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Effects of dietary supplementation of methionine and lysine on milk production and nitrogen utilization in dairy cows[1]

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ABSTRACT

The effect of the content of lysine and methionine in metabolizable protein (MP) on lactation performance and N utilization in Chinese Holstein cows was determined. A control diet (C) was formulated to be adequate in energy but slightly limiting in MP. The concentration of Met and Lys in MP was 1.87 and 5.93%, respectively. The treatments were as follows (% of Met or Lys in MP): L = diet C supplemented with L-lysine-HCl at 0.49% on a dry matter (DM) basis (Met, 1.87; Lys, 7.00); M = diet C supplemented with 2-hydroxy-4-(methylthio)-butanoic acid (HMB) at 0.15% (Met, 2.35; Lys, 5.93); ML = diet C supplemented with 0.49% L-lysine HCl and 0.15% HMB (Met, 2.39; Lys, 7.10). The diets were fed to 60 Chinese Holsteins in mid-lactation (average days in milk = 120, and milk yield = 32.0 kg/d) for 8 wk. Milk yield was increased by supplementation of either Lys (1.5 kg/d) or Met (2.0 kg/d), and supplementation of both Lys and Met further increased milk yield (3.8 kg/d). There was no significant difference in dry matter intake across treatment groups. Cows on treatments M (3.95%) and ML (3.90%) had higher milk fat content than those on C (3.60%) and L (3.67%), but there were no significant differences in milk protein and lactose contents or somatic cell count among treatments. Supplementation of Met or Lys significantly increased Met or Lys concentration in arterial plasma. Treatment ML had a higher conversion of intake N to milk N and lower urea N concentrations in serum, urine, and milk than did treatment C. Supplementing HMB and L-lysine-HCl to provide approximately 2.3% Met and 7.0% Lys of the MP in diets slightly limiting in MP increased milk production, milk protein yield, and N utilization efficiency.

Key words: methionine, lysine, milk performance, nitrogen utilization

INTRODUCTION

The efficiency of N utilization is low in dairy cows, with only 25 to 35% of consumed N secreted into milk (Chase, 1994) and almost all the remaining N excreted into feces and urine (72%; Yan et al., 2006). Most urinary N is excreted as urea and can be lost as ammonia into the atmosphere (Broderick, 2003; Wang et al., 2008). Due to pressure to reduce the environmental footprint of livestock production, there is increasing interest in increasing N utilization efficiency of dairy cows while maintaining milk production and animal health and welfare.

Manure N excretion is a direct function of N intake (Yan et al., 2006), and use of low N diets can be an effective approach to reduce N excretion of dairy cows. In fact, decreasing dietary protein from 18.4 to 15.1% decreased manure N excretion, but also decreased milk production (Broderick, 2003). With each 1-percentage-unit decrease in dietary protein, milk yield decreased by 0.7 to 1.2 kg/d (Broderick, 2003; Wang et al., 2007) and milk protein yield decreased by 100 to 188 g/d (Kalscheur et al., 1999; Broderick, 2003). Alternatively, N utilization can be improved by increasing the conversion of intake protein to MP and by providing absorbed AA in a pattern that closely matches the AA requirements for milk synthesis (Noftsger and St-Pierre, 2003). By considering the AA content of MP, protein may be reduced in dairy rations, improving N efficiency without compromising milk production.

Methionine and Lys are considered to be the 2 limiting AA for milk production (e.g., NRC, 2001). Dietary supplementation of Met and Lys can therefore be an effective approach to improve AA balance for milk production. Because crystalline Met is degraded by ruminal bacteria, much effort has been focused on feeding either an analog of Met, such as Met hydroxy analog, or various encapsulated forms of Met to avoid ruminal degradation. There are many Met analog and protected

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products, including 2-hydroxy-4-(methylthio)-butanoic acid (**HMB**; a pelletable Met hydroxy analog), which has been shown to be more resistant to rumen degradation than unprotected Met both in vitro (Vazquez-Anon et al., 2001) and in vivo (Koenig et al., 2002). Numerous experiments have studied the benefits of adding different forms of synthetic Lys or Met to lactating cow rations. Responses have included increased milk yield (Piepenbrink et al., 2004; Socha et al., 2005), milk fat content (Rode et al., 1998; Noftsger et al., 2005), milk protein content (Noftsger et al., 2005; Socha et al., 2005), and protein yield (Rode et al., 1998; Piepenbrink et al., 2004; Socha et al., 2005). These differences between trials may be due to variations in stage of lactation of animals, amount of supplemental Lys or Met, proportions of other AA in MP, and experimental design (Latin square or continuous lactation trial).

There is little information in the literature on the effect of supplemental L-lysine-HCl and HMB as the AA sources for lactating dairy cows, particularly when MP is marginal. The objective of this study was to evaluate the effect of supplementation of Met as HMB, Lys as L-lysine HCl, or supplementation with both Met and Lys, in diets marginal in MP on milk production, N utilization, and metabolite concentration in blood in lactating dairy cows.

MATERIALS AND METHODS

Animals, Diets, and Experiment Design

The use of the animals was approved by the Animal Care Committee, Zhejiang University, Hangzhou, China. Thirty-two multiparous [BW = 600 (SD 19.0) kg] and 28 primiparous [BW = 560 (SD 16.0) kg] Chinese Holstein cows in mid-lactation [average DIM = 120 (SD 7.0), milk yield = 30.2 (SD 2.69) kg/d] were used in a 4-treatment randomized block design for 8 wk. Animals were blocked into groups of 4 according to DIM, milk yield, and parity, and allocated to treatments randomly within group. Cows were housed in a tie-stall barn and fed and milked at 0630, 1430, and 2100 h. All animals had free access to drinking water. Feed was offered ad libitum to 5% orts. The experiment lasted for 8 wk from February to April 2008, following a 1-wk adaptation to the experiment.

Four isocaloric diets (Table 1) were formulated to meet the requirements of NE_L but to be slightly deficient in MP for Holstein dairy cows (NRC, 2001; Wang et al., 2007). With a DMI at 21 kg/d, the NE_L was sufficient for 31 kg/d of milk, but estimated MP was adequate for 26.5 kg/d of milk based on a 65% efficiency of utilization for lactation (NRC, 2001). In the control diet (**C**), Met and Lys were calculated to be 1.84 and 5.90% (NRC, 2001) or 1.87 and 5.93% of MP (CPM Dairy; Tedeschi et al., 2008), both lower than recommended by NRC (2001). The other diets consisted of the following supplementations to C (DM basis): methionine-supplemented diet (**M**), 0.15% of a feed-grade source of liquid HMB (2-hydroxy-4-(methylthio)-butanoic acid) with a minimum guarantee of 88% chemical purity (Alimet, Novus Int. Inc., St. Louis, MO); lysine-supplemented diet (**L**), 0.5% L-lysine-HCl; and methionine- and lysine-supplemented diet (**ML**), a combination of 0.15% HMB and 0.5% L-lysine-HCl. The CPM Dairy prediction for the proportion of Met and Lys in MP was 2.35 and 5.93% for M, 1.87 and 7.00% for L, and 2.39 and 7.10% for ML.

Table 1. Ingredient composition of the experimental diets

Ingredient, % of DM	Treatment[1]			
	C	M	L	M+L
Alfalfa pellet[2]	10.2	10.2	10.1	10.1
Corn silage[3]	22.2	22.1	22.0	22.0
Grass hay[4]	13.1	13.1	13.0	13.0
Ground corn grain	23.9	23.9	23.8	23.8
Soybean meal, 42.5%CP	5.0	5.0	5.0	5.0
Whole cottonseed	2.5	2.5	2.5	2.5
Cottonseed meal	3.5	3.5	3.5	3.5
Sesame meal	4.0	4.0	4.0	4.0
Wheat bran	4.0	4.0	4.0	3.9
DDGS[5]	5.0	5.0	5.0	5.0
Apple pomace	3.0	3.0	3.0	3.0
Dicalcium phosphate	1.0	1.0	1.0	1.0
Limestone	0.90	0.90	0.90	0.89
Saleratus	0.70	0.68	0.70	0.69
Salt	0.50	0.47	0.50	0.47
Premix[6]	0.50	0.50	0.50	0.50
Alimet[7]	—	0.15	—	0.15
L-Lysine-HCl[8]	—	—	0.50	0.50

[1]C = control, M = methionine supplemented, L = lysine supplemented, M+L = methionine and lysine supplemented.

[2]Alfalfa pellet contained 90% DM, 18.4% CP, 10.9% RDP, 44.3% NDF, and 33.0% ADF on DM basis.

[3]Corn silage contained 22.7% DM, 7% CP, 4.6% RDP, 67.2% NDF, and 41.0% ADF on DM basis, chopped to a length of about 2.5 cm.

[4]Grass hay contained 91% DM, 7.0% CP, 4.2% RDP, 63.0% NDF, and 37.5% ADF on DM basis, chopped to a length of about 4 cm.

[5]Dried distillers grains plus solubles.

[6]Formulated to provide (per kg of DM) 1,000,000 IU of vitamin A, 200,000 IU of vitamin D, 1,250 IU of vitamin E, 14,000 mg of Zn, 100 mg of Se, 180 mg of I, 3,000 mg of Fe, 40 mg of Co, 3,000 mg of Mn, and 3,000 mg of Cu.

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

[8]Manufactured by Archer Daniels Midland Company, Decatur, IL.

Sampling, Measurement, and Analyses

Feed offered and refused was weighed for 2 consecutive days every other week throughout the trial to calculate DMI. Forage and concentrates were sampled every other

week. Ort samples were collected every other week and composited by animal in proportion to the wet weight from each sampling day. All samples were immediately dried in an air-forced oven at 60°C for 48 h and stored in sealed plastic containers at room temperature until analyzed. In preparation for analyses, dried forages and concentrates were ground through a 2-mm screen (Thomas-Wiley Laboratory Mill; Arthur H. Thomas, Philadelphia, PA), and then through a 1-mm screen in a Cyclotec mill (Tecator 1093, Hoganas, Sweden). Sample DM was determined by drying a subsample at 100°C for 24 h. All samples were analyzed for NDF and ADF (Van Soest et al., 1991), and total N (method 988.05; AOAC, 1990). Body weight for each cow was measured every other weekon 2 consecutive days from initiation of treatments to the end of the experiment.

Milk yield was estimated by recording the weight (Waikato Milking Systems NZ Ltd., Waikato, Hamilton, New Zealand) at each milking for 2 consecutive days every week. Weekly, two 50-mL aliquots of milk were collected weekly, proportional (4:3:3) to yield of each milking at 0630, 1400, and 2100 h. One sample was mixed with Bromopol (milk preservative; D&F Control Systems, San Ramon, CA) and analyzed for fat, protein, and lactose by infrared analysis (Laporte and Paquin, 1999) with a 4-channel spectrophotometer (MilkoScan, Foss Electric, Hillerød, Denmark), and for SCC using a cell counter (Fossmatic 400, Foss Electric). Another sample was stored at −20°C for analysis of MUN. For MUN analysis, 4 mL of cold TCA (25%) was added to thawed samples, allowed to stand for 5 min, and then centrifuged at 3,000 × *g* for 20 min at 4°C. The clear supernatant was pipetted carefully through the solidified fat layer and analyzed for MUN, using the diacetyl monoxime-binding assay (Rahmatullah and Boyde, 1980).

Blood and urine samples were sampled approximately 3 h after feeding on the first day of wk 1, 3, 5, and 7. Blood samples were collected from the mammary vein and coccygeal artery, and centrifuged at 3,000 × *g* for 15 min. Serum was frozen at −10°C and later thawed for analysis of BUN (Wang et al., 2007), total protein (Thomas, 1998), albumin (Johnson et al., 1999), NEFA (McCutcheon and Bauman, 1986), cholesterol (Deeg and Ziegenhorm, 1983), high density lipoprotein (Nauck et al., 1998), low density lipoprotein (Lichtenstein et al., 1993), triglyceride (Cole et al., 1997), glucose (Barham and Trinder, 1972), aspartate aminotransferase (Moss and Henderson, 1999), and alanine aminotransferase (Moss and Henderson, 1999). Amino acid concentration was analyzed using commercial kits according to the procedure described by Calder et al. (1999) using kits (Diasys Diagnostic Systems, Shanghai Co. Ltd., Shanghai, P. R. China). Urine samples were acidified immediately using 4 volumes of 0.072 *N* H_2SO_4 and stored at −20°C (Valadares et al., 1999). Later, samples were thawed at room temperature and analyzed for urinary urea N (Rahmatullah and Boyde, 1980).

Statistical Analysis

Statistical analysis was conducted using SAS software (SAS Institute, 2000). All data except for BW were analyzed using the MIXED procedure with covariance type AR(1). The model included treatment, week, and interaction of treatment × week. The effect of week was included as a repeated measure. Parity was included in the model but omitted in the presentation. Means were separated by using the PDIFF option in the LSMEANS statement. Data on BW were analyzed using the general linear model procedure. Results are reported as least squares means. Probability values of $P < 0.05$ defined statistically significant results, with $P < 0.10$ defining a statistical trend.

RESULTS

Effects on Feed Intake and Milk Production

All diets were similar in CP, RDP, RUP, NDF, MP, and NE_L content (Table 2). Overall, DMI did not differ among treatments, averaging 20.8 kg/d (SD 0.60) (Table 3). Milk yield was significantly increased by supplementation of Lys or Met, and further increased with supplementation of both. No interaction of Lys with Met was observed but their combined effects were additive. For C, milk yield was 26.5 kg/d and supplementation with Met increased milk yield to 28.5 kg/d, with no change in DMI, resulting in more efficient feed utilization (milk:DMI ratio = 1.38 vs. 1.27). Adding Lys to the control diet increased milk yield to 28.0 kg/d with no increase in DMI, improving feed efficiency to 1.34. The addition of both Met and Lys increased milk yield to 30.3 kg/d with no change in DMI, increasing milk efficiency to 1.46. There was no interaction of treatment by week in milk yield (Figure 1) or for DMI.

Milk protein content was not different across treatments and ranged from 3.25 to 3.30%, whereas protein yield followed milk yield trends (Table 3). Milk fat content was increased by Met supplementation (from 3.60% for C to 3.95% for M and 3.90% for ML), but not changed by Lys, averaging 3.67%. Dietary treatments had no effect on lactose, SNF, or TS in milk. Supplementation of both Met and Lys numerically decreased SCC content in milk, as did supplementation of either

Table 2. Chemical composition of the experimental diets

	Treatment[1]				
Composition	C	M	L	M+L	SEM
DM,[2] %	52.3	51.7	51.8	52.0	2.30
CP,[2] % of DM	16.5	16.4	16.4	16.4	0.05
NDF,[2] % of DM	37.8	37.7	37.4	37.5	0.33
ADF,[2] % of DM	21.0	20.5	20.1	20.8	0.23
RDP,[3] % of DM	10.3	10.3	10.2	10.2	
RUP,[3] % of DM	6.2	6.1	6.2	6.2	
MP,[3] % of DM	9.70	9.79	9.80	9.85	
Lys:Met	3.10:1	2.55:1	3.60:1	3.00:1	
NE_L,[3] Mcal/kg of DM	1.56	1.56	1.56	1.56	

[1]C = control, M = methionine supplemented, L = lysine supplemented, M+L = methionine and lysine supplemented.

[2]The biweekly samples were chemically analyzed for these nutrient values.

[3]Calculated based on individual feedstuffs in CNSAPH (2000) guidelines.

AA. There was no significant treatment effect on BW during the study. No interaction of Lys with Met was observed on these parameters.

Effects on Serum AA Concentrations

There was no effect of week or week by treatment interaction in serum AA. Dietary supplementation of Lys (diets L and ML) significantly increased Lys concentration ($P < 0.05$; Table 4). Similar results were also found for Met supplementation; that is, Met concentration in serum was higher for M and ML than for C and L ($P < 0.05$). Plasma Met and Lys concentration was increased by 45 to 63% (M and ML) and 28 to 46% (L and ML), respectively, compared with diet C. There were no significant dietary differences in other AA, total essential AA (**EAA**), total nonessential AA (**NEAA**), or total AA, except for His, which was higher for ML than for C ($P < 0.05$; Table 4).

Compared with C, supplementation of both Met and Lys significantly increased the supply of total EAA and all individual EAA to the mammary gland ($P < 0.05$) except for Phe and Thr (Table 5). Supplementation of Met increased the disappearance rate for Arg, His, Met, and Val ($P < 0.05$), and supplementation of Lys increased the disappearance rates for Arg, Lys, and

Table 3. Effects of dietary supplementation of lysine and methionine on DMI and milk production

	Treatment[1]				
Item	C	M	L	M+L	SEM
DMI, kg/d	20.9	20.7	21.0	20.7	0.24
MP - Met, g/d[2]	38	48	38	48	1.50
MP - Lys, g/d[2]	122[b]	122[b]	144[a]	144[a]	5.10
Lys:Met	3.10:1[b]	2.58:1[c]	3.74:1[a]	3.04:1[b]	0.12
Milk production, kg/d					
Milk	26.5[b]	28.5[a]	28.0[ab]	30.3[a]	0.65
FCM[2]	25.7[b]	28.3[a]	27.5[a]	28.5[a]	0.61
Milk protein	0.87[b]	0.92[a]	0.90[ab]	0.98[a]	0.020
Milk composition, %					
Protein	3.27	3.30	3.25	3.25	0.100
Fat	3.60[b]	3.95[a]	3.67[b]	3.90[a]	0.050
Lactose	5.01	5.04	5.15	5.06	0.042
TS	12.79	13.00	12.93	12.60	0.204
SNF	8.99	9.06	9.10	8.91	0.120
SCC, $\times 10^3$/mL	404	361	263	213	50.0
Initial BW, kg	583	570	597	596	18
End BW, kg	591	580	606	609	21
BW change, g/d	143	179	161	230	35
Milk efficiency[3]	1.27[b]	1.38[a]	1.34[a]	1.46[a]	0.03

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

[1]C = control, M = methionine supplemented, L = lysine supplemented, M+L = methionine and lysine supplemented.

[2]Values were predicted by NRC (2001) model based on measured DMI and chemistry analysis of feeds.

[3]Milk efficiency = milk yield/DMI.

Table 4. Effects of dietary supplementation of lysine and methionine on free AA concentrations in arterial serum

	Treatment[1]				
Item	C	M	L	M+L	SEM
Essential AA, μmol/L					
Arg	93.8	91.4	101.6	95.1	7.90
His	176.8[b]	257.4[a]	171.1[b]	193.6[b]	7.24
Ile	74.3	81.3	83.5	83.8	3.56
Leu	100.2	104.3	99.5	102.1	4.39
Lys	69.9[b]	61.5[b]	101.7[a]	89.7[ab]	4.55
Met	29.4[b]	48.0[a]	22.3[b]	42.5[a]	3.01
Phe	39.4	39.4	37.8	41.2	1.33
Thr	179.7	175.4	174.0	184.5	5.65
Val	163.3	165.1	177.3	180.3	4.64
Total essential AA	926.6	1,023.8	968.8	1,012.8	33.60
Nonessential AA, μmol/L					
Ala	183.2	189.7	175.2	171.9	5.00
Glu	108.4	120.5	110.6	102.0	8.00
Gly	336.8	309.3	339.7	313.3	10.00
Pro	82.3	75.0	76.9	72.7	3.70
Ser	106.7	118.8	112.5	102.8	5.88
Tyr	45.7	42.2	32.4	38.2	1.34
Total nonessential AA	863.0	856.3	847.4	800.8	22.10
Total AA	1,789.6	1,879.9	1,816.2	1,813.6	45.31

[a,b]Means within same row with different superscripts differ ($P < 0.05$).

[1]C = control, M = methionine supplemented, L = lysine supplemented, M+L = methionine and lysine supplemented.

Val ($P < 0.05$). Arteriovenous difference in total EAA concentration tended to be higher for M and L than for C. However, supplementation of both Met and Lys had an additive effect on total EAA. Dietary treatments had no significant effect on arteriovenous differences on individual NEAA or total NEAA concentration, except for Glu, for which Lys supplementation had a higher disappearance rate than did C ($P < 0.05$).

Effects on Plasma Metabolites and Nitrogen Utilization Efficiency

There were no significant effects of supplementation of Lys, Met, or both, on plasma concentrations of glucose, albumin, cholesterol, high density lipoprotein, low density lipoprotein, triglyceride, aspartate aminotransferase, or alanine aminotransferase (Table 6). However, cows receiving ML had numerically higher concentrations of total protein in serum and tended to have lower glucose and NEFA concentrations. Serum concentration of NEFA was decreased by Met supplementation ($P < 0.05$).

Dietary supplementation of Met or both Met and Lys significantly increased N utilization efficiency (milk N/N intake; Table 7). This variable was similar for L and C, although it was numerically higher for L than for C. Diet ML had a lower concentration of urea nitrogen in serum, urine, and milk ($P < 0.05$).

DISCUSSION

The milk was recorded on 2 d (6 milkings) a week, and DMI was recorded for 2 consecutive days every other week in this study. We have found this protocol sufficient for monitoring changes in these measurements

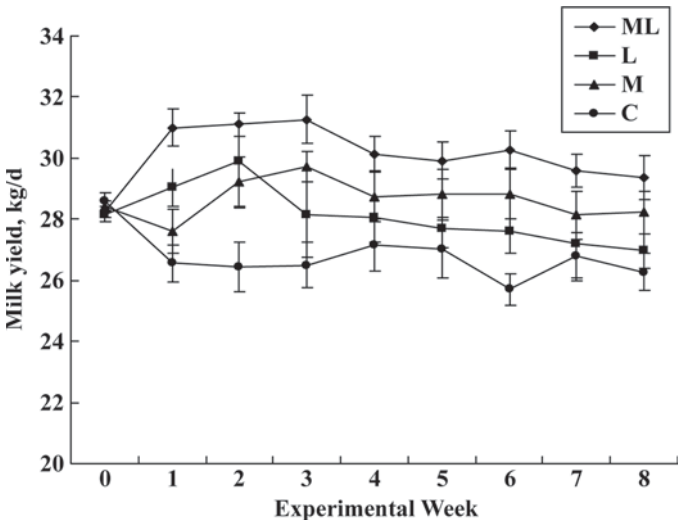

Figure 1. Average weekly milk yield for cows on control (C, ●), treatment M [2-hydroxy-4-(methylthio)-butanoic acid 32 g/d, ▲], treatment L (L-lysine-HCl 108 g/d, ■), and treatment ML [32 g/d 2-hydroxy-4-(methylthio)-butanoic acid and 108 g/d L-lysine-HCl, ♦]. Error bars represent SE values; pooled SEM = 0.65.

Table 5. Arteriovenous difference in free AA concentrations of cows fed diets supplemented with methionine and lysine

	Treatment[1]				
Item	C	M	L	M+L	SEM
Essential AA, μmol/L					
Arg	65.5[b]	75.7[a]	79.6[a]	79.7[a]	5.55
His	133.9[b]	164.3[a]	132.0[b]	146.7[ab]	6.10
Ile	58.5[b]	55.6[b]	59.6[b]	70.6[a]	2.70
Leu	77.9[b]	62.6[b]	64.7[b]	82.1[a]	4.23
Lys	35.7[b]	34.5[b]	67.1[a]	49.5[a]	3.45
Met	12.0[b]	29.8[a]	12.1[b]	29.7[a]	1.99
Phe	20.4	24.2	20.4	27.4	1.39
Thr	140.0	140.9	135.6	149.7	5.67
Val	96.0[ab]	116.8[a]	130.6[a]	152.5[a]	9.31
Total essential AA	639.8[b]	704.4[b]	701.5[b]	787.8[a]	21.38
Nonessential AA, μmol/L					
Ala	139.9	155.7	152.9	143.2	11.84
Glu	65.3[b]	59.3[b]	75.6[a]	70.4[ab]	4.65
Gly	231.7	238.0	245.5	262.8	11.33
Pro	61.3	62.1	61.1	59.3	4.84
Ser	74.6	76.9	76.6	79.5	7.21
Tyr	25.4[b]	28.1[a]	19.7[b]	26.9[a]	1.87
Total nonessential AA	598.2	620.2	631.5	642.0	21.20
Total AA	1,238.0	1,324.5	1,333.0	1,429.9	79.95

[a,b]Means within same row with different superscripts differ ($P < 0.05$).

[1]C = control, M = methionine supplemented, L = lysine supplemented, M+L = methionine and lysine supplemented.

associated with stages of lactation on our research farm where the cows are kept under an indoor system, and the feeding, management and environment conditions are relatively stable. Discussion of lactation performance was based on this protocol.

In the present study, supplementation of L-lysine-HCl and HMB to a diet that was low in these AA as a percentage of MP compared with those recommended by the NRC increased milk and milk protein yields. When estimated by CPM Dairy, the control diet was adequate in energy but limiting in MP. The ME-allowable milk yield was 30 kg/d but the MP-allowable milk yield was 26.4 kg/d compared with the observed milk yield (26.5 kg/d). Tedeschi et al. (2008) reported that CPM Dairy adequately predicted milk production at the farm level, and suggested that improvements need to be made to account for individual animal variation. Compared with cows without supplemental AA, cows receiving both L-lysine-HCl and HMB produced more milk (30.3 vs. 26.5 kg/d) and milk protein (0.98 vs. 0.87 kg/d). The results

Table 6. Effects of dietary supplementation of lysine and methionine protein on plasma metabolic parameters

	Treatment[2]				
Item[1]	C	M	L	M+L	SEM
Glucose, mmol/L	2.63	2.88	2.72	2.60	0.08
Total protein, g/L	53.2	58.8	58.9	61.1	2.20
Albumin, g/L	31.8	34.0	32.6	33.1	1.00
Triglyceride, mmol/L	0.24	0.26	0.24	0.26	0.03
NEFA, μmol/L	99.0[a]	88.3[b]	98.1[a]	90.8[b]	3.10
Cholesterol, mmol/L	5.56	5.48	5.15	6.17	0.35
HDL, mmol/L	3.31	3.27	3.11	3.35	0.10
LDL, mmol/L	1.02	0.99	0.92	1.27	0.10
ALT, IU/L	26.4	25.6	25.6	25.9	1.40
AST, IU/L	121.6	105.7	81.6	82.6	13.6

[a,b]Means within same row with different superscripts differ ($P < 0.05$).

[1]HDL = high density lipoprotein; LDL = low density lipoprotein; ALT = alanine aminotransferase; AST = aspartate aminotransferase.

[2]C = control, M = methionine supplemented, L = lysine supplemented, M+L = methionine and lysine supplemented.

Table 7. Effects of dietary supplementation of lysine and methionine on nitrogen (N) utilization efficiency and urea N concentration in serum, urine, and milk

	Treatment[1]				
Item	C	M	L	M+L	SEM
N conversion[2]	0.260[c]	0.283[b]	0.277[bc]	0.300[a]	0.010
Urea N concentration, mg/dL					
Serum	14.8[b]	13.6[b]	15.0[a]	13.0[c]	0.40
Urine	672.8[b]	676.9[b]	710.0[a]	582.1[c]	11.90
Milk	14.4[a]	13.4[b]	14.6[a]	12.8[c]	0.20

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

[1]C = control, M = methionine supplemented, L = lysine supplemented, M+L = methionine and lysine supplemented.

[2]Milk N/N intake.

observed in this study were similar to those observed when cows were fed rumen-protected Met with or without Lys (Socha et al., 2005; Broderick et al., 2009). The basal diet used by Broderick and Muck (2009) contained 9.6% MP in DM and Lys and Met were 6.7 and 1.9% of MP, similar to the content of the basal diet used in this study. The production responses to AA supplementation obtained in the present study were larger than reported by some other studies (e.g., Armentano et al., 1993). The positive response in milk production to Met supplementation remained relatively constant across the weeks of the experiment (Figure 1), which was different from the result reported by St-Pierre and Sylvester (2005), where there was a progressive response with advancing weeks. This may be due to differences in lactation stage between the 2 experiments. Cows in this study were in mid-lactation, whereas those used by St-Pierre and Sylvester (2005) were in early lactation. This suggests that there may be an interaction between lactation stage and Met supplementation that remains to be studied. Compared with cows offered the control diet, cows fed Met with or without Lys had higher milk fat percentages. The increased milk fat content for cows fed Met with or without Lys agrees with several previous studies (Huber et al., 1984; Lundquist et al., 1985; Broderick and Muck, 2009; Broderick et al., 2009). Vazquez-Anon et al. (2001) reported that supplementation of HMB did not affect nutrient digestibility or VFA concentrations. Although ruminal fermentation was not assessed in this study, this suggests that the increase in milk fat content observed with Met supplementation may be related to metabolic pathways of Met and its methylated compounds (Benefield et al., 2009). Methionine is important for choline synthesis. The requirement for choline can be met by both dietary choline and transmethylation reactions. Two principal methyl donors in animal metabolism are betaine and S-adenosyl-methionine, a metabolite of Met. Choline and Met are interchangeable with regard to their methyl group-furnishing functions (Pinotti et al., 2002). Blocking the synthesis of choline from Met depressed yields of milk and milk fat (Janovick Guretzky et al., 2006). In this study, perhaps a portion of absorbed Met was obligatorily used for choline synthesis, benefitting milk fat content. Because of the short-term nature of this study, major effects of HMB on fat metabolism were probably minor. Some researchers have reported little effect of HMB on milk fat percentage (Stokes et al., 1981; Rulquin et al., 2006).

In high-producing dairy cows, the AA supply from microbial protein alone cannot meet the requirement for milk production (Volden et al., 1998; Volden, 1999), and there is a need to supplement these cows with protein sources high in RUP. A break-point analysis reported by NRC (2001) indicated that for maximal milk yield, Lys and Met proportions in MP are about 7.2 and 2.4%, respectively (optimal Lys: Met ratio of 3:1). In an in vitro trial, Liu et al. (2007) reported that expression of the α_{S1}-casein gene was enhanced with increasing ratio of Lys to Met, with the optimal level at 3:1. These findings suggest that the appropriate ratio of Lys:Met in MP can increase nutrient utilization efficiency in dairy cows.

In the current study, supplementation of Met, Lys, or both increased the level of arterial concentrations of Lys, Met, EAA, and total AA, which improved the AA supply to the mammary gland for milk production. Using arteriovenous differences in AA concentration to estimate uptake by the mammary gland, a 15% increase in total AA can result in a 13% increase in milk protein production, compared with a 5% increase with 8% more total AA observed by Volden (1999). Achieving an appropriate AA balance is critical and adding one AA without addressing the shortage of other AA could exaggerate the imbalance of AA supply. Feeding dairy cows with diets containing imbalanced AA could reduce milk production and N utilization efficiency. In the present experiment, the increase in the net mam-

mary uptake of Lys, Met, and total AA reached the maximum when the Lys:Met ratio was 3:1 with supplementation with L-lysine-HCl and HMB (Table 5).

Our data agree with the study of Boston et al. (2000). The optimal ratio of Lys to Met to maintain the maximum milk yield should be based on meeting the total requirement of dietary protein and AA. Compared with cows fed ML, milk production was lower for cows fed C, which was inadequate in both Met and Lys although the ratio of Lys to Met was close to 3:1. These results indicate that based on meeting the requirement, appropriate quantities and the ratio of Lys to Met are critical for maximizing the synthesis of milk protein.

The changes in some metabolite concentrations in blood may reflect the responses of dairy cows to the supply of AA and other nutrients from diets. In previous AA infusion studies, increasing the supply of Met linearly reduced plasma NEFA concentrations (Socha, 1994; Pisulewski et al., 1996). In this study, Met supplementation decreased the NEFA concentration in serum, but Lys supplementation had no effect on NEFA concentration. This result suggested that with Met supplementation, mammary gland uptake of NEFA was increased, resulting in a higher concentration of milk fat. This effect was not observed in other studies (Xu et al., 1998; Socha et al., 2005). The reason for the difference is unclear, but might be related to enhanced fatty acid transport and clearance by choline as a result of increased Met supply. Additionally, treatment duration could be important. In production studies, blood samples are usually collected several weeks after initiation of treatment. In contrast, in infusion experiments, the length of experimental periods is usually from 10 to 14 d (Socha, 1994; Pisulewski et al., 1996). In the study of Pullen et al. (1989), Met hydroxy analog had no effect on incorporation of NEFA into plasma triglyceride, indicating that the analog probably did not alter hepatic lipoprotein synthesis. This result is in line with the present study in which supplementation of Met had no effect on plasma concentrations of high density lipoprotein, low density lipoprotein, or triglyceride.

In dairy cows, urea N in blood, urine, and milk is mainly derived from excessive ammonia absorbed through the rumen wall from degradation of dietary CP in the rumen, and from the deamination of AA, which may be from MP and catabolism of body tissue protein (Linn and Olson, 1995; Wang et al., 2008). In the present study, supplementation of both synthetic Lys and HMB significantly decreased urea N concentration in serum, urine, and milk compared with the control. Similar results were reported by Johnson-Van-Wieringen et al. (2007). Assuming that there were no effects of adding a small amount of protected Lys and Met on CP degradability in the rumen, the decrease in urea N concentration indicates that supplementation of protected Met and Lys to the control diet in the present study may have improved AA balance in MP and resulted in less deamination of absorbed AA. This consideration was further supported by the fact that in the present study, treatment ML had a higher N utilization efficiency (milk N/N intake) than the control. The N utilization efficiency in lactating dairy cows is influenced by the quality and quantity of dietary protein and can be reduced by 0.672 with every 0.1% increment of dietary CP (Yan et al., 2006).

CONCLUSIONS

The addition of HMB and L-lysine-HCl to a Met- and Lys-inadequate diet resulted in an increase in the amount of Met and Lys in MP, and consequently increased milk yield, milk protein yield, and milk fat content. Based on meeting the requirement, an appropriate ratio (3:1) of Lys to Met is critical for maximizing the synthesis of milk protein, hence improving milk and milk protein yield and N utilization efficiency, provided that dietary CP concentration is relatively stable.

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