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Effects of 2-Hydroxy-4-(Methylthio) Butanoic Acid (HMB) and Its Isopropyl Ester on Milk Production and Composition by Holstein Cows*

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ABSTRACT

The esterification of 2-hydroxy-4-(methylthio)-butanoic acid (HMB) to isopropanol (HMBi) decreases the rate and extent of its ruminal breakdown. The modes of action of HMB and HMBi appear to be different. The quantification of the production response to HMBi has not been done. The objectives of this study were (1) to determine the lactation response to HMB, (2) to determine the lactation response to HMBi, and (3) to evaluate whether the response to HMBi is affected by HMB in the diet. Sixty-one Holstein cows (24 primiparous, 37 multiparous) were assigned to 1 of 4 dietary treatments 21 to 28 d after calving. The base diet consisted of [on a dry matter (DM) basis] 32.5% corn silage, 17.5% alfalfa hay, 10% whole cottonseed, and 40% of a pelleted concentrate made primarily of ground corn, soybean meal, and blood meal, and was fed for 16 wk as a control diet. To prepare the dietary treatments, the base diet was supplemented with 0.1% of diet DM with HMB (treatment 2), with 0.15% HMBi (treatment 3), or with 0.045% HMB and 0.15% HMBi (treatment 4). Results showed a significant increase in milk yield (2.9 kg/d), protein content (0.15%), protein yield (115 g/d), fat yield (165 g/d), and lactose yield (182 g/d) from HMBi. Supplementation of HMB had small and nonsignificant effects on milk yield and composition. There were no significant interaction effects of HMB with HMBi on any of the production traits measured in this experiment. Plasma free Met as a proportion of essential amino acids was increased by HMBi, but not by HMB. Dietary supplementation of HMBi increased gross N efficiency expressed as the proportion of ingested N secreted in milk. Consequently, HMBi significantly improved N efficiency.

(**Key words:** 2-hydroxy-4-(methylthio)-butanoic acid, methionine hydroxy analog, milk yield, milk composition)

Abbreviation key: HMB = 2-hydroxy-4-(methylthio)-butanoic acid, **HMBi** = isopropyl ester of HMB, **MP** = metabolizable protein, **PUN** = plasma urea nitrogen, **WOE** = week of experiment.

INTRODUCTION

Methionine and lysine have been reported to be limiting AA for milk yield and protein production (Schwab et al., 1992). Supplementation of Met or Met and Lys postruminally has had positive effects on milk yield, and milk protein concentration (Varvikko et al., 1999; Noftsger and St-Pierre, 2003).

2-Hydroxy-4-(methylthio)-butanoic acid (**HMB**) is a common source of supplemental Met (Schwab et al., 2001) that varies in estimated rumen degradability from 99% (Jones et al., 1988) to 21 to 50% (Koenig et al., 1999; Vázquez-Anon et al., 2001). The most consistent response to feeding HMB has been an increase in milk fat percentage (Huber et al., 1984; Lundquist et al., 1985), although some researchers have reported no effect (Stokes et al., 1981). Milk yield response to HMB has been less consistent (Polan et al., 1970). Most research found no effect of HMB on milk protein concentration (Stokes et al., 1981; Hansen et al., 1991). Most research done before 1988 used the Ca salt of HMB, which is not completely water-soluble. The liquid form of HMB currently in use is completely water-soluble. In this form, only 5% of ingested HMB flows out of the reticulorumen (Noftsger et al., 2005), suggesting a ruminal mode of action (Noftsger et al., 2003).

Recent research reported by Robert et al. (2001b) has shown that the esterification of HMB to various alcohols has profound effects on the apparent ruminal degradation of the HMB molecule. The isopropyl ester of HMB (**HMBi**) was shown to have 40 to 58% bioavailability based on blood kinetics of a pulse ruminal dose (Robert et al., 2001a, 2002), or based on a cow bioassay using milk true protein concentration as a bioavailability index (Schwab et al., 2001). The bioavailability of HMBi have been shown to be independent of whether it is supplied in its liquid form or as a dry supplement using clay powder as a carrier (Robert et al., 2002). Accurate estimates of production responses to the supplementation of HMBi in early and midlactation diets are needed,

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as well as the determination of an optimal level of rumen-available supplementation of HMB. The objectives of the present study were (1) to determine the lactation response in milk yield and components to ruminally available Met (HMB), (2) to determine the lactation response to partially protected Met provided as HMBi, and (3) to evaluate whether HMBi supplied at 0.15% of the diet DM provides enough ruminally available HMB to achieve maximal production response.

MATERIALS AND METHODS

Treatments

Sixty-one Holstein cows (24 primiparous, 37 multiparous) were assigned to 1 of 4 dietary treatments 21 to 28 d after calving. Diets contained the same basal ingredients, with 32.5% corn silage, 17.50% alfalfa hay, 10% whole cottonseed with lint, and 40% of a pelleted concentrate made of ground corn, soybean hulls, blood meal, dehulled-solvent extracted soybean meal, tallow, calcium soaps of fatty acids (Megalac; Church & Dwight Co., Inc., Princeton, NJ), urea, and vitamins and minerals (Table 1). Diets had a calculated metabolizable protein (**MP**) deficit of 93 g/d for a 650-kg animal producing 45.0 kg/d of 3.5% fat and 3.0% protein milk, and eating 26 kg of DM/d (NRC, 2001). Treatment 1 was the control diet, which consisted of the basal diet with no supplement (Table 1). The 3 supplemented treatments consisted of the basal diet plus the following supplementation: treatment 2, 0.1% of diet DM as HMB supplied as a 30% premix of a commercial HMB source (AT-88; Adisseo, Atlanta, GA) with sepiolite as an inert carrier; treatment 3, 0.15% of a feed grade source of HMBi with a minimum guarantee of 90% HMBi monomers also supplied as a 30% premix (MetaSmart; Adisseo) with sepiolite as an inert carrier; and treatment 4, a combination of 0.15% HMBi and 0.0475% HMB supplied by the same 2 premixes previously described. These levels were calculated assuming that HMBi is 70% Met equivalent of which 50% is metabolizable (i.e., ruminal escape × absorption × conversion to Met; NRC, 2001) resulting in an additional supply of 13.65 g/d of additional metabolizable Met in the HMBi treatment to bring the Lys:Met ratio in MP to 3:1. Supplementation for treatment 4 equaled the level of metabolizable Met of treatment 3 (13.65 g/d) and the level of rumen-available HMB (26 g/d) of treatment 2, assuming a 50% rumen dissociation of HMBi. Premixes of HMB and HMBi substituted for some of the soyhulls in the pelleted concentrate of treatments 2, 3, and 4.

Table 1. Ingredient and expected nutrient composition of control diet.

Ingredients	DM, % of total ration
Alfalfa hay	17.50
Corn silage	32.50
Cottonseed, whole with lint	10.00
Corn grain, ground, dry	25.00
Soybean hulls	5.08
Tallow	1.00
Calcium soaps of fatty acids	1.00
Soybean meal, solvent extracted	2.50
Blood meal, ring dried	2.73
Urea	0.31
Salt	0.40
Limestone	0.30
(Di) Calcium phosphate	0.38
Sodium bicarbonate	0.75
Dynamate (K and Mg sulfate)	0.10
Magnesium oxide	0.25
Trace minerals and vitamin premix	0.15
Expected chemical composition[1]	
DM	59.1
CP	16.6
RDP	10.5
RUP	6.2
NDF	31.3
Forage NDF	20.0
ADF	22.5
NFC	41.2
NE_L	1.63
MP[2]	10.9
Ca	0.74
P	0.38
Mg	0.36
K	1.22
S	0.21
Metabolizable Met, % of MP	1.80
Metabolizable Lys, % of MP	6.81

[1]Calculations based on NRC (2001) model using a 650-kg cow producing 45 kg of milk at 3.50% fat, 3.00% true protein, 4.8% lactose, and consuming 26.0 kg of DM/d.

[2]MP = Metabolizable protein.

Animals

Cows were assigned to their treatment on the Monday of their fourth week of lactation. Days of assignment were used to block cows for treatment assignment. Cows were fed the control diet (treatment 1) from parturition until their assignment to treatments. Milk weights and milk samples were taken on 2 consecutive days in each of the 2 wk before the cow assignment to treatment as covariate measurements. All cows were injected at 14-d intervals with r-bST (Posilac; Monsanto, St. Louis, MO) beginning at 63 DIM, which corresponds to the fifth week of experiment (**WOE**). Cows remained on the experiment for 16 wk. Care and handling of the animals was conducted as outlined in the guidelines of The Ohio State University Institutional Animal Care and Use Committee.

Cows were housed in a tie-stall barn for the duration of the trial, and were bedded as needed. They were milked as a group in the parlor, twice a day at 0700 and 1700 h. Diets were mixed once a day in the after-

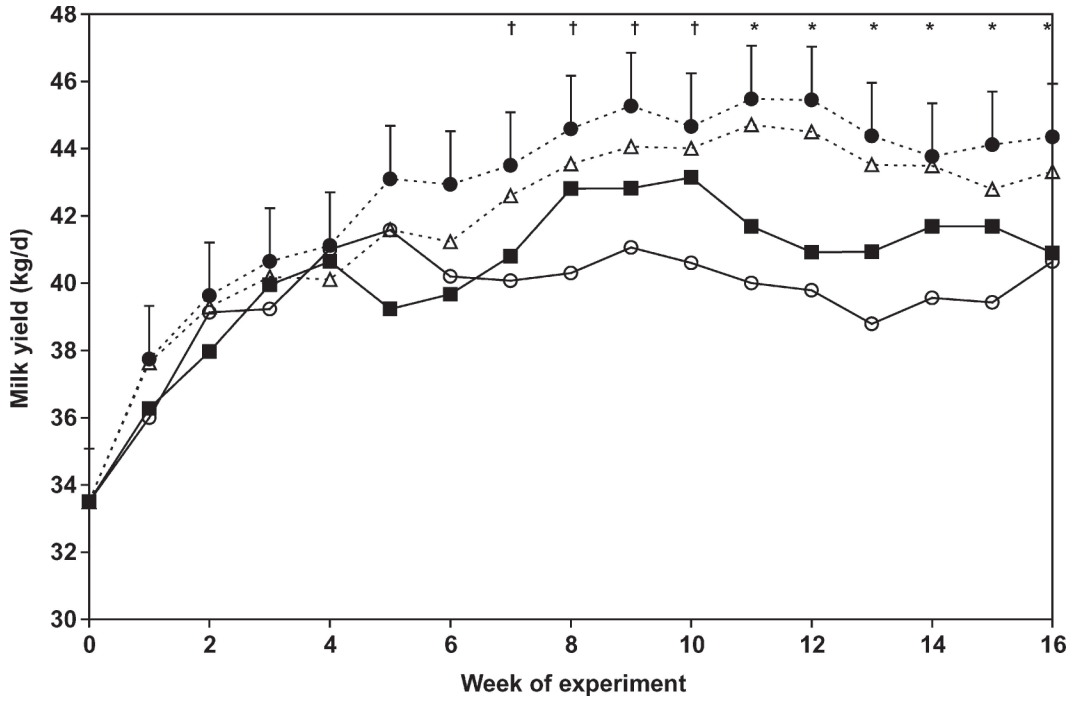

Figure 1. Milk yield of 24 primiparous and 37 multiparous cows fed an unsupplemented diet (○) or a diet supplemented with 0.1% HMB [2-hydroxy-4-(methylthio)-butanoic acid; ■], 0.15% HMBi [isopropyl-2-hydroxy-4-(methylthio)-butanoic acid; △], or 0.0475% HMB and 0.15% HMBi (●); † and * indicate a significant effect of HMBi at $P < 0.1$ and $P < 0.05$, respectively.

noon. Cows were individually fed twice a day at 1730 and 0730 h. Approximately one-half of the total daily feed allowance was fed in the afternoon. The remainder was stored overnight in individual plastic drums and was fed the next morning. Amounts fed were adjusted daily for a 5 to 10% refusal. Adjustments to the TMR were made weekly based on corn silage DM.

Sampling

Corn silage, hay, whole cottonseed with lint, pelleted concentrates, and TMR were sampled weekly throughout the 9 mo of trial. Forage and TMR samples were divided into 2 parts. One subsample was analyzed for proximate analyses within 1 wk of sampling. The second subsample was dried at 55°C, ground, and stored. These subsamples were composited monthly for further analyses. Cottonseed and pelleted concentrate samples were dried at 55°C, ground, stored, and composited on a monthly basis for chemical analyses.

Cows were milked twice daily with milk weights recorded at each milking. Milk samples were collected weekly at 4 consecutive milkings and preserved with 2-bromo-2-nitropane-1, 3-diol and refrigerated until analyzed after the fourth milking. Blood samples were collected via the coccygeal vein and arteries at 0 and 5 wk of trial (before the r-bST injection) for plasma urea nitrogen (**PUN**) and free plasma AA analyses. Blood samples were collected approximately 2 h postfeeding and placed on ice for transport to the laboratory, where they were immediately centrifuged and the plasma removed. Blood plasma was stored at –20°C until analyzed. After thawing, samples were deproteinized using 1 mL of plasma with 100 μL of a 35% aqueous solution of sulfosalicylic acid dehydrate. Cows were weighed and body condition was scored on a scale of 1 to 5 once a week throughout the trial.

Sample Analyses and Calculated N Balance

Ingredient and TMR samples were analyzed for CP (AOAC, 1990), NDF, ADF, and lignin (Van Soest et al., 1991). Wet samples were dried for 48 h at 55°C and ground using a Wiley mill (Arthur H. Thomas, Philadelphia, PA) with a 2-mm screen. Milk samples were analyzed for true protein, fat, lactose, and SCC according to approved procedures (AOAC, 1990) with a B2000 Infrared Analyzer (Bentley Instruments, Chaska, MN); MUN was determined by a diacetyl monoxime assay using a Skalar SAN Plus segmented flow analyzer (Skalar, Inc., Norcross, GA) by DHI Cooperative, Inc. (Columbus, OH). The accuracy of the method has been investigated previously (De Jong et al., 1992).

Plasma samples were assayed for plasma urea N using a standard diacetyl monoxime colorimetric assay (Sigma kit # 535, Sigma Diagnostics, St. Louis, MO) as described in Kauffman and St-Pierre (2001). Free plasma AA were assayed on deproteinized samples using a Beckman system 6300 High Performance Amino Acid Analyzer (Beckman Instruments, Palo Alto, CA) according to the method described in Noftsger et al. (2005).

Fecal N was estimated using the equation of Jonker et al. (1998). Urinary N was calculated from MUN and BW using the equation of Kauffman and St-Pierre (2001). Total milk N was calculated as [(milk true protein/6.38)/0.9375] to account for milk NPN when calculating N partitioning (Noftsger et al., 2005).

Statistical Analyses

Production data were analyzed using the MIXED procedure of SAS (SAS Institute, 2004) according to the following model:

$$Y_{ijklm} = \mu + T_i + P_j + TP_{ij} + b_k + B_j(X_{ijl} - \overline{X}_j) + c_{ijl} + W_m + TW_{im} + PW_{jm} + TPW_{ijm} + E_{ijklm} \quad [1]$$

where Y_{ijklm} is the dependent, continuous variable; T_i is the fixed effect of the ith treatment (i = 1, 2, 3, 4); P_j is the fixed effect of the jth parity (j = 1, 2); TP_{ij} is the fixed effect of the ith treatment by jth parity; b_k is the random effect of the kth block (k = 1, . . ., 18); B_j is the regression coefficient (covariate) for the jth parity; X_{ijl} is the covariate measurement for the lth cow within the ith treatment and the jth parity; $\overline{X}_j$ is the mean covariate measurement for the jth parity; c_{ijl} is the random effect of the lth cow within the ith treatment and jth parity (l = 1, . . ., n_{ij}); W_m is the fixed effect of the mth week of experiment (m = 1, . . ., 16); TW_{im} is the fixed effect of the ith treatment by mth week of experiment interaction; PW_{jm} is the fixed effect of the jth parity by mth week of experiment lactation interaction; TPW_{ijm} is the fixed effect of the ith treatment by jth parity by mth week of experiment interaction; and E_{ijklm} is the residual error.

Errors within cows across weeks, which are repeated measures due to multiple sampling of milk, intake, and blood, were modeled using a first-order autoregressive covariance structure. This structure consistently gave the lowest Bayesian information criteria of 4 covariance structures tested: unstructured, compound symmetry, first-order autoregressive, and simple (Littell et al., 1996). Significance was determined at $P < 0.05$. The interaction of parity with dietary treatments was not significant for any of the variables analyzed. Thus, none of the parity × treatment interactions is reported in this paper. Treatment effects were separated into 3 orthogonal contrasts according to a factorial treatment design. When the interaction of a main effect with week of trial was significant, the SLICE option in MIXED was used to test treatment differences for each of the weeks.

Gross feed efficiency was calculated as weight of milk per unit of DMI and analyzed according to model [1]. To test whether changes in gross feed efficiency were the result of treatment effects on body energy mobilization and replenishment, BW and BCS were also analyzed according to model [1]. Marginal feed efficiency was modeled by fitting [1] with milk production as the dependent variable, with the following term added to the linear model:

$$B_i (Z_{ijlm} - \overline{Z}) \quad [2]$$

where B_i is the regression coefficient for the ith treatment; Z_{ijlm} is the DMI measurement for the lth cow within the ith treatment and the jth parity on the mth week; and $\overline{Z}$ is the overall DMI mean.

The first partial derivative of the milk production function ([1] augmented with [2]) with respect to DMI provides an estimate of marginal feed efficiency for each treatment.

RESULTS

Feed Composition

The chemical analyses of corn silage, hay, whole cottonseed, concentrates, and TMR are reported in Table 2. The corn silage and alfalfa hay used for this experiment were of better quality than the average for normal corn silage and legume forage hay reported by NRC (2001) based on average measured CP and NDF. Dry matter, CP, NDF, ADF, and ash contents were similar across concentrates and diets, and relatively close to the formulated values. There were no systematic changes to forage quality during the trial. This was expected because the corn silage used for the experiment had been harvested from a single field over a short period, and the hay was purchased from a single and historically uniform source.

BW and BCS

Body weights and BCS did not differ by treatment (data not shown). Mean BW were 500.8 and 630.9 kg, and BCS were 3.23 and 2.88 for primiparous and multiparous cows, respectively. Body weights changed linearly with WOE. Primiparous cows gained on average 0.23 kg/wk [BW = 0.23 (± 0.80) × WOE + 499.4 (± 7.7)], whereas multiparous cows lost 1.50 kg/wk [BW = –1.50 (± 0.80) × WOE + 641 (± 7.7)].

Table 2. Chemical analysis of ingredients, concentrates, and experimental diets (% of DM).

	n_1[1]	n_2[2]	DM	CP	NDF	ADF	Lignin	Ash	Ca	P	Mg	K	S
Corn silage	35	35	35.4	7.9	38.5	22.5	3.07	3.81	0.22	0.25	0.17	0.99	0.1
Alfalfa hay[3]	34	34	89.3	21.8	35.6	27.1	7.3	10.7	1.67	0.28	0.31	2.4	0.28
Whole cottonseed	8	8	90.9	26.1	39.7	32.5	ND[4]	ND	0.21	0.88	0.46	1.29	0.24
Concentrate 1	8	8	88.6	20.2	16.5	9.8	ND	ND	1.13	0.44	0.5	0.69	0.2
Concentrate 2	8	8	89.7	20.3	16.1	9.3	ND	ND	1.15	0.43	0.63	0.68	0.24
Concentrate 3	8	8	90.7	19.6	16.6	9.4	ND	ND	1.2	0.43	0.64	0.66	0.24
Concentrate 4	8	8	90	19.4	17.7	9.3	ND	ND	1.16	0.42	0.69	0.67	0.25
Diet 1 – Control	35	8	58.7	16.3	34.8	23.5	ND	7.74	0.74	0.38	0.32	1.31	ND
Diet 2 – 0.1% HMB[5]	35	8	59.1	16.1	35.9	24.3	ND	7.43	0.70	0.36	0.32	1.35	ND
Diet 3 – 0.15% HMBi[6]	35	8	58.7	16.5	35.6	24.1	ND	7.61	0.72	0.37	0.33	1.43	ND
Diet 4 – 0.15% HMBi + 0.045% HMB	35	8	59.1	16.1	35.2	23.9	ND	7.75	0.73	0.36	0.33	1.44	ND

[1]n_1 = Number of independent samples assayed for DM, CP, NDF, and ADF.

[2]n_2 = Number of independent samples assayed for lignin, ash, Ca, P, Mg, K, and S.

[3]One weekly sample of hay was lost.

[4]ND = Not determined.

[5]HMB = 2-Hydroxy-4-(methylthio)-butanoic acid.

[6]HMBi = Isopropyl-2-hydroxy-4-(methylthio)-butanoic acid.

Intake and Milk Production

Results for DMI and milk production measurements are reported in Table 3. Treatments had no effect on DMI, which averaged 23.0 kg/d across all 4 treatments. Milk production was affected by HMBi ($P = 0.04$) but not by HMB ($P = 0.46$). Cows supplemented with HMBi produced an additional 2.9 kg of milk compared with the control cows ($P = 0.04$). Cows supplemented with both HMB and HMBi produced a nonsignificant 0.9 kg/d more milk than cows supplemented solely with HMBi ($P = 0.61$). The response in milk production to HMBi supplementation was progressive across WOE (Figure 1). The difference in milk production between the HMBi supplemented cows and the nonsupplemented ones approached significance ($P < 0.1$) by wk 7 of supplementation and achieved significance ($P < 0.05$) by wk 11.

Milk true protein content was increased by HMBi supplementation ($P = 0.006$) but not by HMB supplementation ($P = 0.45$; Table 3). There was no additional response in milk protein concentration from supplementing additional HMB when HMBi was in the diet because the milk protein content for the HMB + HMBi diet (2.95%, SE = 0.04) was equal to that of the HMBi diet (2.97%, SE = 0.04). The response in milk protein

Table 3. Effect of HMB [2-hydroxy-4-(methylthio)-butanoic acid] and HMBi [isopropyl-2-hydroxy-4-(methylthio)-butanoic acid] supplementation on milk production, composition, SCC, and feed efficiency.

	Treatments (% of DM)					P[1]		
HMB: / HMBi:	0 / 0	0.10% / 0	0 / 0.15%	0.045% / 0.15%	SEM	HMB	HMBi	Interaction
DMI (kg/d)	22.7	22.8	23.5	23.0	0.68	0.80	0.40	0.62
Milk production (kg/d)	39.8	40.7	42.3	43.2	1.23	0.46	0.04	0.99
Fat-corrected milk (kg/d)[2]	37.4	39.5	41.9	42.2	1.13	0.22	0.001	0.39
Milk composition (%)								
True protein	2.81	2.88	2.97	2.95	0.042	0.45	0.006	0.28
Fat	3.61	3.76	3.82	3.86	0.111	0.41	0.19	0.66
Lactose	4.79	4.91	4.86	4.83	0.031	0.13	0.77	0.03
Production (kg/d)								
True protein	1.102	1.158	1.228	1.261	0.035	0.15	0.001	0.72
Fat	1.436	1.553	1.654	1.666	0.050	0.17	0.001	0.28
Lactose	1.904	1.992	2.051	2.089	0.056	0.04	0.001	0.24
Log(SCC)	4.49	4.29	4.12	4.41	0.270	0.87	0.64	0.37
MUN (mg/dL)	12.6	13.6	12.0	11.3	0.45	0.69	0.01	0.01
PUN (mg/dL)[3]	15.4	16.0	14.5	16.0	1.1	0.38	0.71	0.72
Gross feed efficiency[4]	1.79	1.78	1.78	1.89	0.07	0.42	0.46	0.36

[1]Significance of the main effects of HMB, HMBi, and their interaction.

[2]Calculated as (0.4 × milk) + (15 × fat content).

[3]PUN = Plasma urea N.

[4]Calculated as kg of milk/kg of DMI.

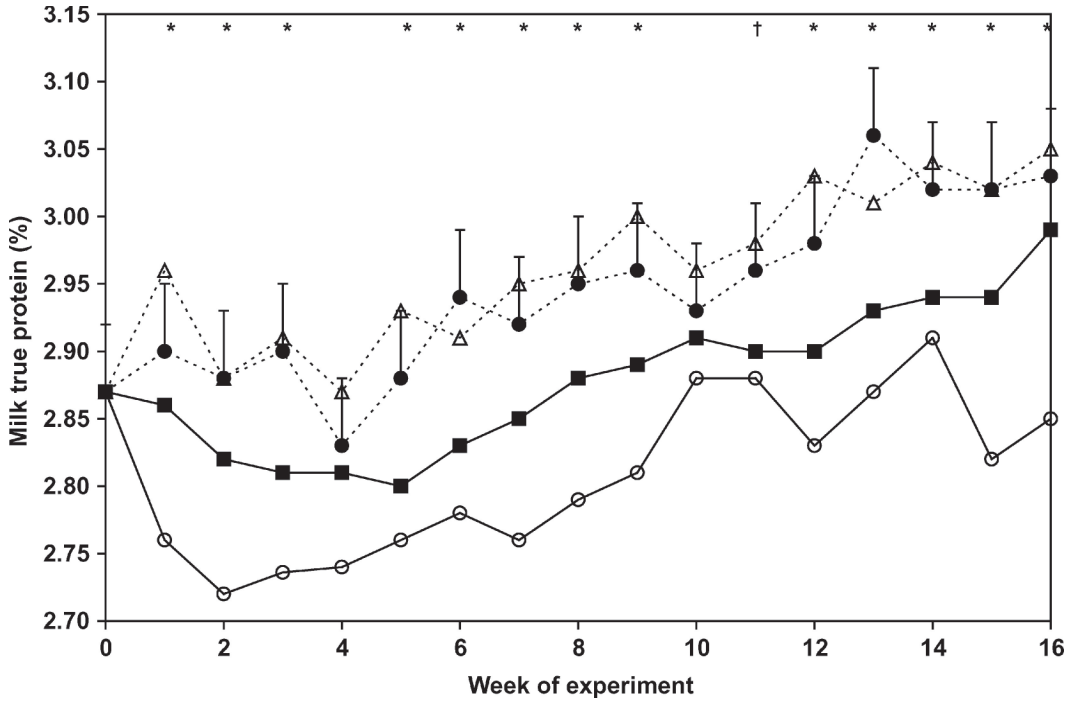

Figure 2. Milk true protein content of 24 primiparous and 37 multiparous cows fed an unsupplemented diet (○) or a diet supplemented with 0.1% HMB [2-hydroxy-4-(methylthio)-butanoic acid; ■], 0.15% HMBi [isopropyl-2-hydroxy-4-(methylthio)-butanoic acid; △], or 0.0475% HMB and 0.15% HMBi (●); † and * indicate a significant effect of HMBi at $P < 0.1$ and $P < 0.05$, respectively.

content to HMBi was nearly immediate and was significant starting in wk 1 of supplementation (Figure 2).

Treatments did not affect milk fat content ($P = 0.46$), which averaged 3.76% across the 4 diets (Table 3). The milk fat content for the 3 supplemented diets was numerically 0.20% (SE = 0.13) higher than for the control diet ($P = 0.14$).

The lactose content of milk was not affected by the main effects of HMB and HMBi (Table 3). The interaction of the 2 main effects, however, was significant ($P = 0.03$), due to a significant increase in lactose content when HMB was fed alone.

Milk true protein yield was increased ($P < 0.001$) by 115 g/d by HMBi, whereas HMB supplementation resulted in a nonsignificant ($P = 0.15$) increase of 44 g/d (Table 3). The response to HMBi supplementation was progressive throughout WOE, and took 5 wk to reach statistical significance ($P < 0.05$, Figure 3). Fat and lactose yields were increased ($P < 0.001$) by HMBi by 166 and 122 g/d, respectively. In contrast, HMB resulted in a nonsignificant ($P = 0.17$) increase of 65 g/d in fat yield and a significant ($P = 0.04$) increase of 63 g/d in lactose yield. Treatments had no effect on SCC (Table 3).

Supplementation of HMBi decreased MUN ($P = 0.01$), and the interaction of HMB and HMBi was also significant ($P = 0.01$), reflecting the significant 1.3 mg/dL decrease of MUN concentration for cows on the HMB plus HMBi diet compared with cows fed the control diet. This interaction became significant by wk 5 of the experiment (data not shown). Plasma urea N at wk 5 of the experiment was not affected by treatments ($P > 0.38$). Milk urea N was a more sensitive measurement of treatment effects than PUN in this experiment because of its multiple samplings within each animal, whereas PUN was sampled only once per animal at 5 wk in the experiment with the measurements at time zero being used as covariates.

Milk had no effect on gross feed efficiency, which averaged 1.81 kg/kg (SE = 0.06) across treatments (Table 3). Marginal feed efficiency was also the same for all 4 treatments and averaged 0.39 kg/kg (SE = 0.05).

N Partitioning and Efficiency

Least squares means for the partitioning of N intake are reported in Table 4. Nitrogen intake did not differ across diets ($P > 0.5$) because diets were isonitrogenous and treatments had no effect on DMI. Predictably, estimated fecal N was not affected by treatments and averaged 204 g/d. Estimated urinary N was reduced by 17.5 g/d by HMBi ($P = 0.08$), but not by HMB ($P = 0.85$). Milk N was significantly increased by HMBi ($P < 0.01$), but not by HMB ($P = 0.15$). Thus, a greater proportion

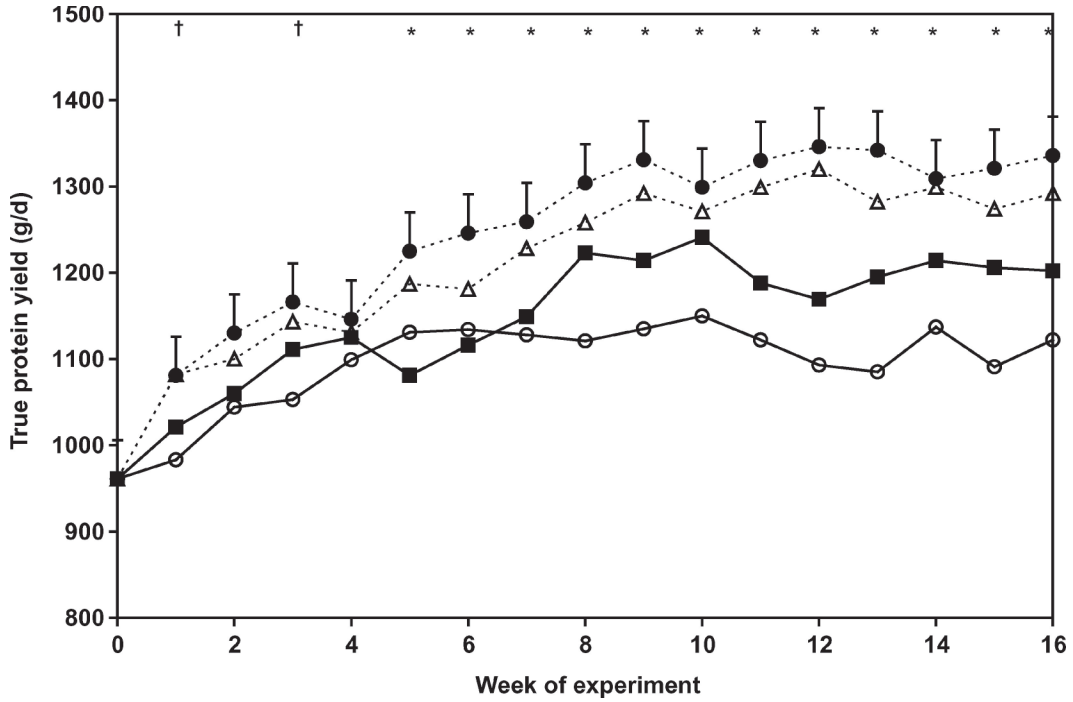

Figure 3. Milk true protein yield of 24 primiparous and 37 multiparous cows fed an unsupplemented diet (○) or a diet supplemented with 0.1% HMB [2-hydroxy-4-(methylthio)-butanoic acid; ■], 0.15% HMBi [isopropyl-2-hydroxy-4-(methylthio)-butanoic acid; △], or 0.0475% HMB and 0.15% HMBi (●); † and * indicate a significant effect of HMBi at $P < 0.1$ and $P < 0.05$, respectively.

of intake N and absorbed N was being partitioned to milk N with HMBi supplementation.

Gross N efficiency was increased by 3.1 percentage units by HMBi supplementation ($P = 0.02$), but not by HMB ($P = 0.17$). Dietary supplementation of HMBi reduced the amount of N excreted per kilogram of milk N produced (environmental N load) from 2.04 to 1.77 ($P = 0.01$). In contrast, HMB had no effect on N efficiency ($P = 0.21$).

Plasma Amino Acids

Least squares means of free plasma amino acid concentrations are presented in Table 5. Dietary HMBi supplementation had a significant effect on plasma Gly concentration ($P < 0.05$) and showed a trend ($P = 0.07$) for an increase in plasma Met concentration. The HMBi effect was significant ($P = 0.03$) when plasma Met was expressed as a percentage of total plasma essential AA.

Table 4. Nitrogen partitioning of diets supplemented with HMB [2-hydroxy-4-(methylthio)-butanoic acid], HMBi [isopropyl-2-hydroxy-4-(methylthio)-butanoic acid], or a combination.

	Treatments (% of DM)					P[1]		
HMB:	0	0.10%	0	0.045%				
HMBi:	0	0	0.15%	0.15%	SEM	HMB	HMBi	Interaction
N intake (g/d)	618	627	631	627	20	0.76	0.63	0.90
Estimated fecal N (g/d)[2]	202	204	204	204	17	0.76	0.63	0.90
Estimated urinary N (g/d)[3]	177	186	166	162	10	0.85	0.08	0.53
N milk (g/d)	184	194	205	211	6	0.15	0.01	0.74
Gross N efficiency (%)[4]	29.8	30.1	32.5	33.6	0.95	0.17	0.02	0.67
Environmental N load[5]	2.06	2.01	1.80	1.73	0.12	0.21	0.01	0.98

[1]Significance of the main effects of HMB, HMBi, and their interaction.

[2]Calculated as (N intake × 0.17) + 97, from Jonker et al., 1998.

[2]Calculated as 0.0259 × BW (kg) × MUN (mg/dL), from Kauffman and St-Pierre, 2001.

[4]Calculated as milk N/N intake × 100.

[5]Calculated as kg of fecal N + kg of urinary N/g of N milk.

Table 5. Effect of HMB [2-hydroxy-4-(methylthio)-butanoic acid], HMBi [isopropyl-2-hydroxy-4-(methylthio)-butanoic acid], or a combination of HMB and HMBi in the diet of lactating dairy cows on free AA concentrations in blood plasma 5 wk after initiation of the supplementation.

	Treatments[1]					P[2]		
	Control	HMB	HMBi	HMB +HMBi	SEM	HMB	HMBi	Interaction
Essential AA (EAA)	(μM)							
Arg	143	153	147	152	13.4	0.58	0.88	0.85
His	78.1	74.4	71.1	82.8	6.5	0.54	0.91	0.24
Ile	102	95	94	104	9.1	0.87	0.99	0.36
Leu	246	240	233	243	19.1	0.90	0.81	0.66
Lys	154	140	145	180	15.4	0.51	0.31	0.12
Met	19.6	20.1	21.6	27.0	2.4	0.22	0.07	0.31
Phe	67.5	67.1	67.1	78.4	5.9	0.36	0.36	0.33
Thr	136	130	142	151	113	0.89	0.31	0.51
Trp	48.4	49.7	49.9	56.4	4.3	0.37	0.34	0.54
Val	374	375	354	349	27.0	0.78	0.50	0.76
Total EAA	1368	1343	1334	1423	93.0	0.68	0.76	0.52
Nonessential AA (NEAA)								
Ala	364	310	352	387	30.1	0.76	0.28	0.14
Asp	29.2	26.1	29.0	41.6	7.9	0.54	0.33	0.32
Asn	5.0	5.6	5.8	6.1	0.7	0.47	0.34	0.81
Gln	43.4	46.1	45.2	40.0	5.4	0.79	0.67	0.45
Glu	236	214	221	264	16.7	0.54	0.30	0.059
Gly	263	264	325	336	33.4	0.85	0.05	0.87
Pro	127	121	126	147	9.1	0.45	0.18	0.13
Ser	154	146	156	182	12.0	0.46	0.12	0.15
Tyr	47.7	46.1	48.0	55.8	4.8	0.52	0.30	0.32
Cit	85.1	87.6	82.2	84.3	5.3	0.66	0.56	0.97
Orn	80.1	59.1	67.3	82.7	10.1	0.78	0.52	0.072
Tau	32.2	32.0	35.1	41.1	3.2	0.37	0.07	0.33
Total NEAA	1470	1361	1496	1674	103.0	0.67	0.12	0.12
Total AA	2838	2704	2830	3097	185.0	0.66	0.36	0.30
	(%)							
Met/EAA	1.42	1.53	1.63	1.80	0.11	0.24	0.028	0.82
Lys/EAA	11.0	10.4	10.9	12.6	0.54	0.33	0.053	0.033

[1]Control = no Met supplementation, HMB = HMB supplementation at 0.10% of DM, HMBi = HMBi supplementation at 0.15% of DM, and HMB + HMBi = HMB supplementation at 0.045% of DM and HMBi at 0.15% of DM.

[2]Significance of the main effects of HMB, HMBi, and their interaction.

DISCUSSION

Milk Yield and Composition

Milk yield response to Met supplementation has not been consistent in the literature. Stage of lactation (Schwab et al., 1992), Met supply by the base diet (Rulquin et al., 1993), and diet adequacy in Lys (NRC, 2001) modulate milk yield and composition responses to Met supplementation. In our trial, cows were in early lactation, the control diet was inadequate in calculated Met supply (1.80% of MP; Table 1), but adequate in calculated Lys supply (6.81% of MP; Table 1). Thus, the significant responses in milk and component yields that we observed from HMBi supplementation are consistent with that expected from a source of supplemental metabolizable Met under the conditions of our study. The feeding of HMB, however, has not been associated with an increase in milk protein content (Stokes et al., 1981; Hansen et al., 1991) but often induces a response in milk fat content (Lundquist et al., 1985; Hansen et al., 1991; Johnson et al., 1999). We did not observe significant effects of HMB on any yield or milk composition measurements with the exception of lactose yield. This differential production response to HMB from the classic response to Met supplementation points to a mode of action for HMB not associated with a direct increase in the supply of metabolizable Met. It has been suggested (Noftsger et al., 2003, 2005) that HMB would act primarily in the rumen by shifting ruminal microbial populations.

Metabolizability of Methionine Sources

There have been prior attempts at determining the effectiveness of various Met sources in delivering Met to dairy cows (Robert et al., 2001a; Schwab et al., 2001). The term "bioavailability" has been used somewhat loosely without a clear definition of what it spe-

cifically entails. Fundamentally, bioavailability refers to the net increase in absorbed Met per unit of raw Met supplied. Current measurement techniques in digestive physiology of ruminants have neither the precision nor the cost effectiveness necessary for the direct measurement of the bioavailability of Met sources. Indirect methods will have to be used in the near future. One such method is based on the increase in milk protein content associated with supplemental Met (Schwab et al., 2001).

Postruminal infusions of Met result in a rapid increase in milk protein content, linear to the amount of Met infused over the range of 0 to 24 g/d (Pisulewski et al., 1996). In the same experiment, infused Met did not result in any significant changes in milk yield, and fat and lactose contents during the short time of supplementation (2 wk). This short-term milk protein response to supplemental Met by cows fed diets with MP relatively low in Met is very consistent in the scientific literature (Rulquin et al., 1993). The short-term protein response associated with the feeding of a supplemental Met source can be compared with that of Met infusion as an estimate of relative bioavailability (metabolizability being a more descriptive term). Metabolizability is then expressed relative to infused Met, which is used as a standard with an assumed metabolizability of 100%. In our experiment, HMBi supplementation at 0.15% of DM resulted in an average increase of 1.15 g/kg in milk protein content. Duodenal infusions of Met resulted in a linear increase in milk protein content of 0.1133 g/kg of milk per g of Met infused (Pisulewski et al., 1996). Assuming that 100% of duodenally infused Met is absorbed (i.e., 100% metabolizability), the 1.15 g/kg increase in milk protein content observed from HMBi translates to a calculated 10.15 g/d in additional metabolizable Met. At a DMI of 23 kg/d and a dietary HMBi monomers concentration of 0.135% (0.15% × 0.9), and accounting for the fact that 1 mole of HMBi corresponds to 0.78 mole of Met, the relative metabolizability of the Met equivalent in HMBi (what others have termed bioavailability) is estimated at $10.15 \div (23 \times 0.15 \times 10 \times 0.9 \times 0.78) \times 100 = 41.9\%$. Clearly, this value is subject to considerable error in its estimation. Nevertheless, it is within the 40 to 58% range previously reported (Robert et al., 2001a; Schwab et al., 2001). The same calculation on the nonsignificant 0.25 g/kg increase in milk protein concentration results in an estimated relative metabolizability of the Met equivalent in HMB of 9.6%. This value is relatively close to the 5.3% of Noftsger et al. (2005) that was based on HMB passage to the omasum, but it is markedly different from the 50% estimate of Koenig et al. (1999) that was based on degradation kinetics using a pulse ruminal dose of HMB.

Nitrogen Efficiency

Our current understanding of AA nutrition in ruminants supports the concept that when essential AA are absorbed in the profile as required by the animal, the requirement for total essential AA is reduced and the efficiency of AA use for protein synthesis is maximized (NRC, 2001). In situations where the supply of one AA limits protein synthesis by the mammary gland, dietary supplementation of this AA in a metabolizable form would improve the profile of absorbed AA, resulting in additional protein synthesis. Our observation of an immediate increase in milk protein concentration with the feeding of HMBi supports this conceptual framework if HMBi results in additional absorbed Met. Other measurements are also supportive of an improvement in AA efficiency for protein synthesis from the addition of HMBi in the diet; PUN was numerically and MUN was significantly lower for HMBi diets (Table 3). A larger proportion of the absorbed N was secreted in milk as true protein, whereas a lower proportion was excreted in the urine with HMBi supplementation (Table 4), pointing to a reduction in the catabolism of N substrates, most likely AA, by the animals.

Plasma Amino Acids

Abomasal infusions of 56.5 g/d (Seymour et al., 1990) as well as duodenal infusions of 0, 6, 12, 18, and 24 g/d of DL-Met (Pisulewski et al., 1996) elevated blood and plasma Met concentrations linearly. Likewise, dietary supplementation with rumen-protected Met generally is associated with higher blood and plasma Met concentrations (Nichols et al., 1998; Blum et al., 1999) but not in all instances (Colin-Schoellen et al., 1995). Plasma concentrations of AA vary markedly between animals and across time. Multiple samplings are required to reach a precision sufficient for detecting changes of biological significance. Expressing concentrations of EAA as a percentage of EAA reduces the CV of the estimated means by more than 50%, thus increasing the ability to detect differences. Plasma Met concentrations expressed as a proportion of essential AA were significantly increased by HMBi but not by HMB (Table 5). This observation is consistent with other measurements indicating that HMBi but not HMB is an effective source of metabolizable Met for dairy cows under the conditions of our experiment. However, the interpretation of plasma AA concentrations with respect to the adequacy of AA supply is still unclear and debated (Johnson et al., 1999).

CONCLUSION

Dietary supplementation of HMB did not affect intake, milk production, and milk composition. Supplementation of HMBi at 0.15% of DM increased milk production, protein content, and production of protein, fat, and lactose. Milk urea N concentration was decreased by dietary HMBi supplementation. Moreover, HMBi improved N use by partitioning a greater proportion of absorbed N into milk protein N. Consequently, HMBi affected N efficiency expressed either in the form of the traditional gross N efficiency, or the recently proposed expression of environmental N load. Lastly, results indicate that HMBi at 0.15% of DM provides sufficient ruminally available HMB to achieve maximal production response.

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