Feeding 2-Hydroxy-4-(Methylthio)-Butanoic Acid to Periparturient Dairy Cows Improves Milk Production but not Hepatic Metabolism*

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ABSTRACT

Forty-eight Holstein cows, entering second or later lactation, were utilized to determine the effects of 2-hydroxy-4-(methylthio)-butanoic acid (HMB) on milk production, hepatic lipid metabolism, and gluconeogenesis during the periparturient period. Cows were fed one of 3 diets as TMR starting 21 d before expected calving. These diets contained 0 (the basal diet), 0.09 (+HMB), or 0.18 (++)HMB% HMB. From parturition to 84 DIM, cows were fed diets that contained 0, 0.13, or 0.20% HMB. Prepartum and postpartum dry matter intakes were similar among cows fed the basal diet, +HMB and ++HMB. There was a quadratic effect on milk yield such that cows fed +HMB had the greatest milk yield; yields of milk by cows fed the basal diet and ++HMB were similar. This led to trends for increased yields of 3.5% fat-corrected milk and total solids when cows were fed +HMB. Percentages of fat, protein, and total solids in milk were not affected by treatment. Despite differences in milk yield, calculated energy balance was not affected by treatment. Plasma concentrations of NEFA, β -hydroxybutyrate, and glucose were not different among treatments. Liver triglyceride content was similar among treatments on d 1 postpartum and was increased for cows consuming +HMB on d 21 postpartum compared with the other dietary treatments. Capacities for metabolism of [1-¹⁴C]palmitate by liver slices in vitro were not affected by treatment; however, conversion of [1-¹⁴C]propionate to CO₂ and glucose decreased as the amount of HMB consumed by cows increased on d 21 postpartum. Cows consuming

+HMB had greater days to first ovulation compared with cows consuming the basal diet and ++HMB as measured by plasma progesterone concentrations. These data suggest that adding HMB to low Met diets to achieve a predicted Met supply of approximately 2.3% of metabolizable protein supply is beneficial for increasing milk production but does not appear to benefit hepatic energy metabolism during early lactation.

(Key words: dairy cow, methionine, gluconeogenesis, hepatic lipodosis)

Abbreviation key: HMB = 2-hydroxy-4-(methylthio)-butanoic acid, TG = triglyceride, VLDL = very low density lipoproteins.

INTRODUCTION

Requirements for energy, glucose, and amino acids increase dramatically during the periparturient period to support the onset of milk production. This requires remarkable coordination of metabolism to meet the demands of lactation (Bell, 1995). Energy requirements are met, in part, by rapid mobilization of fatty acids from adipose tissue, resulting in increased circulating concentrations of NEFA. Although these NEFA can be used by other tissues for energy and for milk fat, the liver extracts NEFA in proportion to its concentration in the bloodstream (Emery, 1992; Reynolds et al., 2003), resulting in hepatic lipodosis because the capacity of ruminant liver for fatty acid oxidation and export as very low density lipoproteins (VLDL) is low relative to fatty acid uptake (Grummer, 1993). The resulting triglyceride (TG) accumulation in the liver has been suggested to reduce the capacity of the liver to detoxify ammonia to urea (Strang et al., 1998), potentially leading to decreased capacity for gluconeogenesis from propionate (Overton et al., 1999). The corollary is that strategies to decrease the accumulation of TG in liver of periparturient dairy cows may alleviate the potential inhibition of these important metabolic processes.

Given the potentially detrimental metabolic effects of accumulation of TG in liver during the periparturient period, several nutrients have been identified as poten-

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tially limiting for hepatic disposal of fatty acids either through oxidation or export (Grummer, 1993; Bauchart et al., 1998). Research conducted in our laboratory (Piepenbrink and Overton, 2003) has demonstrated that supplementation of choline to dairy cows during the periparturient period resulted in coordinated changes in fatty acid and carbohydrate metabolism consistent with alleviation of the impairment of TG accumulation on hepatic metabolism. Phosphatidylcholine plays an important role in VLDL assembly and stability of the lipoprotein particle (Vermeulen et al., 1997). Although we cannot determine whether the effects on fatty acid metabolism reported in our previous experiment (Piepenbrink and Overton, 2003) are related to VLDL synthesis and secretion, Yao and Vance (1988) reported that hepatocytes isolated from rats fed a choline-free diet and supplemented *in vitro* with either choline or Met increased their secretion of phosphatidylcholine and export of TG as VLDL.

Methionine is another potentially limiting nutrient for hepatic metabolism of fatty acids during the periparturient period. Although most commonly noted for its role as one of the two most limiting AA for production of milk and milk protein (Schwab, 1994), suggestions that Met as DL-Met or Met hydroxy analog may have a role in hepatic lipid metabolism and ketosis have existed for more than 30 yr (McCarthy et al., 1968; Waterman and Schultz, 1972). Methionine is required for synthesis of carnitine (Combs, 1992), which is required for the transport of long-chain fatty acids across mitochondrial and peroxisomal membranes, allowing the fatty acids to undergo oxidative processes (Zammit, 1999). Emmanuel and Kennelly (1984) found that 28% of radioactivity from L-[methyl-¹⁴C]Met was recovered as choline in lactating goats, indicating that choline biosynthesis is an important fate of Met fed to ruminants. Furthermore, Met is required for synthesis of apolipoprotein B100 (apoB100; Grummer, 1993), which is essential for synthesis and secretion of VLDL particles. Despite these possible roles, few data exist that describe the effects of Met on hepatic metabolism in ruminants. In an experiment with limited replication, Durand et al. (1992) demonstrated that infusing Met and Lys into the portal vein of early-lactation cows caused the hepatic balance of VLDL to be positive, whereas the balance of VLDL was negative during both the preinfusion and postinfusion periods in these cows. In a subsequent experiment conducted during the first 4 wk of lactation, cows fed ruminally protected Met had similar concentrations of TG and apoB100 in liver compared with controls, whereas those fed both ruminally protected Met and Lys had lower concentrations of TG and higher concentrations of apoB100 in liver compared with controls (Bauchart et al., 1998). Bertics

and Grummer (1999) fed Met hydroxy analog to cows that were subjected to fatty liver induction by feed restriction and found that cows fed Met hydroxy analog had higher plasma concentrations of NEFA, and neither accumulation nor depletion of TG within the liver were affected by feeding Met hydroxy analog. Collectively, these data do not allow evaluation of the role of Met in hepatic fatty acid metabolism independent of Lys supply.

We hypothesized that hepatic fatty acid metabolism and cow performance might be sensitive to Met supply during the periparturient period and early lactation. Therefore, the objective of this experiment was to determine the effects of increasing Met supply on hepatic metabolism, key metabolites in blood, and cow performance by feeding increasing amounts of a Met analog (DL-2-hydroxy-4-methylthiobutanoic acid; **HMB**) to periparturient cows fed diets formulated to be deficient in Met and adequate in Lys.

MATERIALS AND METHODS

The Cornell University Institutional Animal Care and Use Committee approved all procedures involving animals. Forty-eight Holstein dairy cows, entering second or greater lactation, were moved into individual tie stalls approximately 28 d before expected parturition and fed for *ad libitum* intake a common prepartum diet (Basal, Table 1) once daily as TMR. On d 21 before expected parturition, cows were assigned to one of 3 diets containing increasing amounts of HMB (0, 0.09, and 0.18% of DM for the basal diet, +HMB, and ++HMB, respectively; Alimet feed supplement, Novus International, St. Louis, MO) in a completely randomized design. After parturition, cows were fed postpartum diets (Table 1) containing HMB at 0, 0.13, and 0.20% of DM (for the basal diet, +HMB, and ++HMB, respectively) until 84 d of lactation. Cows assigned to each of the 3 prepartum treatments were maintained on their respective treatments during the postpartum period. Pre- and postpartum basal diets were formulated using the CPM Dairy (version 1.0) ration evaluator and were found to be deficient in Met and adequate in Lys as described by NRC (2001). Additions of HMB were made to the basal diet to achieve projected Met concentrations in metabolizable protein of 2.34 and 2.70% for prepartum diets and 2.36 and 2.63% for postpartum diets (+HMB and ++HMB, respectively; Table 2). The +HMB diets were formulated to bring Met concentrations close to NRC (2001) requirements for cows in established lactation, and the ++HMB diet was formulated to provide Met in excess of the recommendations, given the potential additional requirement for Met during hepatic metabolism of fatty acids. The source of HMB

Table 1. Ingredient composition of diets (% of DM).

Ingredient	Prepartum			Postpartum		
	Basal	+HMB	++HMB	Basal	+HMB	++HMB
Grass hay (chopped)	10.8	10.8	10.8	—	—	—
Mixed silage	29.1	29.1	29.1	—	—	—
Alfalfa hay (chopped)	—	—	—	8.6	8.6	8.6
Alfalfa haylage	—	—	—	12.9	12.9	12.9
Corn silage	27.8	27.8	27.8	36.5	36.4	36.4
Ground shelled corn	12.1	12.1	12.1	20.3	20.2	20.2
Soybean hulls	7.9	7.9	7.9	5.4	5.4	5.4
Soybean meal	2.50	2.49	2.49	3.24	3.23	3.23
SoyPlus ¹	4.99	4.99	4.99	7.77	7.76	7.75
Blood meal	1.66	1.66	1.66	2.16	2.15	2.15
EnerG II ²	0.83	0.83	0.83	1.08	1.08	1.08
Minerals and vitamins ³	2.2	2.2	2.2	2.0	2.0	2.0
Alimet ⁴	—	0.10	0.21	—	0.15	0.23

¹Expeller soybean meal (West Central Soy, Ralston IA).

²Calcium salts of palm fatty acid distillate (Bioproducts Inc., Fairlawn, OH).

³Prepartum cows received a mineral and vitamin mix that contained 11.10% Ca, 3.42% P, 10.06% Mg, 0.39% K, 4.78% Na, 3.48% Cl, 7.59% S, 46.43 ppm of Co, 617.84 ppm of Cu, 1913.35 ppm of Fe, 68.42 ppm of I, 4166.36 ppm of Mn, 13.85 ppm of Se, 4860.68 ppm of Zn, 458 KIU/kg vitamin A, 133 KIU/kg vitamin D, and 6222 IU/kg vitamin E. Postpartum cows received a vitamin and mineral mix that contained 13.7% Ca, 4.42% Mg, 0.32% K, 12.15% Na, 3.49% Cl, 4.52% S, 23.09 ppm Co, 361.14 ppm of Cu, 41.75 ppm of I, 2317.55 ppm of Mn, 11.76 ppm of Se, 2656.26 ppm of Zn, 316.5 KIU/kg of vitamin A, 77 KIU/kg of vitamin D, and 1466 IU/kg of vitamin E.

⁴Alimet contains 88% D,L 2-hydroxy-4-methylthiobutanoic acid (HMB) and was provided by Novus International, Inc., St. Louis, MO.

Table 2. Nutrient composition of diets fed to cows starting 3 wk postpartum through 12 wk postpartum (DM basis).

Nutrient	Prepartum			Postpartum		
	Basal	+HMB	++HMB	Basal	+HMB	++HMB
CP, %	14.0	14.0	14.0	16.8	16.6	16.5
ADICP, %	0.9	0.9	0.9	1.1	1.1	0.9
Soluble CP, % CP	42.6	42.6	42.6	41.3	41.3	41.1
NDICP, %	3.2	3.3	3.2	3.6	3.2	3.1
ADF, %	27.9	28.0	27.9	22.3	22.1	21.9
NDF, %	42.8	42.8	42.9	32.7	32.5	32.6
Lignin, %	3.2	3.3	3.3	2.9	2.9	2.9
NFC, %	36.4	36.5	36.3	44.0	44.0	43.8
Crude fat, %	3.2	3.2	3.2	3.3	3.3	3.4
Ash, %	6.8	6.8	6.8	6.8	6.8	6.8
NE _L , Mcal/kg	1.53	1.53	1.53	1.69	1.69	1.69
Ca, %	0.82	0.82	0.81	0.91	0.95	0.91
P, %	0.34	0.34	0.33	0.30	0.30	0.29
Mg, %	0.45	0.45	0.45	0.34	0.34	0.34
K, %	1.26	1.26	1.25	1.22	1.22	1.22
Na, %	0.12	0.12	0.12	0.31	0.31	0.31
S, %	0.30	0.31	0.33	0.26	0.28	0.29
Fe, ppm	218.2	210.5	216.9	198.4	183.4	196.6
Zn, ppm	129.7	129.5	129.5	89.2	88.3	88.9
Cu, ppm	19.4	19.3	19.4	14.7	14.6	14.5
Mn, ppm	115.6	115.2	115.4	76.5	75.9	76.3
Mo, ppm	1.1	1.2	1.3	1.1	1.0	1.8
Met, % of MP ¹	2.01	2.34	2.70	1.86	2.36	2.63
Lys, % of MP ¹	7.36	7.33	7.31	7.22	7.19	7.17

¹Values predicted by CPM Dairy (version 1.0) ration evaluation software based on measured DMI and wet chemistry analysis of feeds.

used in this experiment was estimated to have a ruminal escape value of 40% when given as a single bolus to ruminants (Koenig et al., 2002).

Amounts of feed offered and refused were measured daily throughout the experiment, and weekly analyses of DM content of TMR were used to calculate DMI. Samples of concentrates, silages, and TMR were collected on a weekly basis, and grass and alfalfa hay were collected on a monthly basis and dried to static weight at 60°C. Samples were ground through a 2-mm screen in a Wiley mill, and monthly composites were prepared of the concentrates and silages. Monthly composites of silages, monthly samples of hays, and total experiment composites of concentrate ingredients were analyzed (DairyOne Laboratories, Ithaca, NY) using wet chemistry techniques for DM, OM, CP, soluble CP, ADF, NDF, ether extract, ADF CP, NDF CP, lignin, and minerals.

Cows were milked 3× per day at approximately 8-h intervals, and milk weights were recorded using in-line meters (DeLaval, Kansas City, MO). Milk was sampled from each milking during one 24-h period each week and composited. The composite samples were stored at 4°C with a preservative (bronopol tablet; D & F Control System, San Ramon, CA) until analyzed for fat, protein, and lactose using infrared analysis (AOAC 2000: method 972.160), and SCC by an optical fluorescent method (AOAC 2000: method 978.26) (Dairy One Cooperative Inc.). The calibration reference methods for the infrared milk analysis were as follows: fat by modified Mojonnier ether extraction (AOAC 2000: method 989.05), Kjeldahl true protein (AOAC 2000: method 991.22), and lactose by difference of oven drying total solids (AOAC 2000: method 990.20) minus the sum of modified Mojonnier ether extraction, Kjeldahl true protein (AOAC 2000: method 991.22), estimated nonprotein nitrogen (0.19% on a protein basis), and estimated ash $[(\text{true protein} \times 0.596) + 0.5379]$. Body weights of cows were measured weekly. Two individuals assigned BCS to cows weekly (1 = thin, 5 = fat; Wildman et al., 1982).

Blood samples were collected by venipuncture of the coccygeal vein/artery using heparinized Vacutainer tubes (Becton Dickinson, Franklin Lanes, NJ). Samples were obtained on 1 d during the 7 d before assignment to treatment, and then every other day from approximately 21 d prior to expected parturition to 60 d after parturition. Sampling time (approximately 0900 h) corresponded to the time after orts were removed, after lactating cows returned from the parlor, and prior to feeding. Blood samples were placed on ice immediately following collection. Plasma was harvested after centrifugation of the blood at $2060 \times g$ for 15 min at 5°C. Plasma was stored at -20°C until subsequent analysis for glucose by enzymatic analysis (glucose oxidase) us-

ing a commercial kit (kit 510-A; Sigma Chemical). Plasma concentrations of NEFA were analyzed by enzymatic analysis (NEFA-C; WAKO Pure Chemical Industries, Osaka, Japan) using modifications described by McCutcheon and Bauman (1986) and Sechen et al. (1990). Plasma concentrations of BHBA were quantified (BHBA dehydrogenase) using a commercial kit (kit 310-UV; Sigma Chemical). All spectrophotometric measurements were conducted using a Versa_{max} tunable microplate reader (Molecular Devices, Sunnyvale, CA). Inter-assay variation was maintained at <5%. Only samples obtained through 30 d of lactation were analyzed for metabolites. Postpartum plasma samples were analyzed for progesterone by radioimmunoassay (Elrod and Butler, 1993). The assay limit of detection was 0.2 ng/mL. Inter- and intra-assay coefficients of variation were 11.9 and 15.7%, respectively.

Liver samples were obtained by percutaneous trochar biopsy (Veenhuizen et al., 1991) from each cow on 1 d during the week prior to assignment to treatment, 1 d after calving, and again 21 d after calving. Total liver removed was approximately 3 g for each biopsy. Liver was blotted to remove excess blood and connective tissue. A portion of the liver was frozen in liquid nitrogen for subsequent analysis for content of TG and glycogen (described below). The remaining liver was transported to the laboratory in ice-cold PBS (0.2 mM; pH 7.4) and used immediately to determine hepatic capacities for fatty acid metabolism and gluconeogenesis using an in vitro metabolic incubation system (described below).

Liver Composition

The concentration of glycogen in liver was determined using a procedure that has been previously described (Lo et al., 1970). Liver TG content was determined using a modification of the procedure described by Rukkwamsuk et al. (1999a). Liver (between 35 and 50 mg) was weighed into disposable tubes, and 0.5 mL of 20% KOH (wt/vol) was added. Liver was allowed to digest overnight. Vortexing the tubes after 1 h improved consistency of digestion by ensuring complete submersion of the liver sample. The next day, samples were neutralized with 0.5 mL of a 10% H₂SO₄ (vol/vol), followed by addition of 0.5 mL of 5% CHAPS (3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonate; wt/vol) to emulsify lipids. For analysis of the glycerol in the sample, the Infinity Triglyceride Reagent (Sigma Chemical, St. Louis, MO) was used. Glycerol standards (Sigma #339-11) were diluted with water to obtain a standard curve from 0 to 500 mg/dL of triolein-equivalent glycerol. For the analysis, 96-well plates were used. Water was used as a blank because the

absorbance of the KOH, H₂SO₄, CHAPS mixture was not different from that of water. Prior to use, standards and samples were vortexed. To each well was added 2.5 μ L of water, standard, or sample solution (in triplicate). Then 250 μ L of the Infinity Triglyceride Reagent was added. Plates were mixed for 12 s and then incubated for 7 min at 37°C before being read at 660 nm in a microplate reader (Versamax; Molecular Devices Corp., Sunnyvale, CA). Triglyceride concentration was then calculated based on initial wet tissue weight and volume dilution.

Liver Incubations

Upon return to the laboratory (usually within 1 h of biopsy), liver was sliced using a Krumdieck Tissue Slicer (Alabama Research and Development, Munford, AL) filled with ice-cold PBS. Slices of tissue (54 ± 2 mg) were then weighed into flasks. For conversion of [1-¹⁴C] propionate to CO₂ and glucose, incubation procedures, previously described (Piepenbrink and Overton, 2003) were used, except that propionate incubations were terminated with 0.5 mL 25% TCA (wt/vol) instead of the H₂SO₄.

Conversion of [1-¹⁴C] palmitate to CO₂ was measured as previously described (Piepenbrink and Overton, 2003) except that these flasks were covered with rubber septa without a hanging well for the duration of the incubation period (120 min). At either 0 or 120 min, liver was removed from the medium using forceps to terminate the incubations. The liver was rinsed in 1 mL of 3% BSA in KRB (at 37°C, wt/vol), followed by a rinse in 1 mL of saline (0.9% NaCl). Rinses were added to the incubation flasks, and then a septa containing folded filter paper in the hanging well was placed on top. At this time, 1 mL of a 40% perchloric acid (wt/vol) was injected into the media and 0.1 mL of 30% NaOH (wt/vol) was injected onto the filter paper to collect CO₂. The flasks were placed in an ice bath for 1 h to ensure complete evolution of CO₂ and to limit any further metabolism.

Determination of stored esterified products.

After liver slices were rinsed, they were transferred into 16- $\times$ 125-mm glass extraction tubes that contained 5 mL of a 3:2 mixture (wt/vol) hexane:isopropanol (Hara and Radin, 1978). Tubes were then capped and placed on a wrist-action shaker overnight. The next day, tubes were centrifuged at $1800 \times g$ for 5 min. The solvent solution was poured into disposable tubes, and another 2 mL of the hexane:isopropanol mixture was added to the tubes containing the liver tissue. Tubes were capped and vortexed for 30 s. The initial 5 mL of solvent was returned to the extraction tube, and 2 mL of a 6.7% NaSO₄ (wt/vol) solution was added to cause phase separation,

as well as removal of nonlipid components from the hexane layer. The tubes were vortexed and then centrifuged at $1800 \times g$ for 5 min. The hexane layer was removed to scintillation vials and placed in an evaporator at 37°C for a minimum of 4 h. After evaporation, 10 mL of scintillation cocktail (Scintisafe Econo 2; Fisher Scientific, Pittsburgh, PA) was added to each vial, and radioactivity was measured using liquid scintillation spectroscopy (model 2200 CA Scintillation Counter, Packard Instruments Company).

Determination of conversion of [1-¹⁴C] propionate to glucose. Flask contents were neutralized after CO₂ collection with a saturated NaOH solution by dropwise addition and the supernatant frozen at -20°C until isolation of glucose. To isolate glucose, the modified batch procedure of Azain et al. (1999) was used. Because of the added cation/anion load on the resins from using TCA and NaOH instead of the H₂SO₄ and Ba(OH)₂ solutions, it was found that the amount of resin added needed to be increased to give a more consistent recovery of glucose. For this, 2.2 mL of 50% (wt/vol) slurry of the anion exchange resin (AG 1-X8 resin, acetate form; Bio-Rad, Hercules, CA) in water was added in the first step. Tubes were capped and vortexed vigorously for 30 s. This was repeated 2 more times, allowing resin to settle momentarily between mixing bouts. Afterward, 2.0 mL of the cation exchange resin (AG 50W-8 resin, hydrogen form; Bio-Rad) was added. Again, the tubes were capped and vortexed as above. Tubes were then centrifuged at $1390 \times g$ for 15 min. The resins then formed a semi-solid pellet that allowed the aqueous layer to be poured into scintillation vials. The vials were then placed into a 37°C water bath, and a steady stream of air was used to evaporate the water. The next day, 1 mL of water was added to reconstitute the glucose. Ten milliliters of scintillation cocktail (Scintisafe Econo 2) was added to each vial, and radioactivity was measured using dual label liquid scintillation spectroscopy (model 2200 CA, Packard Instrument Company). By counting an equal volume of the internal standard ([³H]L-1-glucose) that was added to each flask prior to neutralization, correction of aqueous volume trapped in the resin and losses during processing of the flasks following incubations could be determined. Recovery of the internal standard averaged $38 \pm 10\%$.

Statistical Analysis

Two cows fed ++HMB developed mastitis due to *Staphylococcus aureus* infection and were removed from the study at approximately 3 wk postpartum. Their remaining data were treated as missing values. Before statistical analysis, daily measurements (DMI, milk production) were reduced to weekly means. To

estimate energy balance, the NRC (2001) equations were used. For in vitro incubations, triplicate values for blanks and live flasks were averaged, and blanks subtracted from values obtained from live flasks. Rates of substrate conversion were calculated based on the known amount of radioactivity added to each flask that was converted to end products over the 2-h incubation period.

Prepartum and postpartum data were analyzed separately because treatments were equally spaced during the prepartum period and unequally spaced during the postpartum period. Performance and blood metabolite data were subjected to repeated measures ANOVA using the MIXED procedure of SAS (SAS Users Guide, 2001). Pretreatment values for all measurements, except milk yield and composition, were utilized during analysis of covariance. In the case of energy balance, pretreatment BW was used in analysis of covariance. Week or day was used in the repeated statement with cow within treatment as the error term and the autoregressive covariate structure (Littell et al., 1998). Degrees of freedom were estimated using the Satterthwaite specification. Single degree of freedom orthogonal contrasts were used to test the linear and quadratic effects of HMB supply; the models used to analyze postpartum data employed adjusted coefficients due to the uneven spacing of treatments. Liver data collected on d 1 and 21 postpartum were subjected to ANOVA using the MIXED procedure of SAS (SAS User's Guide, 2001); the orthogonal contrasts used to analyze the d 1 liver data were based on equally spaced treatments, and those used to analyze the d 21 liver data were based on unequally spaced treatments, as data collected on d 1 postpartum are reflective of treatments applied during the prepartum period.

For determining reproductive status as affected by treatment, concentrations of plasma progesterone were used to determine days to first ovulation. Ovulation was assumed to occur when progesterone values increased by more than 2 ng for more than 2 consecutive samples. For cows that did not have an increase in progesterone before blood sampling ended, a value of 60 d was used. The data for days to first ovulation were subjected to ANOVA using the MIXED procedure of SAS (SAS Users Guide, 2001). Least squares means are reported with the highest standard error for each variable. Significance was declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.15$. Only significant treatment $\times$ time interactions will be discussed.

RESULTS AND DISCUSSION

Nutrient composition of the pre- and postpartum diets fed in this study is presented in Table 2, and the

nutrient composition of individual feeds and protein mixes is presented in Tables 3 and 4, respectively. Both prepartum and postpartum diets were slightly lower than expected in CP and net energy content. Part of this discrepancy was due to variability in the content of CP and energy in forages over the course of the study. Blood meal and protected soybean meal were included in the diets to maintain a high level of Lys in the diets. As predicted by CPM Dairy, Lys accounted for more than 7.15% of metabolizable protein supply for all 6 diets.

Differences in prepartum or postpartum DMI among cows fed the three levels of HMB were not significant ($P > 0.15$; Table 5). Rode et al. (1998) reported an increase in DMI the week before parturition when cows were fed approximately 20 g/d HMB; however, postpartum differences in DMI of cows fed either 0 or 50 g/d of HMB during the postpartum period were not significant. Decreased DMI during both the prepartum and postpartum periods was observed in another study when approximately 20 g/d of HMB was fed to cows prior to parturition and 50 g/d of HMB was fed during the postpartum period (Johnson et al., 1999). Cows fed ++HMB in our experiment would have consumed, on average, 23 g/d of HMB during the prepartum period and 40 g/d of HMB during the postpartum period. Reasons for differences in DMI response to HMB supplementation are not clear; however, HMB was supplied from the same source in our experiment and that of Rode et al. (1998), whereas the HMB fed in Johnson et al. (1999) was supplied from a different source. Furthermore, transient reduction in DMI was reported when HMB was offered in topdress form but not when HMB was mixed into the TMR (Higginbotham et al., 1987). One other potential factor affecting response to HMB is Met adequacy of the basal diet, which was not reported in the abstracted data.

Feeding HMB led to a quadratic effect ($P < 0.03$) on milk yield such that cows consuming the +HMB diets produced approximately 3 kg more milk per day during the first 84 DIM (Table 5 and Figure 1). These cows also had significantly ($P < 0.05$) increased yields of lactose and 3.5% FCM and tended ($P < 0.09$) to yield greater amounts of total solids in milk. Cows fed the three dietary treatments had similar ($P > 0.15$) concentrations of fat, true protein, and lactose, and yields of fat and true protein.

Increased yields of milk and milk fat have been observed consistently when feeding Met hydroxy analog (Loerch and Oke, 1989). This effect can also occur when cows are fed rumen-protected Met beginning prior to calving and continuing through early lactation (Overton et al., 1996). In a study in which 1-[^{14}C]palmitate was infused intravenously to measure NEFA me-

Table 3. Nutrient composition of forages, corn grain, soybean meal, and soybean hulls (DM basis).

	Corn silage	Grass silage	Alfalfa silage	Grass hay	Corn grain	Soybean meal	Soybean hulls
CP, %	8.2	14.0	22.3	7.7	9.0	52.8	11.4
ADICP, %	0.8	1.3	1.5	0.9	0.6	1.1	1.3
Soluble CP, % of CP	58	63	68	22	22	25	27
NDICP, %	2.2	2.9	3.1	2.4	1.3	5.6	4.4
ADF, %	26.2	39.0	33.8	43.0	5.5	6.2	49.0
NDF, %	41.5	58.7	41.9	66.8	11.1	9.2	66.4
Lignin, %	2.6	5.9	7.6	6.9	0.7	1.3	1.1
NFC, %	46.2	19.0	26.4	19.7	75.6	35.6	20.0
Crude fat, %	3.2	3.8	3.7	1.8	4.2	1.3	1.6
Ash, %	3.1	7.4	8.8	6.4	1.4	6.7	5.0
NE _L , Mcal/kg	1.68	1.22	1.41	0.97	2.09	1.90	1.54
Ca, %	0.25	1.18	1.54	0.49	0.05	0.41	0.65
P, %	0.24	0.26	0.35	0.17	0.27	0.76	0.12
Mg, %	0.25	0.33	0.37	0.16	0.11	0.32	0.24
K, %	0.98	1.8	2.31	1.45	0.37	2.4	1.51
Na, %	0.004	0.03	0.036	0.013	0.004	0.007	0.007
S, %	0.09	0.17	0.24	0.09	0.09	0.39	0.11
Fe, ppm	102	78	166	241	35	127	456
Zn, ppm	23	22	25	17	20	47	39
Cu, ppm	5	7	11	8	2	14	7
Mn, ppm	18	33	36	57	8	35	20
Mo, ppm	0.87	1.2	1.6	1.2	0.44	3.8	1.2

Table 4. Feed and nutrient composition of protein mixes used in the diets (% of DM).

Item	Prepartum mixes			Postpartum mixes		
	Basal	+HMB	++HMB	Basal	+HMB	++HMB
Feed						
SoyPlus ¹	66.7	65.8	64.8	70.6	69.7	69.1
Blood meal ²	22.2	21.9	21.6	19.6	19.3	19.2
Ener GH ³	11.1	11.0	10.8	9.8	9.7	9.6
Alimet ⁴	—	1.3	2.7	—	1.3	2.1
Nutrient						
CP	51.4	50.7	51.5	53.9	52.1	50.9
ADICP	0.8	0.7	0.9	2.5	2.5	1.0
Soluble CP	7	6	6	8	8	6
NDICP	11.5	12.5	12.4	13.5	9.9	9.1
ADF	7.2	8.9	7.5	10.7	8.4	6.4
NDF	13.9	13.1	15.0	14.8	13.6	14.1
Lignin	0.5	0.8	1.2	1.5	1.4	1.4
NFC	34.6	36.7	33.8	33.4	31.8	31.7
Crude fat	4.8	5.2	5.6	4.8	5.4	5.7
Ash	6.8	6.8	6.5	6.6	7.0	6.7
TDN	87	89	88	87	86	87
NE _L , Mcal/kg	2.07	2.12	2.09	2.07	2.05	2.07
NE _M , Mcal/kg	2.20	2.25	2.23	2.18	2.16	2.18
Ca	1.31	1.32	1.14	1.22	1.55	1.18
P	0.55	0.55	0.53	0.55	0.57	0.54
Mg	0.24	0.23	0.23	0.23	0.22	0.23
K	1.61	1.54	1.49	1.55	1.56	1.55
Na	0.099	0.094	0.107	0.089	0.093	0.083
S %	0.32	0.54	0.81	0.39	0.6	0.7
Fe, ppm	737	633	720	666	522	649
Zn, ppm	44	41	42	42	33	39
Cu, ppm	9	8	9	9	8	7
Mn, ppm	30	25	28	26	20	24
Mo, ppm	1.6	3.4	4.6	2.3	1.2	8.2

¹Expeller soybean meal (West Central Soy, Ralston, IA).²Ring-dried of porcine origin (Papillon Agricultural Products, Easton, MD).³Ca salts of palm fatty acid distillate (Bioproducts Inc., Fairlawn, OH).⁴88% D,L-2-hydroxy-4-methylthiobutanoic acid (Novus International, Inc., St. Louis, MO).

Table 5. Dry matter intake, milk yield, and milk composition of cows consuming three levels of 2-hydroxy-4-methylthiobutanoic acid (HMB) from 21 d prepartum through 84 d postpartum.

Item	Treatment			SEM	Effect, <i>P</i> <	
	Basal	+HMB	++HMB		Linear	Quadratic
Prepartum DMI, kg/d	12.9	12.8	12.7	0.4	0.76	0.96
Postpartum DMI, kg/d	18.6	19.8	19.8	0.6	0.15	0.65
Milk, kg/d	42.0	45.0	41.9	1.1	0.78	0.03
Fat, %	4.20	4.00	4.08	0.09	0.27	0.25
Fat, kg/d	1.72	1.76	1.69	0.04	0.72	0.19
3.5% FCM ¹ , kg/d	46.0	48.0	45.6	0.9	0.96	0.05
True protein, %	2.80	2.77	2.88	0.08	0.52	0.41
True protein, kg/d	1.16	1.22	1.19	0.04	0.52	0.43
Lactose, %	4.70	4.69	4.75	0.04	0.40	0.40
Lactose, kg/d	1.97	2.11	1.99	0.05	0.56	0.04
Total solids, %	12.46	12.22	12.45	0.14	0.85	0.16
Total solids, kg/d	5.17	5.44	5.19	0.12	0.72	0.09

¹3.5% FCM = Fat-corrected milk = 0.4324*(kg milk) + 16.2162*(kg fat).

tabolism, Met hydroxy analog was shown to increase the amount of ¹⁴C secreted in milk (Pullen et al., 1989) indicating greater use of NEFA for milk fat when HMB was fed. Polan et al. (1970) reported that the optimal dose of Met hydroxy analog was approximately 25 g/d. Markedly decreased yields of milk and FCM were reported when Met hydroxy analog was fed in excess of 45 g/d; however, based on the composition of the basal diet fed in their experiment (limited inclusion of protein sources known to be high in Lys), we would speculate that Lys probably was limiting. Rode et al. (1998) also measured increased yields of milk and FCM, and increased milk fat content, in response to supplying Met as HMB. Although milk protein content was not affected by feeding HMB in their experiment, the yield of milk protein was increased. Cows consuming HMB in the study of Johnson et al. (1999) tended to have

lower yields of milk protein; however, differences among treatments for milk yield and milk protein content were not significant.

Energy balance was not significantly different among cows fed the 3 treatments during either the prepartum or postpartum periods (*P* > 0.15; Figure 2). The lack of differences among treatments probably resulted from the slight, nonsignificant increase in feed intake (Table 5) as cows consumed greater amounts of HMB and small, nonsignificant, differences in milk composition (Table 5), even though cows consuming +HMB had greater milk production than cows consuming ++HMB or the basal diet.

Body weights (Figure 3; Table 6) and BCS (Figure 4; Table 6) were not different (*P* > 0.15) during both the

Figure 1. Milk yield of cows consuming 3 levels of 2-hydroxy-4-methylthiobutanoic acid (HMB) from 21 d prepartum through 84 d postpartum. There was a significant quadratic effect (*P* = 0.05, SEM = 1.00) and a tendency for a treatment by time effect (*P* < 0.15). **Figure 2.** Calculated energy balance of cows consuming three levels of 2-hydroxy-4-methylthiobutanoic acid (HMB) from 21 d prepartum through 84 d postpartum. Body weight measured prior to treatment assignment was used in analysis of covariance. There were no differences in energy balance between cows on the three treatments (*P* > 0.15, SEM = 1.27).

Figure 3. Body weights of cows consuming 3 levels of 2-hydroxy-4-methylthiobutanoic acid (HMB) from 21 d prepartum through 84 d postpartum. Weights obtained before assignment to treatment at approximately 28 d before the expected calving date of each cow were used during analysis of covariance. Effects of treatment and the interaction of treatment and time were not significant ($P > 0.15$; SEM = 7.8).

Figure 4. Body condition scores of cows consuming three levels of 2-hydroxy-4-methylthiobutanoic acid (HMB) from 21 d prepartum through 84 d postpartum. Body condition scores obtained before assignment to treatment at approximately 28 d before the expected calving date of each cow were used during analysis of covariance. Effects of treatment and the interaction of treatment and time were not significant ($P > 0.15$; SEM = 0.08).

prepartum and postpartum periods among cows assigned to the 3 dietary treatments. Cows lost approximately 150 kg of BW and more than one BCS unit by wk 5 to 6 postpartum. Subsequent changes in BW and BCS were minimal. Cows consuming HMB in the study of Rode et al. (1998) lost more body condition during lactation. Johnson et al. (1999) reported no difference in BCS after the first week of lactation.

Concentrations of plasma NEFA, BHBA, and glucose were not affected by treatment ($P > 0.15$) during the prepartum period; however, concentrations of NEFA tended ($P < 0.12$) to be decreased quadratically during the postpartum period (Figure 5; Table 7). Concentrations of glucose in plasma tended ($P < 0.06$) to be affected quadratically by treatment such that cows fed +HMB had slightly increased concentrations of glucose in plasma compared with the other 2 treatments (Table 8). Concentrations of plasma BHBA were not affected

by treatment ($P > 0.15$) during either the prepartum or postpartum period (Table 7), implying that HMB did not affect partial oxidation of fatty acyl-CoA in hepatic mitochondria under the conditions of this experiment.

Concentrations of NEFA were not different among cows in one study (Socha, 1994) but were increased in another (Carson et al., 1998) when rumen-protected Met and Lys were fed. In a study using pregnant, non-lactating cows restricted to 25% of ad libitum intake to induce fatty liver, prefeeding plasma NEFA concentrations were increased when cows consumed HMB (Bertics and Grummer, 1999). The reasons for differences in responses to Met or its analog are not clear. Although circulating concentrations of NEFA generally are predictive of irreversible loss (Pullen et al., 1989), differences in NEFA concentration can be brought about by changes in release from adipose tissue, changes in utilization by the mammary gland or other tissues, or both.

Table 6. Body weights and BCS of cows consuming 3 levels of 2-hydroxy-4-methylthiobutanoic acid (HMB) from 21 d prepartum through 84 d postpartum.

Item	Treatment			SEM	Effect, $P <$	
	Basal	+HMB	++HMB		Linear	Quadratic
BW ¹ , kg						
Prepartum ¹	753	758	747	5	0.44	0.16
Postpartum ²	620	610	614	9	0.55	0.57
BCS ²						
Prepartum ¹	3.74	3.71	3.67	0.04	0.28	0.85
Postpartum ²	2.88	2.75	2.76	0.08	0.29	0.59

¹BW were measured weekly.

²BCS was assigned weekly using a five-point system where 1 = thin and 5 = fat (Wildman et al., 1982).

Figure 5. Plasma concentrations of nonesterified fatty acids (NEFA) from cows consuming three levels of 2-hydroxy-4-methylthio-butanoic acid (HMB) from 21 d prepartum through 84 d postpartum. Plasma samples obtained before assignment to treatment were used during analysis of covariance. The interaction of treatment and time was not significant ($P > 0.15$; SEM = 78.1).

Concentrations of plasma acetone indicated that rumen-protected Met seemed to decrease ketonemia in one study (Bauchart et al., 1998). This treatment, however, did not reduce liver TG content. In contrast to our results, prefeeding plasma glucose also was reduced by feeding HMB in the feed restriction study of Bertics and Grummer (1999).

Concentrations of TG in liver were not affected ($P > 0.15$) by treatment on d 1 postpartum; however, cows fed +HMB tended ($P < 0.15$) to have increased concentrations of liver TG on d 21 postpartum (Table 8). Concentrations of glycogen tended ($P < 0.08$) to be affected quadratically on d 1 postpartum, such that cows fed +HMB had the lowest concentration of liver glycogen (Table 8). Concentrations of liver glycogen and 21 d

postpartum tended ($P < 0.10$) to be decreased linearly by feeding increasing amounts of HMB (Table 8).

Bertics and Grummer (1999) concluded that there were no effects on liver TG of feeding Met hydroxy analog to nonlactating cows that were induced to develop fatty liver by feed restriction. The analog had no effect on the amount of TG in the liver or the amount of TG that was depleted from the liver 3 and 6 d after termination of feed restriction. Liver lipid content at 1 wk postpartum was not different due to feeding rumen-protected Met and Lys in one study (Socha, 1994), although other researchers (Bauchart et al., 1998) reported that the content of liver TG decreased only when both rumen-protected Met and Lys were fed to cows and not when rumen-protected Met was fed alone.

The capacity of liver slices to convert [1- 14 C]palmitate to CO_2 was not different ($P > 0.15$) among cows fed the 3 dietary treatments on either d 1 or 21 postpartum (Table 9). Likewise, the feeding of HMB did not affect the capacity of liver slices to incorporate [1- 14 C]palmitate into esterified products stored intracellularly on d 1 postpartum (Table 9). On d 21 postpartum, the capacity of liver slices to incorporate [1- 14 C]palmitate into esterified products stored intracellularly was affected quadratically ($P < 0.05$) by treatment, such that cows fed ++HMB had the greatest capacity to convert [1- 14 C]palmitate to stored esterified products. Because conversion to CO_2 was not affected by treatment, we infer that in vivo rate of oxidation of NEFA by liver probably was not affected by dietary supply of HMB. In vitro capacity for [1- 14 C]palmitate esterification and storage was modestly affected by HMB supplementation but only on d 21 postpartum. In the study of Pullen et al. (1989), Met hydroxy analog had no effect on the incorporation of NEFA into plasma TG, indicating that the analog probably did not alter hepatic lipoprotein synthesis. Given that neither liver TG nor in vitro capacities for metabolism of [1- 14 C]palmitate by liver

Table 7. Concentrations of plasma metabolites from cows consuming three levels of 2-hydroxy-4-methylthio-butanoic acid (HMB) from 21 d prepartum through 84 d postpartum.

Item	Treatment			SEM	Effect, $P <$	
	Basal	+HMB	++HMB		Linear	Quadratic
NEFA, $\mu\text{Eq/L}$						
Prepartum ¹	247	283	241	31	0.91	0.32
Postpartum ²	676	685	593	32	0.11	0.12
BHBA, mg/dL						
Prepartum ¹	5.90	6.62	5.82	0.68	0.93	0.36
Postpartum ²	19.61	15.92	16.34	2.01	0.21	0.53
Glucose, mg/dL						
Prepartum ¹	46.1	44.5	43.6	1.6	0.26	0.87
Postpartum ¹	37.2	38.6	35.4	1.2	0.34	0.06

¹Mean of samples collected every other day from d 20 before expected parturition until parturition.

²Mean of samples collected every other day from parturition until d 30 postpartum.

Table 8. Concentrations of triglyceride (TG) and glycogen in liver from cows consuming three levels of 2-hydroxy-4-methylthiobutanoic acid (HMB) from 21 d prepartum through 84 d postpartum.

Item	Treatment			SEM	Effect, <i>P</i> <	
	Basal	+HMB	++HMB		Linear	Quadratic
TG, % of wet weight						
d 1	7.17	6.69	6.74	0.85	0.71	0.80
d 21	8.42	13.62	11.07	1.91	0.23	0.15
Glycogen, % of wet weight						
d 1	0.86	0.37	0.64	0.18	0.40	0.08
d 21	1.50	1.09	0.81	0.29	0.10	0.92

slices on d 1 were affected by Met supplied as HMB, and effects on metabolism of [1-¹⁴C]palmitate by liver slices on d 21 postpartum were modest at best, these data suggest collectively that hepatic metabolism of fatty acids is not sensitive to Met supplied as HMB.

The capacity of liver slices to convert [1-¹⁴C]propionate to CO₂ tended (*P* < 0.12) to be increased linearly on d 1 postpartum; however, a similar pattern for conversion of [1-¹⁴C]propionate to glucose on d 1 postpartum was not significant (*P* > 0.15; Table 9). On d 21 postpartum, the capacity of liver slices to convert [1-¹⁴C]propionate to CO₂ and glucose decreased linearly (*P* < 0.03) as cows were fed increasing amounts of HMB. Because the ratio of radiolabeled endproducts (glucose:CO₂) was not affected by treatment (*P* > 0.15; Table 9), these data reflect increased capacity on d 1 and decreased capacity on d 21 for metabolism of propionate rather than a repartitioning of propionate carbon among pathways of oxidation and gluconeogenesis. The pattern on d 21 was similar to the trend (*P* < 0.10) for

linearly decreased concentrations of glycogen in liver as the amount of HMB fed to cows increased. Furthermore, liver slices from cows fed ++HMB that had the greatest capacity to store [1-¹⁴C]palmitate as esterified products had the lowest capacity to convert [1-¹⁴C]propionate to CO₂ and glucose. Although cows fed +HMB had the greatest concentration of TG on d 21 postpartum, cows fed ++HMB also had modestly elevated concentrations of TG compared with control cows (Table 8). Cadorniga-Valino et al. (1997) reported that isolated hepatocytes infiltrated with lipid had decreased capacity to convert [2-¹⁴C]propionate to glucose. Cows fed the basal treatment in our study had comparable liver TG concentrations and in vitro capacity for conversion of [1-¹⁴C]propionate to glucose to cows fed any of the three treatments on d 1 postpartum; cows fed +HMB and ++HMB had modestly elevated liver TG concentrations and decreased in vitro capacity for conversion of [1-¹⁴C]propionate to glucose. Collectively, these data suggest that a threshold of liver TG concentration exists between

Table 9. Capacities for metabolism of [1-¹⁴C]palmitate (2.0 mM) and [1-¹⁴C]propionate (10 mM) by liver slices from cows consuming three levels of 2-hydroxy-4-methylthiobutanoic acid (HMB) from 21 d prepartum through 84 d postpartum.

Item	Treatment			SEM	Effect, <i>P</i> <	
	Basal	+HMB	++HMB		Linear	Quadratic
Conversion of [1- ¹⁴ C]palmitate, nmol/(g wet wt × h)						
CO ₂						
d 1	28.0	25.3	26.7	3.2	0.74	0.53
d 21	24.7	20.5	22.3	1.9	0.28	0.28
Intracellularly stored esterified products						
d 1	354	362	379	20	0.20	0.78
d 21	311	297	339	10	0.20	0.05
Conversion of [1- ¹⁴ C]propionate, μmol/(g wet wt × h)						
CO ₂						
d 1	6.33	6.60	7.17	0.37	0.12	0.74
d 21	5.39	4.46	3.85	0.46	0.02	0.89
Glucose						
d 1	3.63	3.58	4.41	0.44	0.17	0.40
d 21	3.61	2.81	2.23	0.50	0.03	0.86
Ratio of glucose to CO ₂						
d 1	0.58	0.55	0.58	0.05	0.88	0.61
d 21	0.68	0.64	0.65	0.13	0.81	0.86

Table 10. Recorded incidence of health disorders by treatment.

Disorder	Basal		+HMB		++HMB	
	# Cows	# Episodes	# Cows	# Episodes	# Cows	# Episodes
Twins	0	0	2	2	0	0
Retained placenta/ metritis	1	1	0	0	1	1
Displaced abomasum	3	3	2	2	1	1
Mastitis	6	7	2	4	5	9

approximately 8 and 11% liver TG at which impairment of gluconeogenic capacity from propionate begins to occur.

Despite the apparent decrease in hepatic capacity for propionate metabolism as the amount of HMB fed to cows increased, cow performance relative to the controls was not impaired (Table 5), and cows fed +HMB that produced the most milk and most lactose also had the greatest concentrations of plasma glucose during the postpartum period (Table 7). In this study, we measured maximal capacity for gluconeogenesis from supra-physiological concentrations of propionate rather than total glucose production by liver. Danfær et al. (1995) demonstrated that infusing ruminants with either propionate or AA could change the substrate utilized for gluconeogenesis without altering total hepatic glucose production. Even if feeding HMB directly or indirectly altered the capacity of liver to metabolize propionate, it is likely that overall capacity of liver to produce glucose from all glucogenic substrates was not affected, at least for cows fed +HMB.

There was a quadratic effect ($P < 0.04$) for days to first ovulation as determined by concentrations of progesterone in plasma. Average days to first ovulation were 43 for cows consuming +HMB, whereas days to first ovulation were 28 and 34 for the basal diet and ++HMB, respectively (SEM = 4). Rukkwamsuk et al. (1999b) observed a correlation between concentrations of liver TG at 2 wk after calving with days to first ovulation. In this study, the longest number of days to first ovulation occurred in cows that were consuming +HMB and also produced the most milk. These cows also had higher concentrations of liver TG at 21 d postpartum. Table 10 was compiled using reported disorders recorded in individual cow health records. Cases of each disorder appear to be relatively evenly distributed across the 3 treatments.

Recent research has indicated that HMB, when dosed to cows (Koenig et al., 2002) or added to the feed at 2 of 4 feedings used in fermenters in continuous culture (Vázquez-Añón et al. 2001), has a significant ruminal escape value. The degree to which HMB escapes rumi-

nal degradation seems to be related to liquid dilution rate (Vázquez-Añón et al., 2001). This, coupled with the findings of Wester et al. (2000), indicates that HMB is quickly absorbed along the digestive tract and converted to Met by most tissues and, therefore, should be a good source of Met. For cows in established lactation, typical responses to increasing the supply of Met when Lys is adequate are some combination of increased milk protein content, increased milk protein yield, or both (NRC, 2001). However, the positive milk protein response to additional Met is ameliorated when inadequate Lys is supplied to the small intestine (Rulquin et al., 1993; NRC, 2001). Collectively, experiments in which supplies of Met were varied beginning prior to parturition and continuing through early lactation suggest that responses to additional Met are not necessarily manifested through milk protein during this time frame. In experiments in which Met supply alone was altered, increased Met supply resulted in increased yields of milk (present study; Rode et al., 1998) and FCM (Overton et al., 1996; Rode et al., 1998). Supplying additional Met only in other experiments (Socha et al., 1994; Johnson et al., 1999) did not increase yields of milk and milk components. In another experiment in which supplementation of Met was initiated at 22 d postpartum, the response of FCM to additional Met was either positive or negative and dependent on the basal diet (Overton et al., 1998).

Supplementation of both Lys and Met to dairy cows, beginning prior to parturition and continuing through early lactation, resulted in increased energy-corrected milk yield (Socha et al., 1994) and milk protein yield (Carson et al., 1998; Socha et al., 1994); however, effects on both milk protein yield and milk fat yield to Lys and Met were variable and dependent on AA status of the prepartum diet (Carson et al., 1998). Clearly, research is warranted to investigate further these interactions of periparturient diet and supply of Met and Lys on milk yield and milk component synthesis.

We hypothesized that Met supplied as HMB would improve hepatic capacity to metabolize NEFA such that the rate of accumulation of NEFA as liver TG would

decrease and production responses potentially would follow. We optimized milk yield in this experiment when Met was supplied as HMB at approximately 2.3% of metabolizable protein supply during the periparturient period and early lactation when Lys was considered to be adequate, which concurs substantially with published recommendations for cows during established lactation (NRC, 2001). However, given the substantial lack of effect of Met as HMB on hepatic fatty acid metabolism, the mechanism for the increased milk yield does not appear to be related to improved energy metabolism in liver.

In addition to supplying postruminal Met, numerous effects of HMB on ruminal fermentation have been reported, ranging from increased gas production (Robert et al., 1998), CP digestion, nonammonia N flow, microbial protein efficiency (Sloan et al., 2000), bacterial protein synthesis (Vázquez-Añón et al., 2001), decreased ruminal free fatty acids and increased polar lipids (Patton et al., 1970), increased acetate/propionate ratios (Ellis et al., 1986), and increased fiber, protein, and ether extract digestibility (Polan et al., 1970). Although the mechanisms by which HMB stimulates ruminal fermentation are not well defined, effects appear to be dependent on dose of HMB. Using in vitro systems, when HMB was supplied at high doses (0.45, 0.22, and 1.45% of DM), ruminal effects were nullified (Robert et al., 1998; Sloan et al., 2000; Vázquez-Añón et al., 2001). The dual effect of HMB on stimulating ruminal fermentation and supplying postruminal Met might explain the milk yield response measured in this experiment. The increased milk yield by cows consuming +HMB might be caused by the combination of adequate supply of Met derived from HMB to the cow and the direct effect of HMB on ruminal fermentation. In cows consuming ++HMB (0.20% of DM), the effect of HMB on ruminal fermentation may have been negated. Alternatively, any benefits of increased postruminal Met derived from HMB may have been limited by postruminal Lys supply (Rulquin et al., 1993; NRC, 2001). However, in this study we did not collect data that would have allowed us to measure the postruminal supplies of Met and Lys or to find that ruminal fermentation was indeed altered by treatment.

CONCLUSIONS

Milk yield increased in a quadratic fashion in response to supplementation with HMB during the periparturient period. However, the lack of meaningful effects of HMB supply on metabolic parameters and long-chain fatty acid metabolism indicate that the mechanism was not related to alterations in hepatic long-chain fatty acid metabolism in periparturient cows as

hypothesized. Future research should seek to determine the mechanism underpinning the increase in milk yield when moderate amounts of HMB are included in the diet.

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