Effects of providing two forms of supplemental methionine to periparturient Holstein dairy cows on feed intake and lactational performance¹

R. S. Ordway,^{*2} S. E. Boucher,[†] N. L. Whitehouse,[‡] C. G. Schwab,[‡] and B. K. Sloan[§]

^{*}Blue Seal Richer Dairy Nutrition Inc., Sandy Creek, NY 13145

[†]William H. Miner Agricultural Research Institute, Chazy, NY 12921

[‡]Department of Animal and Nutritional Sciences, University of New Hampshire, Durham 03824

[§]Adisseo Inc., Alpharetta, GA 30022

ABSTRACT

Eighteen primiparous and 42 multiparous Holstein cows were blocked according to parity and expected calving date and assigned randomly to 1 of 3 dietary treatments: 1) a basal diet (negative control), 2) the basal diet plus 2-hydroxy-4-methylthio butanoic acid isopropyl ester (MetaSmart, Adisseo Inc., Antony, France), or 3) the basal diet plus rumen-protected Met (Smartamine M, Adisseo Inc., Alpharetta, GA). Treatments were initiated 21 d before expected calving and continued through 140 d postpartum. Diets were similar in ingredient and chemical composition, except for the content of Met in metabolizable protein. MetaSmart [0.35% prepartum and 0.54% postpartum in diet dry matter (DM)] and Smartamine M (0.06% prepartum and 0.10% postpartum in diet DM) were added to the basal diet in amounts needed to achieve a 3.0:1 ratio of Lys to Met in metabolizable protein. Prepartum DM intake (DMI; 13.5 kg/d), body weight (687 kg), body condition score (3.81), postpartum milk yield (42.0 kg/d), milk fat yield (1,549 g/d), milk fat content (3.66%), milk true protein yield (1,192 g/d), and milk urea N content (12.9 mg/dL) were not different among treatments. Postpartum DMI and body condition score were greater and the ratios of milk:DMI and milk N:feed N were less for cows fed the MetaSmart diet than for cows fed the control and Smartamine M diets. Milk protein content was greater for the Smartamine M (2.87%) and MetaSmart (2.81%) treatments than for the control treatment (2.72%). Concentrations of Met and Met + Cys in total plasma AA were different among treatments, with values for the Smartamine M treatment being the highest, followed by the MetaSmart and control treatments. The results indicated that both MetaSmart and Smartamine M are effective in provid-

ing metabolizable Met, but clarification of their relative contributions to metabolizable Met is still needed.

Key words: methionine, methionine analog, amino acid, dairy cow

INTRODUCTION

The efficiency with which the dairy cow utilizes MP for protein synthesis is assumed to indicate how well the essential AA (EAA) profile in MP meets the profile of EAA required by the animal as well as by the total amount of EAA in MP (NRC, 2001). Research has indicated that Met is often a limiting AA for ruminants fed corn silage- or haylage-based diets, such as those typically fed in the United States (Schwab et al., 1992; NRC, 2001). Increasing the supply of Met to the small intestine has been shown to be effective in increasing protein synthesis in the mammary gland (Guinard and Rulquin, 1995; Pisulewski et al., 1996; St-Pierre and Sylvester, 2005; Rulquin et al., 2006; Socha et al., 2008). Research has indicated that milk protein content is sensitive to changes in the adequacy of Met in MP (NRC, 2001); therefore, increasing the concentration of Met in MP may lead to increased milk protein production. Additionally, Phillips et al. (2003) demonstrated that supplementing Met during the prepartum and postpartum period appears to have a sparing effect on body protein loss. The use of supplemental Met to spare BP loss in early lactation may not allow for additional MP-Met for milk protein synthesis, but instead may serve an even more important function in maintaining the health and longevity of the animal. A further benefit of using rumen-protected Met to improve the profile of EAA in MP is that the overall amount of RUP in the diet can be reduced (NRC, 2001). Reducing the amount of dietary N may result in an overall reduction in the amount of N excreted into the environment. Given the environmental concerns and regulations related to food production in the United States, this benefit might be valuable and useful.

Supplemental sources of Met have been developed in an effort to increase postruminal supplies of Met. These

Received March 30, 2009.

Accepted June 11, 2009.

¹This is Scientific Contribution Number 2400 from the New Hampshire Agricultural Experiment Station.

²Corresponding author: ryan.ordway@blueseal.com

sources include rumen-protected Met, Met hydroxy analogs such as 2-hydroxy-4-methylthio butanoic acid (HMB), and more recently, the isopropyl ester of HMB (MetaSmart, Adisseo Inc., Antony, France). The latter has been shown to increase plasma Met concentrations more than HMB (Robert et al., 2003; Graulet et al., 2005). Although results have been variable, studies have demonstrated that feeding HMB may increase milk (Piepenbrink et al., 2004) and milk fat yields (Patton et al., 1970), but not milk protein concentration.

Lysine and Met have been shown to be the 2 most often limiting AA for milk protein production in lactating dairy cows (NRC, 2001). The NRC (2001) suggests that the required amounts of Lys and Met in MP for optimal milk protein production are 7.2 and 2.4%, respectively. Because these amounts are difficult to achieve, it is recommended that the first step in balancing diets for Lys and Met is to maintain a Lys-to-Met ratio in MP of 3.0:1.0 and to try to achieve levels as close to 7.2 and 2.4% as possible. Only a limited number of studies (Phillips et al., 2003; Socha et al., 2005; Johnson-VanWieringen et al., 2007; Preynat et al., 2009a,b) have been conducted that have examined the effects of feeding rumen-protected Met to prepartum and early-lactation dairy cows, and no studies have been conducted that have examined the effects of feeding MetaSmart to prepartum and early-lactation dairy cows. The objective of this study was to compare the effects on animal performance of feeding Smartamine M (Adisseo Inc., Alpharetta, GA) and MetaSmart in amounts that result in a predicted 3.0:1 ratio of Lys to Met in MP to prepartum and early-lactation dairy cows fed a Met-deficient diet according to the NRC (2001) model. We hypothesized that both products would increase milk protein production. However, because approximately 50% of the HMB in MetaSmart appears to be available for use by rumen microorganisms (Schwab et al., 2001), we further hypothesized that a greater response in milk fat production would be observed for cows fed MetaSmart.

MATERIALS AND METHODS

Experimental Design and Dietary Treatments

Eighteen primiparous and 42 multiparous Holstein cows were blocked according to parity (primiparous or multiparous) and expected date of calving and assigned to 3 dietary treatments according to a balanced complete block design. The 3 experimental treatments consisted of 1) a basal diet (negative control: no supplemental Met and Met deficient), 2) the basal diet plus MetaSmart in amounts required to achieve a predicted ratio of Lys to Met in MP of 3.0:1.0, or 3) the basal

diet plus Smartamine M in amounts required to achieve a predicted ratio of Lys to Met in MP of 3.0:1.0 at 21 d before expected calving and continuing through 140 d postpartum. All cows received the same prepartum (Table 1) and postpartum (Table 1) Met-deficient basal diets.

Smartamine M and MetaSmart were supplied by Adisseo Inc. MetaSmart was supplied as a dry powder consisting of 30% of active ingredient on sepiolite, which was used as a carrier. Adjusted Met supplementation levels, based on Met equivalency of Smartamine M or MetaSmart, were added to the prepartum and postpartum diets of each cow to result in predicted concentrations of Lys and Met in MP of 3.0:1 according to the NRC (2001) diet evaluation (Table 2). The final treatment mixtures consisted of laboratory-weighted mixtures of the test products. The amounts of Smartamine M and MetaSmart in the final dietary treatments, as weighed for each cow for each feeding, varied according to 1) test product (owing to differences in assumed Met equivalency), 2) MP equivalency, and 3) DMI. It was assumed that MetaSmart contained no less than 70% HMB and that 50% of the HMB in MetaSmart was absorbed and converted to metabolizable Met. It was also assumed that Smartamine M contained 75% Met and that 80% of the Met was absorbed (Rulquin and Kowalczyk, 2003).

The basal diet (Table 1) was fed as a TMR and prepared by weighing each ingredient and mixing in a mobile tumble drum mixer (Data Ranger, American Calan Inc., Northwood, NH). Treatments were top-dressed onto the TMR of each cow and cows were fed 20% of their total daily feed allotment and treatment at 0600 h, 50% at 1400 h, and 30% at 2100 h for ad libitum feed intake, using fresh feed at each feeding. Feed offered was adjusted daily to achieve 5 to 10%orts. Orts were collected and weighed daily at 1130 h so that DMI could be calculated daily.

Efficiency of milk production to DMI was calculated by dividing the kilograms of milk produced by the kilograms of DM consumed. Efficiency of milk N production was calculated by dividing the kilograms of milk protein produced by the kilograms of feed protein consumed. Energy balance was calculated by using equations from the NRC (2001): Net energy for lactation intake (NE_L , predicted from the NRC, 2001, model $\times$ DMI) $-$ [milk energy ($0.0929 \times \text{fat } \% + 0.0563 \times \text{true protein } \% + 0.0395 \times \text{lactose } \%$) $+$ maintenance energy ($0.08 \text{ Mcal/kg of BW}^{0.75}$)].

Management of Cows

All procedures related to animal care were conducted with approval of the University of New Hampshire In-

Table 1. Ingredient and nutrient composition of prepartum and postpartum diets fed to cows receiving 2 forms of Met

Item, % of DM	Prepartum			Postpartum		
	Control	MetaSmart	Smartamine	Control	MetaSmart	Smartamine
Alfalfa hay	8.0	8.0	8.0	4.6	4.6	4.6
MMG silage ¹	13.2	13.1	13.2	11.4	11.3	11.4
Corn silage	33.4	33.2	33.4	33.5	33.3	33.4
Soyhulls	12.8	12.7	12.7	7.9	7.8	7.9
Corn meal	7.1	7.1	7.1	15.7	15.6	15.7
Barley meal	19.1	19.1	19.1	11.0	11.0	11.0
Soybean meal	3.7	3.7	3.7	7.7	7.7	7.7
Urea	0.4	0.4	0.4	0.4	0.4	0.4
Blood meal	—	—	—	2.2	2.2	2.2
Megalac ²	—	—	—	1.8	1.8	1.8
Vitamin-mineral mix ^{3,4}	2.3	2.3	2.3	3.8	3.8	3.8
MetaSmart ⁵	—	0.35	—	—	0.54	—
Smartamine M ⁶	—	—	0.06	—	—	0.10

¹MMG silage = mixed mostly grass silage.

²Megalac was supplied by Church and Dwight Co. Inc. (Princeton, NJ).

³The prepartum vitamin-mineral mix contained (DM basis): 1,137 kIU/kg of vitamin A, 284 kIU/kg of vitamin D, 7,580 IU/kg of vitamin E, 0.04% Ca, 0.03% P, 18.6% Mg, 0.02% K, 6.8% S, 26 mg/kg of Se, 6,554 mg/kg of Mn, 6,553 mg/kg of Zn, 4,301 mg/kg of Fe, 1,289 mg/kg of Cu, 86 mg/kg of Co, 11.6% Cl, and 7.5% Na.

⁴The postpartum vitamin-mineral mix contained (DM basis): 294 kIU/kg of vitamin A, 68 kIU/kg of vitamin D, 1,131 IU/kg of vitamin E, 13.5% Ca, 1.1% P, 6.6% Mg, 0.03% K, 0.27% S, 10 mg/kg of Se, 1558 mg/kg of Mn, 1,648 mg/kg of Zn, 1,055 mg/kg of Fe, 219 mg/kg of Cu, 31 mg/kg of Co, 9.2% Cl, and 6.0% Na.

⁵MetaSmart was supplied as a dry powder containing 30% of active ingredient on a sepiolite carrier. A 70% Met equivalence for MetaSmart with a bioavailability of 50% was assumed. MetaSmart was added to the diet in an amount needed to generate a Lys-to-Met ratio of 3.0:1. MetaSmart was supplied by Adisseo Inc. (Antony, France).

⁶A 75% Met equivalence for Smartamine M with a bioavailability of 80% was assumed. Smartamine M was added to the diet in an amount needed to generate a Lys-to-Met ratio of 3.0:1. Smartamine M was supplied by Adisseo Inc. (Alpharetta, GA).

stitutional Animal Care and Use Committee. The first 4 blocks of cows were injected every 14 d with bST (Monsanto Company, St. Louis, MO) beginning 84 d after calving. The remaining 16 blocks of cows were not injected with bST because of changes in the standard operating procedures at the Fairchild Dairy Teaching and Research Center (Durham, NH).

Cows were housed in a naturally ventilated tie-stall barn, fed individually, and milked in a milking parlor equipped with automatic takeoffs and milk meters. Prepartum cows were fed at 0700 and 1400 h.

Lactating cows were fed and milked 3 times each day at 8-h intervals. Weather permitting, lactating cows were exercised for 15 to 20 min before the afternoon milking in a dry lot. Body weight and BCS (scale of 1 to 5, with 1 being emaciated and 5 being obese; Wildman et al., 1982) were recorded each week beginning 21 d before the expected calving date and continuing through 140 d postpartum.

Feed, Milk, and Blood Sampling

All feed ingredients were sampled 14 d before the beginning of the experiment and analyzed for DM (60°C in a forced-air oven for 4 h for silages and near-infrared spectroscopy for grains and hay and residual moisture on silages; AOAC, 2000; method 991.03), CP (AOAC

2000; method 990.03; Leco Application Note 203-821-146), ADF (AOAC 2000; method 973.18), and NDF (Van Soest et al., 1991) using wet chemistry by Dairy One Forage Testing Laboratory (Ithaca, NY; www.dairyone.com/forage/procedures) to assist in determining the final formulation of the diets. Thereafter, silages were sampled daily during the a.m. feeding and were analyzed for DM by using a microwave oven (model R-209HK, Sharp, Mahwah, NJ) according to the procedure of the National Forage Testing Association (Undersander, 2004). Total mixed ration samples were taken at each feeding each day from Sunday at 1200 h through Friday at 0600 h and composited (Sunday at 1200 h through Wednesday at 0600 h, and Wednesday at 1200 h through Friday at 0600 h) and evaluated for DM and particle size by using the Penn State Particle Size Separator (Heinrichs, 1996). Ort samples were collected at 1100 h Monday through Friday and composited (Monday through Wednesday and Thursday through Friday) for DM analysis and particle size.

Each month, concentrate feeds and silages were sampled on 2 consecutive days, every other week, composited as collected, and a portion of the composited feed was analyzed for CP (AOAC 2000; method 990.03; Leco Application Note 203-821-146), ADF (AOAC 2000; method 973.18), and NDF (Van Soest et al., 1991) using wet chemistry by the Dairy One Forage

Table 2. Evaluation (NRC, 2001) of prepartum and postpartum diets fed to cows receiving 2 forms of Met¹

Chemical component	Prepartum			Postpartum		
	Control	MetaSmart	Smartamine	Control	MetaSmart	Smartamine
NDF, % of DM	38.7	38.7	38.7	32.9	32.9	32.9
ADF, % of DM	24.4	24.4	24.3	20.4	20.4	20.4
RDP, % of DM	10.1	10.2	10.2	10.0	10.0	10.0
RUP, % of DM	3.6	3.6	3.6	6.3	6.4	6.4
CP, % of DM	13.7	13.8	13.8	16.3	16.4	16.4
Energy balance, Mcal/d	8.6	8.4	7.5	-4.4	-3.6	-4.6
RDP balance, g/d	-8	-3	-1	23	24	21
RUP balance, g/d	431	432	381	-160	-136	-250
MP supplied, g/d	1,196	1,195	1,133	2,412	2,525	2,360
MP balance, g/d	325	326	288	-129	-107	-199
Lys, g/d	87	87	82	168	174	164
Met, g/d	24	29	27	43	58	55
His, g/d	26	26	25	64	66	62
Lys, % of MP	7.26	7.28	7.24	6.96	6.89	6.95
Met, % of MP	1.99	2.43	2.38	1.79	2.30	2.33
His, % of MP	2.20	2.18	2.21	2.64	2.61	2.63
Lys:Met in MP	3.6:1	3.0:1	3.0:1	3.9:1	3.0:1	3.0:1

¹NRC evaluation of diets was based on final production data and feed analyses. MetaSmart and Smartamine M were supplied by Adisseo Inc. (Antony, France, and Alpharetta, GA, respectively).

Testing Laboratory for ration adjustment, using the NRC (2001) model. A composite sample of each of the forages and grains at the conclusion of the study was sent to the Dairy One Forage Testing Laboratory, where wet chemistry was used for analysis of DM (4 h at 60°C in forced-air oven; VWR Scientific, West Chester, PA) for silages and near-infrared spectroscopy (AOAC 2000; method 991.03) was used for analysis of grains, hay, and residual moisture on silages. Wet chemistry was used for analysis of CP (AOAC 2000; method 990.03; Leco Application Note 203-821-146), ADF (Ankom A200 Filter Bag Technique, Ankom Application Note 01/02, "Method for Determining Acid Detergent Fiber") NDF (Ankom A200 Filter Bag Technique, Ankom Application Note 01/02, "Method for Determining Neutral Detergent Fiber"), acid detergent insoluble CP (AOAC 2000; method 2001.11; Foss Application Note AN 300), neutral detergent insoluble CP (AOAC 2000; method 2001.11; Foss Application Note AN 300), NE_L, NFC [100 - (CP + NDF + ash + ether extract)], lignin (AOAC 2000; method 973.18), starch (YSI 2700 Select Biochemistry Analyzer, YSI, Yellow Springs, OH; YSI Application Note 319), sugar (Hall et al., 1999), fat (Soxtec System HT6, Foss Tecator, Eden Prairie, MN; Foss Tecator Application Note AN301; Subnote AN 3414), ash (AOAC 2000; method 942.05), and minerals (IRIS Advantage Inductively Coupled Plasma Radial Spectrometer, Thermo Jarrell Ash, Franklin MA).

A portion of the composited forage and grain samples was also dried to approximately 90% DM in a forced-air oven at 55°C (VWR Scientific, West Chester, PA), allowed to equilibrate for 2 to 4 h to room temperature (23°C), and ground to pass through a 1-mm screen us-

ing a Wiley mill (Thomas Scientific, Swedesboro, NJ) for analysis of AA at the conclusion of the study. A composite sample of each of the ground forage and grain samples was sent for analysis of AA, using a Beckman system 6300 High-Performance Amino Acid Analyzer (Beckman-Coulter Instruments, Fullerton, CA), by the Experimental Station Chemical Laboratories, University of Missouri-Columbia.

Milk samples were collected from each cow during all 3 milkings 1 d each week from d 4 postpartum through 140 d postpartum. A milk sample was not taken until cows reached a minimum of 4 DIM. Samples were refrigerated until composited by milk weight for the sample date. Samples were preserved with 2-bromo-2-nitropropane-1,3-diol (1 tablet/40 mL of milk). Samples were analyzed for CP, true protein, fat, lactose, and MUN by mid-infrared spectrophotometric analysis with a Foss 4000 instrument (Foss North America, Eden Prairie, MN; Dairy One Cooperative Inc., Ithaca, NY).

Blood samples were drawn at 112 and 119 DIM, 2 h after the 0600 h feeding, via venipuncture of the coccygeal vein into 10-mL evacuated tubes containing sodium heparin (Becton Dickinson and Co., Rutherford, NJ). Samples were immediately placed in an ice bath and centrifuged within 45 min at 3,300 × *g* for 20 min at 5°C. An aliquot of 4 mL of plasma was deproteinized (4 vol of plasma was vortexed with 1 vol of 15% sulfosalicylic acid) and then placed in the centrifuge for 10 min at 5°C before centrifuging at 3,000 × *g* for 20 min at 5°C. The supernatant was collected and 0.25-mL aliquots were placed into Nunc CryoTube vials (Nalge Nunc International, Roskilde, Denmark) and stored at -80°C until analyzed for plasma free AA

as described by Noftsger et al. (2005), using a Beckman system 6300 High Performance Amino Acid Analyzer (Beckman-Coulter Instruments) by the Experimental Station Chemical Laboratories, University of Missouri-Columbia. Between the 112- and 119-d samplings, the deproteinized plasma samples taken at 112 d were stored at -20°C until composited with the 119-d deproteinized samples. Plasma AA concentrations ($\mu\text{g/mL}$) were adjusted to account for the use of 15% sulfosalicylic acid, which was used in a 1:4 ratio with plasma to deproteinize the original plasma sample.

Statistical Analysis

Production and intake data were analyzed using the MIXED procedure (SAS Institute, 2001) according to the following model:

$$Y_{ijklm} = \mu + T_i + P_j + TP_{ij} + B_k + C_{ijkl} + W_m + TW_{im} + TPW_{ijm} + e_{ijklm},$$

where Y_{ijklm} is the dependent, continuous variable; μ is the overall mean; T_i is the fixed effect of the i th treatment ($i = 1, 2, 3$); P_j is the fixed effect of the j th parity ($j = 1, 2$); TP_{ij} is the fixed effect of the i th treatment by the j th parity; B_k is the random effect of the k th block ($k = 1, \dots, 20$); C_{ijkl} is the random effect of the l th cow within the i th treatment, within the j th parity, and within the k th block ($l = 1, \dots, n_{ijk}$); W_m is the fixed effect of the m th week of the experiment ($m = 1, \dots, 20$); TW_{im} is the fixed effect of the i th treatment by the m th week of experiment interaction; TPW_{ijm} is the fixed effect of the i th treatment by the j th parity by the m th week of experiment interaction; and e_{ijklm} is the residual error.

In this model, the random effect of cow within treatment subclasses was used as the error term for the effect of treatments. Residual errors, which are errors within cow across time and represent error from repeated measurements in the experimental unit (cow), were modeled using a first-order autoregressive covariance structure. Autoregressive (1) resulted in the smallest Bayesian information criterion of the 3 covariate structures tested: autoregressive (1), compound symmetry, and unstructured (Littell et al., 1996). Data for multiparous cows were analyzed with and without covariates: previous 305-d mature equivalent milk yield, milk protein yield and percentage, and milk fat yield and percentage. Analyzing the data without covariates resulted in similar Bayesian information criterion values; therefore, covariates were not included in the final statistical model. Degrees of freedom were calculated using the Kenward-Roger option of the MIXED procedure.

Data for AA concentrations were analyzed using the MIXED procedure (SAS Institute, 2001) according to the following model:

$$Y_{ijkl} = \mu + T_i + P_j + TP_{ij} + B_k + C_{ijkl} + e_{ijkl},$$

where Y_{ijkl} is the dependent, continuous variable; μ is the overall mean; T_i is the fixed effect of the i th treatment ($i = 1, 2, 3$); P_j is the fixed effect of the j th parity ($j = 1, 2$); TP_{ij} is the fixed effect of the i th treatment by the j th parity; B_k is the random effect of the k th block ($k = 1, \dots, 20$); C_{ijkl} is the random effect of the l th cow within the i th treatment, within the j th parity, and within the k th block ($l = 1, \dots, n_{ijk}$); and e_{ijkl} is the residual error.

The UNIVARIATE procedure of SAS (SAS Institute, 2001) was used to determine whether the data set contained data points that were considered outliers. An observation that was greater than 2.5 standard deviations from the mean for milk production and DMI was considered an outlier. The results of the outlier analysis indicated that the milk production and DMI data from 3 multiparous cows were considered to be outlying from the rest of the data set. As a result, all the data points from these cows were removed from the final statistical analyses.

Overall treatment differences were examined using least squares means with the lowest standard error. Significance was declared at $P \leq 0.10$ and a trend in the data was declared at $P \leq 0.15$. Week and block were included in the final statistical model for all analyses. The DIFF option of SAS (SAS Institute, 2001) was used to test treatment differences among least squares means, and the SLICE option was used to analyze differences among weekly treatment means.

RESULTS

Ingredient and Chemical Composition of Diets

The chemical composition of the consumed diets (Table 2) was determined by analyzing each individual feed ingredient for its chemical composition and then entering the feed analysis results into the NRC (2001) model. The chemical composition of the forages is listed in Table 3 and the AA composition of the feed ingredients is presented in Table 4.

Prepartum DMI, BW, and BCS

The average number of days on trial before calving was (mean $\pm$ SD) 18.8 ± 0.89 d and was not different among treatments. Prepartum DMI, BW, and BCS were not different among treatments (Table 5). Prepartum

Table 3. Chemical composition of forages fed to prepartum and postpartum cows

Item, % of DM	Alfalfa hay	MMG silage ¹	Corn silage
DM	91.8	28.0	30.2
CP	16.2	16.5	7.9
Soluble CP, % of CP	43.0	58.3	56.7
NE _L , Mcal/kg	1.3	1.4	1.6
ADF	35.3	39.9	26.6
NDF	45.7	57.2	45.1
NFC ²	29.2	20.1	38.9
NSC ³	—	—	33.3
Starch	—	—	30.3
Sugar	—	—	3.0
Ca	1.7	0.8	0.2
P	0.2	0.3	0.2
Mg	0.2	0.2	0.1
K	2.3	2.7	1.4
Na	0.07	0.04	0.01
S	0.2	0.2	0.1

¹MMG silage = mixed mostly grass silage.²NFC = 100 - (CP + NDF + ash + ether extract).³NSC = starch + sugar.

DMI as a percentage of BW was not different among treatments and averaged $1.97 \pm 0.076\%$ (Table 5).

Postpartum DMI, BW, and BCS

A treatment effect on postpartum DMI was observed (Table 5). Supplementation with MetaSmart resulted in greater DMI compared with the control and Smartamine M treatments (22.9 ± 0.41 vs. 22.0 ± 0.41 and 21.4 ± 0.41 kg/d, respectively); however, supplementation with Smartamine M was not observed to elicit an

effect on DMI compared with the control treatment. A parity $\times$ treatment interaction was observed for postpartum DMI, indicating that primiparous and multiparous cows responded differently to dietary treatments (Table 5). For primiparous cows, DMI was greater for cows fed MetaSmart compared with the control and Smartamine M treatments (Table 5). For multiparous cows, MetaSmart supplementation did not increase DMI compared with the control or Smartamine M treatment; however, multiparous cows supplemented with Smartamine M had lower DMI compared with cows in the control treatment.

Dry matter intake was also analyzed weekly for all cows and cows within parity (Figure 1). When DMI was analyzed by week for all cows, it was observed that cows receiving MetaSmart had greater DMI than did control cows during wk 4 to 5, and had greater DMI than cows receiving Smartamine M during wk 4 to 7, wk 10, and wk 13 to 14. Primiparous cows receiving MetaSmart had greater DMI during wk 4 to 7 and wk 15 postpartum than did cows receiving the control diet. Cows receiving MetaSmart also had greater DMI during wk 4 to 7, wk 9 to 10, and wk 14 than did cows receiving Smartamine M. Multiparous cows receiving Smartamine M had lower DMI during wk 2, 11, and 13 compared with control cows but were not different from cows receiving MetaSmart.

Differences in overall postpartum BW were not observed to be different among treatments and averaged 578 ± 12.7 kg during the first 140 d postpartum (Table 5), but a parity $\times$ treatment interaction was observed. Body weights for primiparous cows were greater for

Table 4. Amino acid composition¹ of feeds fed to prepartum and postpartum cows

AA	Corn silage	MMG ² silage	Alfalfa hay	Corn meal	Barley meal	Soyhulls	Soybean meal	Blood meal ³
Arg	2.17	2.58	4.41	5.23	5.26	4.55	7.01	4.01
His	1.63	1.44	2.04	4.06	2.35	2.43	2.65	6.93
Ile	3.18	3.98	3.97	3.37	3.52	3.51	4.42	0.33
Leu	8.81	7.24	7.11	14.28	7.25	6.33	7.67	13.56
Lys	2.44	3.59	5.11	4.94	3.73	6.07	6.16	9.96
Met	1.56	1.37	1.47	2.32	1.82	1.13	1.39	1.28
Phe	3.59	4.11	4.68	6.21	5.04	3.64	4.97	7.57
Thr	3.05	3.23	4.08	4.18	3.47	3.42	3.70	4.34
Trp	0.54	0.52	1.40	1.10	1.09	0.69	1.23	2.07
Val	4.61	5.32	5.24	6.74	5.21	4.33	4.81	9.21
Ala	7.86	7.99	4.91	8.59	4.17	4.03	4.08	8.32
Asp	5.49	7.01	11.39	8.59	6.47	8.75	11.06	10.52
Cys	1.29	0.88	1.34	2.26	2.39	1.73	1.48	0.75
Glu	11.05	7.08	9.29	19.68	23.24	10.88	18.08	8.40
Gly	3.66	4.47	4.48	4.65	4.21	7.71	4.12	4.56
Pro	5.69	4.34	7.01	9.17	10.25	5.11	5.06	3.60
Ser	2.78	2.41	3.74	4.76	3.82	4.51	4.07	4.30
Tyr	2.03	2.02	2.87	3.31	2.78	3.73	3.42	2.66

¹Percentage of ingredient CP.²MMG silage = mixed mostly grass silage.³Ring-dried blood meal.

Table 5. Effects of feeding 2 forms of Met¹ on least squares means of DMI, BW, and BCS for prepartum and postpartum cows

Item	Control	MetaSmart	Smartamine	SE ²	<i>P</i> -value		
					Treatment	Parity	Parity × trt ³
n	20	18	19				
Prepartum							
DMI, kg/d	13.8	13.7	13.0	0.45	NS	<0.0001	NS
Primiparous							
Multiparous							
BW, kg	689	692	679	16.5	NS	<0.0001	NS
Primiparous	615	650	615	27.0			
Multiparous	763	734	744	19.0			
DMI, % BW	2.00	1.98	1.92	0.076	NS	NS	NS
BCS	3.79	3.85	3.80	0.051	NS	<0.0001	NS
Postpartum							
DMI, kg/d	22.0 ^b	22.9 ^a	21.4 ^b	0.41	0.01	<0.0001	0.04
Primiparous	18.4 ^b	20.7 ^a	18.2 ^b	0.68			
Multiparous	25.6 ^a	25.2 ^{ab}	24.5 ^b	0.47			
BW, kg	580	588	567	12.7	NS	<0.0001	0.06
Primiparous	515 ^b	554 ^a	512 ^b	20.9			
Multiparous	644 ^a	622 ^{ab}	620 ^b	14.4			
DMI, % BW	3.79	3.90	3.76	0.065	NS	<0.001	NS
BCS	3.26 ^b	3.37 ^a	3.28 ^b	0.053	0.09	<0.0001	NS

^{a,b}Means in same row with different superscripts differ ($P < 0.10$).

¹MetaSmart and Smartamine M were supplied by Adisseo Inc. (Antony, France, and Alpharetta, GA, respectively).

²Highest SE of treatment means is shown.

³Parity × trt = parity × treatment interaction.

cows supplemented with MetaSmart (554 ± 20.9 kg) compared with both control cows (515 ± 20.9 kg) and Smartamine M-supplemented cows (512 ± 20.9 kg). Multiparous cows fed the control (644 ± 14.4 kg) diet had greater BW compared with those fed Smartamine M (620 ± 14.4 kg), with the MetaSmart treatment (622 ± 14.4 kg) being intermediate and similar to both the control and Smartamine M treatments. Cows supplemented with MetaSmart (3.37 ± 0.053) had greater BCS than both control cows (3.26 ± 0.053) and Smartamine M-supplemented cows (3.28 ± 0.053 ; Table 5). Postpartum DMI as a percentage of BW was not different among treatments and averaged 3.82%.

Milk Production and Composition

Milk yield, milk fat concentration, milk true protein yield, lactose yield, MUN yield, and MUN concentration were not different among treatments (Table 6). When analyzed on a weekly basis, cows receiving Smartamine M produced less milk during wk 9 to 11 and wk 13 (trend for wk 12: $P = 0.11$) compared with control cows (data not shown).

Fat-corrected milk (3.5% FCM) averaged 43.7 ± 0.80 kg/d and was not different among treatments; however, a parity × treatment interaction was observed (Table 6). Primiparous cows supplemented with MetaSmart had greater yields of 3.5% FCM (39.0 ± 1.32 kg/d) compared with those supplemented with Smartamine M (36.5 ± 1.32 kg/d), whereas cows in the control

treatment (36.9 ± 1.32 kg/d) were similar to those in both the MetaSmart and Smartamine M treatments. Supplementation with MetaSmart and Smartamine M did not increase 3.5% FCM yields compared with the control treatment (49.4 and 49.3 vs. 51.3 ± 0.91 kg/d, respectively) in multiparous cows.

Milk fat yield was similar among treatments (Table 6); however, a parity × treatment effect was observed. Supplementation with MetaSmart ($1,382 \pm 49.2$ g/d) in primiparous cows did not increase milk fat yield compared with the control treatment ($1,320 \pm 49.2$ g/d) but did increase milk fat yield compared with supplementation with Smartamine M ($1,288 \pm 49.2$ g/d). Smartamine M did not increase milk fat yield compared with the control treatment in primiparous cows. Supplementation with MetaSmart ($1,744 \pm 33.6$ g/d) and Smartamine M ($1,757 \pm 33.6$ g/d) in multiparous cows did not increase milk fat yield compared with the control treatment ($1,806 \pm 33.6$ g/d). Control cows produced more milk fat than MetaSmart-supplemented cows, with no difference being observed between the control and Smartamine M treatments. As expected, supplementation with MetaSmart and Smartamine M increased milk protein concentrations compared with the control treatment (2.81 and 2.87 vs. $2.72 \pm 0.031\%$, respectively; Table 6).

Feed Efficiency and N Conversion

Feed efficiency (kg of milk/kg of DMI) and conversion of feed N to milk N (g of feed N/g of milk N)

Figure 1. Effects of feeding 2 forms of Met (MetaSmart, Adisseo Inc., Antony, France; Smartamine M, Adisseo Inc., Alpharetta, GA) on postpartum DMI for primiparous cows, multiparous cows, and all cows by week. Differences are indicated by an asterisk (*).

differed among treatments (Table 6). Supplementation with MetaSmart resulted in a lower feed efficiency compared with the Smartamine M and control treatments (1.92 vs. 1.98 and 2.00 ± 0.032 , respectively).

Smartamine M supplementation improved the conversion of feed N into milk N compared with the control and MetaSmart treatments (0.34 vs. 0.33 and 0.32 ± 0.004 , respectively).

Table 6. Effects of feeding 2 forms of Met¹ on least squares means for production variables

Item	Control	MetaSmart	Smartamine	SE ²	P-value		
					Treatment	Parity	Parity × trt ³
n	20	18	19				
Milk yield, kg	43.4	43.5	42.0	0.99	NS	<0.0001	NS
3.5% FCM	44.1	44.2	42.9	0.80	NS	<0.0001	0.09
Fat, g/d	1,563	1,563	1,522	29.8	NS	<0.0001	0.10
Protein, ⁴ g/d	1,170	1,215	1,192	26.8	NS	<0.0001	NS
Lactose, g/d	2,095	2,106	2,025	45.6	NS	<0.0001	NS
MUN, g/d	5.68	5.68	5.68	0.176	NS	<0.0001	NS
Fat, %	3.65	3.65	3.68	0.074	NS	NS	NS
Protein, ⁴ %	2.72 ^b	2.81 ^a	2.87 ^a	0.031	0.001	NS	NS
Lactose, %	4.84	4.84	4.83	0.018	NS	<0.0001	0.03
MUN, mg/dL	12.7	12.7	13.2	0.29	NS	NS	NS
Milk:DMI ⁵	2.00 ^a	1.92 ^b	1.98 ^a	0.032	0.09	NS	NS
Milk N:feed N ⁶	0.33 ^b	0.32 ^b	0.34 ^a	0.004	0.003	NS	NS
Energy balance, ⁷ Mcal/d	−3.85 ^b	−2.86 ^a	−4.15 ^b	0.409	0.03	NS	NS

^{a,b}Means in same row with different superscripts differ ($P < 0.10$).

¹MetaSmart and Smartamine M were supplied by Adisseo Inc. (Antony, France, and Alpharetta, GA, respectively).

²Highest SE of treatment means is shown.

³Parity × trt = parity × treatment interaction.

⁴Protein = true protein.

⁵Ratio of milk (kg) to DMI (kg).

⁶Milk protein N yield (kg) per kg of feed N intake.

⁷Calculated using equations from NRC (2001): Net energy for lactation intake (NE_L predicted from NRC, 2001, model × DMI) − [milk energy ($0.0929 \times \text{fat \%} + 0.0563 \times \text{true protein \%} + 0.0395 \times \text{lactose \%}$) + maintenance energy ($0.08 \text{ Mcal/kg BW}^{0.75}$)].

Energy balance, as predicted by the NRC (2001) model, was observed to be negative for all treatments for the duration of the study (Table 6). When energy balance was calculated by treatment (energy required for milk and maintenance subtracted from net energy intake), MetaSmart supplementation resulted in the least severe energy deficit and was different from both the control and Smartamine M treatments (−2.9 vs. −3.9 and -4.2 ± 0.41 Mcal/d, respectively; Table 6).

Plasma AA

Effects of MetaSmart and Smartamine M supplementation on total plasma AA concentrations and as a percentage of total AA are presented in Table 7. Supplementation of both MetaSmart and Smartamine M increased the concentration of Met (4.26 and 5.96 vs. 3.34, respectively), Met + Cys (9.23, and 11.11 vs. 7.97, respectively), and total sulfur AA (14.87 and 16.93 vs. 12.46, respectively) in total plasma AA compared with the control treatment. A similar effect was observed when Met (1.43 and 2.10 vs. 1.15, respectively), Met + Cys (3.12 and 3.92 vs. 2.73, respectively), and total sulfur AA (5.01 and 5.98 vs. 4.25, respectively) were expressed as a percentage of total AA compared with the control treatment. However, Smartamine M supplementation resulted in an even greater increase in the concentrations of Met, Met + Cys, and total sulfur AA

in total plasma AA as well when expressed as a percentage of total AA compared with MetaSmart.

DISCUSSION

Dietary Effects

Particle size was estimated for forages, TMR, and orts. Heinrichs and Kononoff (2002) suggested that approximately 3 to 8% of the total TMR remains on the top sieve (19 mm) when using the Penn State Particle Separator or sorting may occur. They also suggested that there should be no more than a 20% difference between the amount of TMR and orts remaining on the top sieve. In the current study, approximately 27% of the prepartum TMR and 22% of the postpartum TMR and approximately 38% of the prepartum orts and 44% of the postpartum orts remained on the top sieve. The difference between the prepartum TMR and orts was within the 20% allowable range; however, there was a 22% difference between the postpartum TMR and orts. This difference was attributed to the low-quality alfalfa hay that was included in the postpartum diet, which cows did appear to sort. Because the basal diet was similar for all treatments, any negative effects of particle size would have affected all treatment groups the same; therefore, the difference was not considered detrimental in this study.

Table 7. Effects of feeding 2 forms of Met¹ on postpartum plasma AA concentrations

Item	Control	MetaSmart	Smartamine M	SE ²	P-value		
					Treatment	Parity	Parity × trt ³
n	14	15	15				
µg/mL ³							
Met	3.34 ^c	4.26 ^b	5.96 ^a	0.250	<0.0001	NS	NS
Met + Cys	7.97 ^c	9.23 ^b	11.11 ^a	0.353	<0.0001	NS	NS
Total sulfur	12.46 ^c	14.87 ^b	16.93 ^a	0.582	<0.0001	0.05	NS
Total essential AA	145.85	140.38	135.84	6.949	NS	NS	NS
Total AA	293.18	295.73	284.14	9.025	NS	NS	NS
% of total AA							
Met	1.15 ^c	1.43 ^b	2.10 ^a	0.078	<0.0001	NS	NS
Met + Cys	2.73 ^c	3.12 ^b	3.92 ^a	0.102	<0.0001	NS	NS
Total sulfur	4.25 ^c	5.01 ^b	5.98 ^a	0.160	<0.0001	0.03	NS
Total essential AA	49.46	47.16	47.67	1.113	NS	NS	NS

^{a-c}Means in same row with different superscripts differ ($P < 0.10$).

¹MetaSmart and Smartamine M were supplied by Adisseo Inc. (Antony, France, and Alpharetta, GA, respectively).

²Highest SE of treatment means is shown.

³Parity × trt = parity × treatment.

Prepartum and postpartum diets were evaluated using the NRC (2001) model assuming the average chemical composition of the feeds used throughout the duration of the study. The NRC (2001) model predicted a negative energy balance of -4.4 , -3.6 , and -4.6 Mcal/d for the postpartum control, MetaSmart, and Smartamine M diets, respectively, and also predicted energy to limit milk production (Table 2). Actual milk yields compared with NRC (2001)-predicted NE_L-allowable and MP-allowable milk yields were 43.4 vs. 37.0 and 40.2 kg/d, respectively, for the control treatment; 43.5 vs. 38.4 and 40.6, respectively, for MetaSmart; and 42.0 vs. 35.4 and 37.0, respectively, for Smartamine M. The prepartum and postpartum diets were formulated to be adequate in energy and protein; however, according to NRC (2001) model predictions using the results of the study, the postpartum diet was predicted to be deficient in ME. This result was not unexpected given that cows during early and peak lactation are mobilizing body energy and protein reserves and cannot generally meet their energy requirements solely through their dietary intakes, and the milk production and BCS results indicate that animals were indeed mobilizing body reserves to meet lactation demands.

Previous Research Findings with Smartamine M and MetaSmart

Several studies have been conducted that have examined the effects of feeding HMB on postpartum production; however, only a few studies have examined the effects on production of feeding rumen-protected Met (Griel et al., 1968; Phillips et al., 2003; Piepenbrink et al., 2004; Socha et al., 2005; Johnson-VanWieringen

et al., 2007; Preynat et al., 2009a,b) during both the prepartum and postpartum periods. To our knowledge, no studies have been conducted that have examined the effects of specifically feeding MetaSmart (the isopropyl ester of HMB) during both the prepartum and postpartum periods.

Effects on DMI and Milk Production

Socha et al. (2005) observed that there was no effect of feeding Smartamine M or Smartamine ML during the prepartum period on prepartum DMI during the last week of gestation. However, a trend ($P > 0.15$) was observed for decreased postpartum DMI when only Smartamine M was fed. Cows fed Smartamine M averaged 14.2 kg/d of DMI during the week before parturition, whereas control cows and cows fed Smartamine M plus Smartamine ML averaged 15.1 and 15.2 kg/d, respectively.

In the current study, differences in mean prepartum DMI were not observed; however when the data were analyzed by week, prepartum DMI during the last 2 wk before calving for Smartamine M was significantly less than for the control and MetaSmart treatments, which is in agreement with the trend observed by Socha et al. (2005). Overall dietary CP content for the prepartum diet averaged 13.8% in the current study compared with 15.6% for the prepartum diet of Socha et al. (2005). The NRC (2001)-predicted flows of MP-Lys and MP-Met were also lower in the current study for Smartamine M compared with those reported by Socha et al. (2005) for Smartamine M (82 and 27 g/d vs. 101 and 37 g/d, respectively). When DMI as a percentage of BW was analyzed as a means of adjusting for any numerical dif-

ferences in BW, no differences between treatments were observed. In addition, no treatment $\times$ week, parity $\times$ week, or parity $\times$ treatment $\times$ week interactions were observed. These results indicate that any numerical differences in DMI prepartum were most likely the result of differences in BW between the cows, because when adjusted for BW, there were no differences in DMI.

A parity $\times$ treatment interaction was observed for postpartum DMI, with primiparous cows on the MetaSmart treatment consuming more feed than cows in either the control or Smartamine M treatment, and with multiparous cows in the MetaSmart and control treatments consuming more feed than cows on the Smartamine M treatment (Table 5). When DMI was calculated as a percentage of BW, no differences existed among treatments and there were no treatment $\times$ week, parity $\times$ week, or parity $\times$ treatment $\times$ week interactions. Again, these results indicate that the observed differences in DMI were due to numerical differences in BW, but when DMI was adjusted for BW, there were no differences between treatments.

The lower DMI observed for multiparous Smartamine M compared with the control treatment during wk 2, 13, and 14 may help to explain why milk yields for multiparous cows in the control treatment during wk 9 to 11 and wk 13 (trend for wk 12; $P = 0.11$) were greater than for multiparous cows fed Smartamine M. It appears that the lower DMI for cows consuming Smartamine M were accompanied by lower milk yields. A similar effect was observed by Socha et al. (2005), who found that cows receiving Smartamine M consumed less feed than cows receiving no Smartamine M or Smartamine ML. These researchers also observed that cows receiving Smartamine M had numerically lower milk yields compared with cows receiving no Smartamine M or Smartamine ML.

Effects on Milk Components

We originally hypothesized that feeding MetaSmart would increase milk fat concentration as a result of an increase in the acetate-to-propionate ratio as well as an increase in the uptake of TAG by the mammary gland for reasons discussed previously. However, the results of this study disprove our hypothesis that feeding MetaSmart would increase milk fat synthesis but are in agreement with the results observed by St-Pierre and Sylvester (2005) and Phipps et al. (2008) when feeding MetaSmart to lactating dairy cows.

As predicted, milk protein concentrations were greater for MetaSmart and Smartamine M compared with the control treatment. There was no difference between MetaSmart and Smartamine M; however, there was a numerical trend for Smartamine M to increase milk

protein concentration more than MetaSmart (2.87 vs. 2.81, $P = 0.12$). It was originally assumed that 50% of the HMB in dietary MetaSmart was absorbed and converted to metabolizable Met (Schwab et al., 2001) and that 80% of the Met in Smartamine M was absorbed (Rulquin and Kowalczyk, 2003). Based on these assumptions, MetaSmart and Smartamine M diets were formulated to supply similar amounts of metabolizable Met. However, because a trend existed for Smartamine M to increase milk protein concentration more than MetaSmart, it would appear that the Met bioavailabilities of the 2 products may be different from assumed. However, despite this observation, results demonstrate that MetaSmart is effective in providing metabolizable Met based on its ability to increase milk protein concentrations and plasma Met concentrations compared with the control diet.

In the current study, feeding MetaSmart and Smartamine increased milk protein concentration compared with the control diet; however, no increases in milk protein yield were observed, which is in agreement with other studies in which fed rumen-protected Met supplements were fed to cows both prepartum and postpartum (Socha et al., 2005; Preynat et al., 2009b). In a study conducted by Phillips et al. (2003), prepartum cows were fed 2 different diets containing either 11 or 14% CP with or without 20 g/d of HMB and then fed a common 17% CP diet postpartum with or without 50 g/d of HMB. Both prepartum and postpartum diets were made adequate in Met by using Smartamine. This was done so that the supplemental HMB was added in addition to a Met-adequate diet to determine whether there would be additional effects of HMB supplementation at the animal or organ level. No response in milk protein concentration or yield was observed at either the prepartum protein level or with or without supplemental HMB. Unlike in the current study, in which supplemental Met in the form of either MetaSmart or Smartamine was added to a diet deficient in metabolizable Met, Phillips et al. (2003) added HMB to diets already adequate in Met. However, similar results were observed concerning a lack of response in milk protein production to supplemental Met feeding in prepartum and postpartum cows. Given that both studies observed increases in plasma Met concentrations, it could be concluded that the addition of supplemental Met to postpartum cows did provide a source of metabolizable Met; however, the cow appeared to use the Met in early lactation for uses other than milk protein production. In the current study, it is plausible that the lack of response in milk protein production was the result of the Met being utilized by tissues and organs other than the mammary gland to satisfy requirements by those tissues. Because there is a finite pool of AA in the body,

muscle tissue, gastrointestinal tissue, and the mammary gland must all interact with one another to utilize the pool of available AA most effectively to maintain the health of the animal. The objective of this study was to examine the effects on milk protein production when supplementing rumen-protected Met to prepartum and postpartum cows and was not designed to examine the effects of rumen-protected Met on metabolic systems and health parameters in the cow. As a result, we can only hypothesize that the lack of response in milk protein production was due to a partitioning of Met away from the mammary gland to other tissue and organ systems that had a greater need for Met in the periparturient period to maintain the health of the animal. As nutritional scientists, we tend to put production responses above all other responses, especially when economic considerations are taken into account. However, maintaining the health of the cow also has its economic benefits, and we must consider health responses in addition to production responses when evaluating the effects and benefits of using supplemental Met sources during the periparturient period.

Determining Bioavailability Values

Future research should be conducted to determine a more accurate value for the Met bioavailability of MetaSmart. Although the current study did not directly measure changes in plasma Met concentrations, it is possible that evaluating changes in plasma Met concentrations will improve the precision when evaluating the Met contribution of a rumen-protected Met product compared with changes in milk protein concentration (Ordway, 2005). In the current study, Smartamine M supplementation resulted in a larger numerical, but not statistical, increase in milk protein concentration compared with MetaSmart, which may be the result of an overprediction of the Met bioavailability of MetaSmart. Perhaps if a lower Met bioavailability estimate had been assumed, the numerical difference in milk protein concentration might not have been observed.

CONCLUSIONS

MetaSmart and Smartamine M are effective in providing metabolizable Met, as indicated by increased milk protein concentrations and plasma sulfur AA concentrations. However, MetaSmart did not increase milk fat production, as was originally hypothesized. In addition, overall milk yields and milk protein yields (g/d) were not increased by feeding MetaSmart or Smartamine M. As a result, clarification of their relative contributions to metabolizable Met is still needed. Future research should concentrate not only on determining the best

methodology for predicting the bioavailability of methionine products, but also on reducing the variation associated with predicting bioavailability of Met analogs and Met products.

ACKNOWLEDGMENTS

The authors thank Amy McLaughlin (Appleton Farms, Ipswich, MA) for assistance with administering treatments and collecting samples. The authors also thank the staff at the Fairchild Dairy Teaching and Research Center (Durham, NH) for feeding and caring for the animals. The authors also thank Adisseo Inc. (Antony, France, and Alpharetta, GA) for their generous financial support.

REFERENCES

- AOAC. 2000. Official Methods of Analysis. 19th ed. Association of Official Analytical Chemists, Washington, DC.
- 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.
- Griel, L. C., R. A. Patton, R. D. McCarthy, and P. T. Chandler. 1968. Milk production response to feeding methionine hydroxy analog to lactating dairy cows. *J. Dairy Sci.* 51:1866–1868.
- Guinard, J., and H. Rulquin. 1995. Effects of graded amounts of duodenal infusions of methionine on the mammary uptake of major milk precursors in dairy cows. *J. Dairy Sci.* 78:2196–2207.
- Hall, M. B., W. H. Hoover, J. P. Jennings, and T. K. Miller-Webster. 1999. A method for partitioning neutral detergent soluble carbohydrates. *J. Sci. Food Agric.* 79:2079–2086.
- Heinrichs, A. J., and P. J. Kononoff. 2002. Evaluating particle size of forages and TMRs using the new Penn State Forage Particle Separator. *Coop. Ext. Tech. Bull. DAS 02-42*. College of Agriculture Science, Pennsylvania State University, University Park.
- Heinrichs, J. 1996. Evaluating particle size of forages and TMRs using the Penn State particle size separator. *Coop. Ext. Tech. Bull. DAS 96-20*. College of Agriculture Science, Pennsylvania State University, University Park.
- Johnson-VanWieringen, L. M., J. H. Harrison, D. Davidson, M. L. Swift, M. A. G. von Keyserlingk, M. Vazquez-Anon, D. Wright, and W. Chalupa. 2007. Effects of rumen-undegradable protein sources and supplemental 2-hydroxy-4-(methylthio)-butanoic acid and lysine-HCl on lactation performance in dairy cows. *J. Dairy Sci.* 90:5176–5188.
- Littell, R. C., G. A. Milliken, W. W. Stroup, and R. D. Wolfinger. 1996. SAS System for Mixed Models. SAS Inst. Inc., Cary, NC.
- Noftsker, 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.
- NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.
- Ordway, R. S. 2005. An evaluation of supplemental methionine sources for lactating dairy cows. PhD Thesis. University of New Hampshire, Durham.
- Patton, R. A., R. D. McCarthy, and L. C. Griell Jr.. 1970. Observations on rumen fluid, blood serum, and milk lipids of cows fed methionine hydroxy analog. *J. Dairy Sci.* 53:776–780.
- Phillips, G. J., T. L. Citron, J. S. Sage, K. A. Cummins, M. J. Cecava, and J. P. McNamara. 2003. Adaptations in body muscle and fat in transition dairy cattle fed differing amounts of protein and methionine hydroxy analog. *J. Dairy Sci.* 86:3634–3647.
- Phipps, R. H., C. K. Reynolds, D. I. Givens, A. K. Jones, P. A. Geraert, E. Devillard, and R. Bennett. 2008. Short communication: Effects of 2-hydroxy-4-(methylthio) butanoic acid isopropyl ester on milk

- production and composition of lactating Holstein dairy cows. *J. Dairy Sci.* 91:4002–4005.
- Piepenbrink, M. S., A. L. Marr, M. R. Waldron, W. R. Butler, T. R. Overton, M. Vazquez-Anon, and M. D. Holt. 2004. Feeding 2-hydroxy-4-(methylthio)-butanoic acid to periparturient dairy cows improves milk production but not hepatic metabolism. *J. Dairy Sci.* 87:1071–1084.
- Pisulewski, P. M., H. Rulquin, J. L. Peyraud, and R. Verite. 1996. Lactational and systemic responses of dairy cows to postruminal infusions of increasing amounts of methionine. *J. Dairy Sci.* 79:1781–1791.
- Preynat, A., H. Lapiere, M. C. Thivierge, M. F. Palin, J. J. Matte, A. Desrochers, and C. L. Girard. 2009a. Effects of supplements of folic acid, vitamin B₁₂, and rumen-protected methionine on whole body metabolism of methionine and glucose in lactating dairy cows. *J. Dairy Sci.* 92:677–689.
- Preynat, A., H. Lapiere, M. C. Thivierge, M. F. Palin, J. J. Matte, A. Desrochers, and C. L. Girard. 2009b. Influence of methionine supply on the response of lactational performance of dairy cows to supplementary folic acid and vitamin B₁₂. *J. Dairy Sci.* 92:1685–1695.
- Robert, J. C., E. Madiot, C. Richard, and B. Bouza. 2003. Influence of HMBi, HMB, and combination of both on ruminal metabolism in vivo. *J. Dairy Sci.* 86(Suppl. 1):277. (Abstr.)
- Rulquin, H., B. Graulet, L. Delaby, and J. C. Robert. 2006. Effect of different forms of methionine on lactational performance of dairy cows. *J. Dairy Sci.* 89:4387–4394.
- Rulquin, H., and J. Kowalczyk. 2003. Development of a method for measuring lysine and methionine bioavailability in rumen-protected products for cattle. *J. Anim. Feed Sci.* 12:465–474.
- SAS Institute. 2001. SAS/STAT User's Guide, Version 8.2 Edition. SAS Inst. Inc., Cary, NC.
- Schwab, C. G., C. K. Bozak, N. L. Whitehouse, and V. M. Olson. 1992. Amino acid limitation and flow to the duodenum at four stages of lactation. 2. Extent of lysine limitation. *J. Dairy Sci.* 75:3503–3518.
- Schwab, C. G., N. L. Whitehouse, A. M. McLaughlin, R. K. Kadariya, N. R. St-Pierre, B. K. Sloan, and J. C. Robert. 2001. A comparison of 2-hydroxy-4-methylthio butanoic acid and its isopropyl ester with protected methionine in their ability to provide methionine for milk protein synthesis in Holstein cows. *J. Dairy Sci.* 84(Suppl. 1):146. (Abstr.)
- 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.
- Socha, M. T., C. G. Schwab, D. E. Putnam, N. L. Whitehouse, B. D. Garthwaite, and G. A. Ducharme. 2008. Extent of methionine limitation in peak-, early-, and mid-lactation dairy cows. *J. Dairy Sci.* 91:1996–2010.
- 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.
- Undersander, D. 2004. Procedures for dry matter determination. http://foragetesting.org/index.php?page=lab_procedures Accessed Sept. 23, 2004.
- 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.
- Wildman, E. E., G. M. Jones, P. E. Wagner, R. L. Boman, H. F. Troutt Jr., and T. N. Lesch. 1982. A dairy cow body condition scoring system and its relationship to selected production characteristics. *J. Dairy Sci.* 65:495–501.