. A. Mesbah. 1992. Amino acid limitation and flow to duodenum at four stages of lactation. 1. Sequence of lysine and methionine limitation. J. Dairy Sci. 75:3486–3502.

Schwab, C. G., R. S. Ordway, and N. L. Whitehouse. 2003. Amino acid balancing in the context of MP and RUP requirements. Pages 10–25 in Proc Four-State Applied Nutrition and Management Conference.

Sklan, D., R. Ashkenazi, A. Braun, A. Devorn, and K. Tabori. 1992. Fatty acids, calcium soaps of fatty acids, and cottonseeds fed to high yielding cows. J. Dairy Sci. 75:2463–2472.

Sniffen, C. J., J. D. O'Connor, P. J. Van Soest, D. G. Fox, and J. B. Russell. 1992. A net carbohydrate and protein system for evaluating cattle diets. 2. Carbohydrate and protein availability. J. Anim. Sci. 70:3562–3577.

St-Pierre, N. R., and J. T. Sylvester. 2005. Effects of 2-hydroxy-4-(methylthio) butanoic acid (HMB) and its isopropyl ester on milk production and composition by Holstein cows. J. Dairy Sci. 88:2487–2497.

Stokes, M. R., J. H. Clark, and L. M. Steinmetz. 1981. Performance of lactating dairy cows fed methionine or methionine analog at two concentrations of dietary crude protein. J. Dairy Sci. 64:1686–1694.

Sylvester, J. T., N. R. St-Pierre, B. K. Sloan, J. L. Beckman, and S. M. Noftsger. 2003. Effect of HMB and HMBi on milk production, composition, and N efficiency of Holstein cows in early and mid-lactation. J. Dairy Sci. 86(Suppl. 1):60. (Abstr.)

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 Horn, H. H., A. C. Wilkie, W. J. Powers, and R. A. Norstedt. 1994. Components of dairy manure management systems. J. Dairy Sci. 77:2008–2030.

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.

Vogels, G. D., and C. van der Grift. 1970. Differential analyses of glyoxylate derivatives. Anal. Biochem. 33:143–157.

Windschitl, P. M., and M. D. Stern. 1988. Influence of methionine derivatives on effluent flow of methionine from continuous culture of ruminal bacteria. J. Anim. Sci. 66:2937–2947.

Wu, Z., and L. D. Satter. 2000. Milk production during the complete lactation of dairy cows fed diets containing different amounts of protein. J. Dairy Sci. 83:1042–1051.