



## Effect of feeding methionine supplements with different rumen escape values on performance of high producing dairy cows in early lactation

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### Abstract

A study was undertaken to compare a liquid form of methionine hydroxy analog (MHA; Novus Intl., Atlanta, GA, USA) and D,L-methionine, two methionine supplements with different rumen degradation escape values, on early lactational and reproductive performance by high producing dairy cows. Forty pregnant Holstein cows housed in a free-stall barn, were blocked by parity, date of calving, and previous 305-day mature equivalent milk production, and at calving were assigned randomly to one of two total mixed rations (TMR) containing MHA, or D,L-methionine, and group-fed for ad libitum intake. Cows spent  $33 \pm 15.0$  days in the fresh group, after which they were moved to the high producing group where they stayed up to 8-week postpartum. The TMR were formulated to meet approximately 100% of required methionine, lysine, and other essential amino acids. An adequate amount of D,L-methionine was fed in order to provide a similar amount of methionine posturally as provided by MHA, assuming a rumen degradation escape value of 40% for MHA and 22% for D,L-methionine. The TMR had forage to concentrate ratio of 40 to 60% for fresh group cows and 42 to 58% for high group cows. There were no differences between treatments in milk yield, content of milk fat, CP and true protein, linear somatic cell count, change in body condition score, and days to first service. In conclusion, D,L-methionine performed as well as MHA in promoting milk yield and contents of milk fat and protein when fed at levels aimed at

*Abbreviations:* AA, amino acid(s); ADF, acid detergent fiber; ADL, acid detergent lignin; CP, crude protein; D,L-Met, D,L-methionine; DM, dry matter; HMB, D,L-2-hydroxy-4-(methylthio)-butanoic acid; MHA, methionine hydroxy analog; N, nitrogen; NDF, neutral detergent fiber; NDICP, neutral detergent insoluble crude protein; NEFA, non-esterified fatty acids; TMR, total mixed ration(s)

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supplying similar amounts of methionine postruminally as would be supplied by MHA fed at the recommended level.

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## 1. Introduction

Methionine and lysine are considered the most limiting amino acids (AA) when high producing dairy cows are fed a variety of corn-based diets in early and mid-lactation (Schwab et al., 1992; Rulquin et al., 1993; NRC, 2001). In several studies, postruminal infusion of methionine has been shown to improve milk yield and milk fat (Rulquin et al., 1993), or yield of milk protein, especially casein (Rulquin et al., 1993; Pisulewski et al., 1996). Supplementing the diets of dairy cows with ruminally protected methionine and ruminally protected lysine has been shown to increase milk yield (Robinson et al., 1995; Wu et al., 1997; Xu et al., 1998), milk lactose (Robinson et al., 1995), and more frequently milk protein (Robinson et al., 1995, 1998, 1999; Wu et al., 1997; Xu et al., 1998). Improvement in milk yield from ruminally protected methionine and ruminally protected lysine supplementation, when observed, is generally limited to cows in early lactation (Xu et al., 1998).

In order to be useful, the ruminally protected AA or analogs must be released in the small intestines for absorption. The most common AA analog is D,L-2-hydroxy-4-(methylthio)-butanoic acid (HMB), more commonly known as methionine hydroxy analog (MHA). While MHA is not a true protected AA, it shows resistance to degradation by rumen bacteria (Salsbury et al., 1971; Belasco, 1972; Patterson and Kung, 1988), and when absorbed can be metabolized to methionine (Wester et al., 2000).

Free AA may also make useful feed supplements even when fed in an unprotected form to high yielding dairy cows. In recent studies, it was shown that even though free AA are rapidly degraded in the rumen (Patterson and Kung, 1988), free methionine and lysine had relatively high rumen degradation escape values when they were fed to high producing dairy cows (Velle et al., 1997, 1998; Volden et al., 1998).

The objective of this study was to compare a liquid form of MHA (Novus Intl., Atlanta, GA) and D,L-methionine (D,L-Met), two methionine supplements with different rumen degradation escape values, on milk yield, milk composition, reproductive performance, and health of high producing multiparous Holstein cows in early lactation.

## 2. Materials and methods

### 2.1. TMR and treatments

Total mixed rations (TMR) containing supplemental methionine (Table 1) were formulated using the CPM Dairy<sup>®</sup> nutrition model (version 1.0; Cornell–Penn–Miner, Cornell University, Ithaca, NY, USA). The fresh group total mixed ration was formulated for a cow 20 days in milk, with a body condition score of 2.75, body weight of 612 kg, daily DM

Table 1

Composition of TMR containing methionine hydroxy analog (MHA) or D,L-methionine (D,L-Met)

	Fresh group cows		High group cows	
	MHA	D,L-Met	MHA	D,L-Met
<b>Ingredient composition (g/kg DM)</b>				
Corn silage	179.6	179.4	205.2	205.0
Alfalfa grass silage	182.7	182.4	191.1	191.0
Mixed cool season grass hay	38.7	38.8	26.9	26.9
Whole cottonseed	61.2	61.1	62.6	62.6
Beet pulp	55.0	55.0	45.1	45.1
Ca-PFAD <sup>a</sup>	7.1	8.1	6.9	6.9
Sodium bicarbonate	17.0	16.7	14.4	14.4
Ground corn	98.2	98.0	100.0	99.8
Lactose sugar	12.4	12.4	10.2	10.2
Sugar cane molasses	36.7	36.7	30.6	30.6
Concentrate mix <sup>b</sup>	311.4	311.5	307.2	307.5
<b>Chemical composition (g/kg DM)</b>				
Crude protein	181.8	181.1	181.7	181.8
Soluble protein	53.9	54.2	54.8	55.2
Rumen undegradable protein	68.9	69.3	68.1	68.7
Rumen degradable protein	112.9	111.8	113.6	113.1
Methionine	2.5	2.5	2.5	2.5
Lysine	7.9	7.9	7.9	7.8
Neutral detergent fiber	325.5	330.8	325.2	330.2
Neutral detergent fiber crude protein	23.4	24.0	23.0	23.3
Fat	45.8	46.5	46.3	47.0
Non-fiber carbohydrates	383.2	379.2	386.1	381.1
Ash	87.1	86.5	83.8	83.3
Calcium	11.4	11.7	11.2	11.7
Phosphorus	4.4	4.4	4.5	4.4
<b>Chemical composition (g/kg rumen undegradable protein)</b>				
Methionine	15.1	15.1	15.1	15.1
Lysine	51.7	51.6	51.7	51.5
<b>Chemical composition (g/kg essential amino acids)</b>				
Methionine	47.7	48.0	47.5	47.7
Lysine	147.4	147.3	146.5	146.2
<b>Chemical composition (g/kg metabolizable protein)</b>				
Methionine	22.0	22.2	21.8	22.0
Lysine	69.4	69.4	68.5	68.3

<sup>a</sup> Calcium salts of palm oil fatty acid distillates (Church & Dwight Co. Inc., NJ).<sup>b</sup> The MHA concentrate mix contained on DM basis, 169.6 g/kg steam-rolled corn, 86.4 g/kg wheat flour (Red Dog), 196.4 g/kg solvent extracted soybean meal (490 g/kg CP as fed), 64.4 g/kg excel soy, 6.4 g/kg corn gluten meal (600 g/kg CP as fed), 83.9 g/kg canola meal, 210.4 g/kg wheat middlings, 52.0 g/kg soybean hulls, 32.0 g/kg blood meal, 32.1 g/kg fishmeal (Sea Lac), 10.9 g/kg dehydrated alfalfa meal, 8.8 g/kg salt, 17.7 g/kg calcium carbonate, 12.8 g/kg calcium sulfate, 1.2 g/kg 854-Dairy 5× trace mineral mix, 12.9 g/kg selenium supplement, and 2.1 g/kg MHA. The D,L-Met concentrate mix contained on DM basis, 168.4 g/kg steam-rolled corn, 84.8 g/kg wheat flour (Red Dog), 194.9 g/kg solvent extracted soybean meal (490 g/kg CP as fed), 64.0 g/kg excel soy, 6.3 g/kg corn gluten meal (600 g/kg CP as fed), 83.3 g/kg canola meal, 215.9 g/kg wheat middlings, 51.6 g/kg soybean hulls, 31.7 g/kg blood meal, 31.8 g/kg fishmeal (Sea Lac), 10.8 g/kg dehydrated alfalfa meal, 8.7 g/kg salt, 17.5 g/kg calcium carbonate, 12.6 g/kg calcium sulfate, 1.2 g/kg 854-Dairy 5× trace mineral mix, 12.7 g/kg selenium supplement, and 3.8 g/kg D,L-Met.

intake of 21.7 kg, and producing 41.0 kg of milk per day containing 3.80% fat and 3.30% CP. The high group TMR was formulated for a cow 70 days in milk, with a body condition score of 3.00, body weight 612 kg, daily DM intake of 26.5 kg, and producing 45.5 kg of milk per day containing 3.80% fat and 3.30% CP. The TMR consisted on DM basis of approximately 40% forage and 60% concentrate for fresh group cows and 42% forage and 58% concentrate for high group cows.

All TMR were formulated to meet 100% of required methionine, lysine and other essential AA. The amounts of lysine and methionine in metabolizable protein were close to the recommended levels of 7.2 and 2.4%, respectively (NRC, 2001), while maintaining a lysine to methionine ratio of 3:1 when AA were expressed as a percentage of metabolizable protein or essential AA. Before AA supplements were added, TMR for fresh and high group cows were determined to be deficient in methionine (NRC, 2001), containing 1.98 and 1.91%, respectively, of methionine in metabolizable protein.

The supplemental methionine was added to the diets as provided by MHA or D,L-Met. Sufficient amounts of D,L-Met were fed in order to provide approximately a similar amount of methionine postruminally as MHA, assuming a rumen degradation escape value of 22% for D,L-Met and 40% for MHA (Table 2). However, the amount of rumen degradable methionine supplied by D,L-Met was higher than the amount supplied by MHA. The rumen degradation escape value of 22% for D,L-Met was chosen based on the work of Volden et al. (1998), while the rumen degradation escape value of 40% for MHA was based on the manufacturer's recommendation. MHA comprised 0.06% of the total diet, while D,L-Met comprised 0.12% of the total diet for the respective TMR for both fresh and high group cows.

Corn silage ensiled in silage bags made of 9 mm thick plastic (Ag-Bag, Int., Warrenton, OR, USA) was fed during the first 2 months of the study and corn silage ensiled in a bunk

Table 2

Estimated intake and methionine (DM basis) being supplied by methionine hydroxy analog (MHA) or D,L-methionine (D,L-Met) in TMR containing MHA or D,L-Met<sup>a</sup>

	Fresh group cows		High group cows	
	MHA	D,L-Met	MHA	D,L-Met
Total DM intake (kg)	21.71	21.74	26.47	26.49
Concentrate intake (kg)	6.76	6.77	8.13	8.15
Met <sup>b</sup> from MHA product				
g	14.20	–	17.07	–
g/kg concentrate	2.1	–	2.1	–
Met from D,L-Met product				
g	–	25.73	–	30.97
g/kg concentrate	–	3.8	–	3.8
Rumen degradable Met from MHA product (g)	8.53	–	10.24	–
Rumen degradable Met from D,L-Met product (g)	–	20.07	–	24.16
Rumen escape Met from MHA product (g)	5.68	–	6.83	–
Rumen escape Met from D,L-Met product (g)	–	5.66	–	6.81

<sup>a</sup> Estimated using the CPM Dairy<sup>®</sup> nutrition model (version 1.0; Cornell–Penn–Miner, Cornell University).

<sup>b</sup> Methionine.

silage was fed during the remaining 4 months of the study. Ground corn was prepared by grinding corn grain to pass through a 1.18 cm screen in a feed mill hammer mill.

Feed was mixed in a Reel-Auggie<sup>®</sup> mixer wagon (model 3300; Knight, Brodhead, WI, USA). During mixing, dietary ingredients were put into the mixer wagon in the following order: concentrate mixes, ground corn, whole cottonseed, beet pulp, grass hay, alfalfa grass silage, corn silage, and molasses, and allowed to mix for about 5 min before feeding.

## 2.2. Cows and management

Forty pregnant Holstein cows housed in a free-stall barn at the Miner Institute were paired and assigned to 20 blocks, and at calving the two animals in each block were assigned randomly to one of two TMR containing MHA, or D,L-Met. Parity, date of calving and previous 305-day mature equivalent milk production were used as blocking criteria, such that animals in the same block had similar calving dates (i.e. were calving within a few weeks from each other), were of the same parity and had similar 305-day mature equivalent milk production. In order to monitor the dietary cation–anion balance of pregnant cows, urine was sampled weekly from four cows selected at random and pH was determined using a pH meter (Oakton pHTester, Cole-Parmer Instrument Co., Singapore). Following calving, all multiparous cows received 500 ml of 23% calcium solution, 500 ml of 50% dextrose solution and 5 cc oxytocin intravenously, as well as 10 cc of Vitamin E-selenium, 10 cc of Vitamin B complex and 2 cc of *Escherichia coli* vaccine intramuscularly. Cows were checked 20–30 days post-calving by a veterinarian and if no health problems were found, they were bred on subsequent heat by artificial insemination without instituting a voluntary waiting period. Management practices such as the use of prostaglandins were similar for both treatment groups. After  $33 \pm 15.0$  days, cows were moved from the fresh group to the high producing group within their treatment assignments. All cows received 500 mg-injections of bST every 2 weeks (Posilac<sup>®</sup>; Monsanto, St. Louis, MO, USA) beginning when cows were 100 days in milk or were confirmed pregnant and greater than 70 days in milk, whichever came first. Cows were housed in a free-stall barn and group-fed for ad libitum intake once a day at 08:00 h for 8-week postpartum or until first breeding service. The animal stalls were bedded with sawdust, which was changed once weekly. The animal pens were of the same size with similar animal density, bunk space, stall design, flooring, water accessibility, and all animals had free and equal access to feed. Cows were milked three times per day in a double-six herringbone milking parlor at 04:30, 12:30, and 20:30 h.

Cows were scored for body condition (Wildman et al., 1982) biweekly for 8-week postpartum. Body condition scores were based on a five-point scale with 0.25-unit intervals where 1 = emaciated, and 5 = obese. Reproductive performance was assessed as days to first service and first service conception rate. Animals were monitored for clinical ketosis, displaced abomasums, lameness, mastitis, metritis, milk fever, retained placentas, and scours during the study.

## 2.3. Sampling and chemical analyses

Alfalfa grass silage that was ensiled in silage bags was analyzed prior to the beginning of the study and monthly thereafter. Corn silage that was ensiled in silage bags was analyzed

prior to the beginning of the study and a month later, until it was finished, at which animals were fed corn silage ensiled in the bunk silo. Corn silage ensiled in the bunk silo was analyzed when the bunk was opened and monthly thereafter. Mixed cool season grass hay, whole cottonseed, beet pulp, ground corn, and concentrate mixes were analyzed at the beginning of the study and when any new deliveries were made. The TMR were sampled every week and dried to determine DM concentration for use in estimating group intake by animals in each pen.

The pH of silage samples was determined using a digital pH meter. The silage samples were processed according to Fenner (1984) for the determination of lactic acid and volatile fatty acids (acetic, propionic and butyric acids) using gas chromatography (Supelco Inc., 1975). The equipment used were a Varian 3700 gas chromatograph (Varian Inc., Walnut Creek, CA, USA), a 4% carbowax 20M/80/120 carbopack B-DA column (Supelco, Bellefonte, PA, USA) at the temperature setting of 175 °C, and a Perkin-Elmer LC-100 intergrator (Perkin-Elmer Corp., Norwalk, CT, USA). The flow rates for the nitrogen, hydrogen, and air, respectively, were 24, 30, and 300 ml/min. In addition, samples of silages, feed by-products and concentrate mixes used in the experiment were sent to Cumberland Valley Analytical Services (Hagerstown, MD, USA) where, after being dried (60 °C) and ground to pass through a 1-mm screen using a cyclone mill (Udy Co., Fort Collins, CO, USA), they were analyzed for DM by drying a 1 g sample in duplicate at 100 °C in a conventional oven for 24 h, ash by burning a 2 g sample in duplicate at 600 °C for 2 h in a muffle furnace (Method 942.05; AOAC, 1995), fat (Method 920.39; AOAC, 1995), N (Method 984.13; AOAC, 1995) and CP calculated as  $N \times 6.25$ , neutral detergent fiber with residual ash (NDF, using  $\alpha$ -amylase and sodium sulfite), acid detergent fiber (ADF), and acid detergent lignin (ADL) (Van Soest et al., 1991). Neutral detergent insoluble CP (NDICP) was determined by analyzing the residual CP in NDF. Soluble N was determined as the difference in N concentration of sample before and after soaking in borate phosphate buffer at pH 6.8 (Krishnamoorthy et al., 1982). The non-fiber carbohydrates were calculated as the difference between 100 and the sum of CP, NDF (minus NDICP), fat, and ash. Sulfur was analyzed using a Leco Model SC-432 (Leco, St. Joseph, MI, USA). Analysis of calcium, magnesium, potassium, sodium, iron, zinc, copper, and manganese were done using atomic absorption spectrophotometry (Method 968.08; AOAC, 1995). Phosphorus was analyzed by colorimetry (Method 965.17; AOAC, 1995), and chloride ion was determined using a Brinkman Metrohm 716 Titrino titration unit with a silver electrode (model 716; Brinkman Instruments Inc., Westbury, NY, USA). Corn silage and ground corn samples also were analyzed for sugars and starch at West Virginia University (Morgantown, WV, USA) using a modified enzymatic digestion procedure of Smith (1969) that used potassium ferricyanide instead of copper sulfate to bind glucose. Unbound potassium ferricyanide was read at a wavelength of 422 nm using a Hitachi UV-Vis Spectrophotometer (model U-1500; Hitachi Instruments Inc., San Jose, CA, USA).

Cows were milked three times a day and milk yield recorded from which a weekly average for each cow was calculated for 8-week postpartum. Milk samples were collected once a week during the afternoon milking and sent to Dairy One (Ithaca, NY, USA) for analyses of fat and CP by infrared procedures (Foss 4000; Foss Technology, Eden Prairie, MN, USA), and analysis of somatic cell count by infrared procedures (Foss 5000; Foss Technology, Eden Prairie, MN, USA). Milk samples collected from other commercial dairy farms were

used as external standards in analysis of milk components. These standards were stored under refrigeration at 0–4 °C for a maximum of 14 days. Standards were analyzed for fat (Method 905.02; AOAC, 1995), N (Method 991.22; AOAC, 1995), and somatic cell count (Method 975.16; AOAC, 1995) to provide the wet chemistry values that were used to calculate standard curves used in the respective infrared procedures.

Ten blocks, a total of 10 cows per treatment, were randomly selected and blood collected 3–4 h postfeeding at 7 days prepartum, at calving, and at 14 and 28 days postpartum from the tail vein using one 9.5 cc volume serum separator vacutainer tube. Following blood collection, samples were chilled in ice and centrifuged for 15 min at  $500 \times g$  and the serum analyzed for non-esterified fatty acids (NEFA) using a commercial diagnostic kit (Wako Pure Chemical Ind., Ltd., Osaka, Japan). Urine was also collected from all cows 14 days postpartum and ketones measured using Ketostix<sup>®</sup> reagent strips (Bayer Corp., Elkhart, IN, USA).

#### 2.4. Statistical analysis

Because in dairy cows milk production and other related parameters may display strong autocorrelation, data for milk yield and composition, and body condition score, were analyzed as repeated measures using the PROC MIXED procedure of the SAS [version 6.12, 1996]; cow, block, treatment and week served as class variables. The statistical model used, which is shown below, included treatment, week, treatment by week interaction, and treatment by block was the random effect used to test treatment.

$$Y_{ijk} = \mu + \tau_i + \tau\gamma_{ij} + \beta_k + \tau\beta_{ik} + e_{ijk}$$

where,  $\mu$  is the overall mean,  $\tau_i$  the effect of the  $i$ th treatment ( $I = 1-2$ ),  $\tau\gamma_{ij}$  the interaction between treatment and block ( $\gamma_j$  the effect of the  $j$ th block ( $j = 1-20$ )),  $\beta_k$  the  $k$ th week ( $k = 1-8$ ),  $\tau\beta_{ik}$  the interaction between treatment and week, and  $e_{ijk}$  is the residual error, which together with block were assumed to be randomly distributed.

The NEFA in blood collected 7 days prepartum were used as a covariate in the analysis of NEFA in blood collected at calving, and at 14 and 28 days postpartum. Treatment and block served as class variables in the analysis of NEFA and days to first service data.

### 3. Results

All TMR contained similar nutrient concentrations (DM basis), which were approximately 328 g/kg NDF, 182 g/kg CP, 2.5 g/kg methionine, and 7.5 g/kg lysine. Chemical analyses of major TMR ingredients and concentrate mixes are shown in Table 3. The chemical composition of the forages and major concentrate ingredients was consistent with reported values (NRC, 2001).

CPM Dairy<sup>®</sup> nutrition model showed that adding methionine supplement to the TMR as MHA or D,L-Met, increased the predicted metabolizable protein and methionine allowable milk, respectively, by 0.5 and 1.2%, and increased the predicted metabolizable protein and methionine allowed true protein, respectively, by 0.5 and 1.3%.



Composition (mg/kg DM)												
Iron	81	148	208	62	56	159	118	289	291	31	294	243
Zinc	19	24	33	28	37	47	91	159	163	19	31	37
Manganese	27	22	37	34	14	35	141	106	111	8	33	97
Copper	8	8	15	9	8	13	9	25	30	2	8	18

<sup>a</sup> Ensiled in silage bags made of 9 mm thick plastic (Ag-Bag Int., Warrenton, OR, USA).  
<sup>b</sup> Ensiled in a bunk silo.  
<sup>c</sup> Reed canary grass, timothy grass and orchard grass in ratio of 40:40:20, respectively, harvested at seed heading stage of maturity.  
<sup>d</sup> Solvent extracted.  
<sup>e</sup> Sugar and starch were determined by enzymatic procedure (Smith, 1969).

Table 4

Milk yield, milk composition, and body condition score of lactating Holstein cows fed TMR containing methionine hydroxy analog (MHA) or D,L-methionine (D,L-Met) during weeks 1–8 postpartum<sup>a</sup>

	MHA	D,L-Met	S.E.M.	<i>P</i>
Milk yield (kg per day)	48.9	49.8	1.59	0.671
3.5% FCM yield (kg per day)	52.9	55.3	1.99	0.503
Milk fat				
g/kg	40.4	42.2	0.93	0.281
kg per day	1.96	2.08	0.083	0.954
Milk CP				
g/kg	31.3	32.1	0.43	0.910
kg per day	1.51	1.58	0.042	0.981
Milk true protein				
g/kg	29.6	30.3	0.42	0.919
kg per day	1.43	1.50	0.039	0.983
Milk urea nitrogen (mg/dl)	12.8	12.9	0.38	0.821
Linear somatic cell count <sup>b</sup>	4.09	4.56	0.284	0.563
Change in body condition score	−0.21	−0.11	0.063	0.283

<sup>a</sup> The average DMI by cows fed the two TMR was  $24.5 \pm 2.25$  kg per day per animal.

<sup>b</sup> Calculated by expressing the somatic cell count values to their natural logarithm.

The dairy cows used in this study were at a high level of productivity. Cows produced about 45.5 kg of milk during weeks 1–4 postpartum and 53.3 kg during weeks 5–8 postpartum. Even though there were no statistical differences in milk yield between cows that were fed TMR containing MHA with those fed TMR containing D,L-Met during weeks 1–8 postpartum ( $P = 0.671$ ), the later group of cows produced 0.9 kg more milk per day (Table 4). Previous 305-day mature equivalent milk production by the cows fed the TMR containing MHA and those fed TMR containing D,L-Met were similar, being 14,953 and 14,788 kg (S.E.M. = 200.8;  $P = 0.568$ ), respectively, indicating that blocking of cows effectively eliminated bias of milk production. Overall, there were no treatment differences in FCM yield ( $P = 0.503$ ), milk fat content ( $P = 0.281$ ), milk CP ( $P = 0.910$ ), milk true protein ( $P = 0.919$ ), milk urea N ( $P = 0.821$ ), linear somatic cell count ( $P = 0.563$ ), and change in body condition score ( $P = 0.283$ ). The average daily DM intake by the group-fed cows across treatments was  $22 \pm 2.5$  kg per cow for the fresh group cows and  $27 \pm 2.0$  kg per cow for high group cows.

There were no treatment differences in days to first service. The mean days to first service for cows that were fed TMR containing MHA and TMR containing D,L-Met, respectively, were 68.7 and 65.1 (S.E.M. = 6.61;  $P = 0.702$ ). The median of days to first service for cows that were fed TMR containing MHA and TMR containing D,L-Met, respectively, were 66 and 61. The first service conception rate for cows that were fed TMR containing MHA and D,L-Met, respectively, were 36.9 and 35.0%.

There were no treatment differences in NEFA concentrations in blood sampled at calving ( $P = 0.815$ ), and at 14 ( $P = 0.186$ ) and 28 days ( $P = 0.373$ ) postpartum (Table 5). The average pH of urine in prepartum cows was  $5.8 \pm 0.58$ . Analysis of urine collected 14 days

Table 5

Serum concentration of NEFA ( $\mu\text{eq/l}$ ) in blood drawn from lactating Holstein cows fed TMR containing methionine hydroxy analog (MHA) or D,L-methionine (D,L-Met)

Days postpartum	MHA	D,L-Met	S.E.M.	<i>P</i>
0 <sup>a</sup>	833.5	888.2	160.92	0.815
14	524.7	406.8	59.09	0.186
28	385.1	294.8	68.21	0.373

<sup>a</sup> Sampling conducted at calving.

postpartum showed no treatment differences in incidence of urinary ketosis (ketonuria) among cows. The majority of the cows that tested positive to the ketosis test had only traces of ketones (5 mg/dl), and none of the animals developed acute ketosis. Among the 20 cows that were fed TMR containing MHA, four cows had a ketone concentration of 5 mg/dl and two cows had a ketone concentration of 40 mg/dl. Among the 20 cows that were fed TMR containing D,L-Met, three cows had a ketone concentration of 5 mg/dl, one cow had a ketone concentration of 15 mg/dl, and one cow had a ketone concentration of 40 mg/dl. Incidences of other health problems were generally similar for both treatment groups.

#### 4. Discussion

The concentration of MHA in the diet was approximately 0.1% or 1.0 g/kg DM. This level of inclusion was similar to the one recommended by Sloan et al. (2000), who in a study with a continuous culture system reported that the optimum concentration of dietary HMB that facilitates an improvement in protein degradation and assimilation of N into microbial protein was 0.11%. The continuous culture system used by Sloan et al. (2000) operated with a 12% liquid and 4.2% solids dilution rate, and one of the test diets formulated using CPM Dairy<sup>®</sup> nutrition model to exceed 100% of required methionine and lysine, contained ingredients similar to what was used in our study.

In our study, the rumen degradation escape value of 22% used for D,L-Met was based on the work of Volden et al. (1998). In studies in which ruminally unprotected AA were fed, it was shown that methionine and lysine had relatively high rumen degradation escape values (Velle et al., 1997, 1998; Volden et al., 1998). Volden et al. (1998) reported that the mean rumen degradation escape value for free methionine was 22.1%. The rumen degradation escape value of 40% used for MHA in our study was provided by Novus Int., the manufacturer of the product. However, Koenig et al. (1999) in an experiment with Holstein cows fitted with ruminal and duodenal cannulas reported that based on fractional rate constants for ruminal and duodenal disappearance of the liquid form of MHA and passage rate of the liquid,  $50 \pm 2.8\%$  of MHA escaped ruminal degradation. The liquid passage rate reported in the study conducted by Koenig et al. (1999) was  $12.3\text{ h}^{-1}$ , which gives a ruminal residency time of 8:13 h for the liquid phase. There have also been reports of wide variations in the amounts of MHA and D,L-Met that are degraded by rumen microorganisms in vitro, probably because of differences in the ruminal inoculum and conditions of the assay used (Belasco, 1972; Patterson and Kung, 1988). Since the rumen degradation escape value of

22% for free methionine was determined using high producing dairy cows (Volden et al., 1998), it would be therefore logical to assume that this was the more appropriate value to use in our study which was also conducted using high producing dairy cows.

A control treatment was not used in our study for comparison with TMR supplemented with MHA and D,L-Met because the benefits of adding methionine supplements to diets for lactating cows on milk yield, and especially milk protein are well established (NRC, 2001; Sniffen et al., 2001). From the data that was summarized by Rulquin and Verite (1993) and Rulquin et al. (1993, 1995) it was shown that methionine and lysine, respectively, need to be 2.5 and 7.3% of metabolizable AA in diets for lactating dairy cows in order to optimize milk protein and yield. Recently, NRC (2001) summarizing a more extensive database arrived at approximately the same conclusions of 2.4 and 7.2% of metabolizable AA, respectively. Milk protein appears to be dramatically reduced when diets provide less than 2.10–2.20% methionine or 6.0–6.5% lysine (Sniffen et al., 2001; NRC, 2001). However, it is difficult to reach the optimum concentrations without single sources of methionine and lysine. Chalupa et al. (2001) show potential responses of milk protein and milk yield to increased metabolizable methionine and lysine. In our study the predictions made by CPM Dairy<sup>®</sup> nutrition model show that a control treatment would have lower metabolizable protein and methionine allowable milk, and lower metabolizable protein and methionine allowable true protein compared to diets supplemented with MHA or D,L-Met. Differences in milk yield and protein between cows fed a control diet and those fed diets supplemented with MHA or D,L-Met would therefore be expected to be large (Sniffen et al., 2001). Failure to observe treatment differences in milk yield, milk composition, reproductive performance, and health problems could be attributed to sufficient amount of free D,L-Met escaped ruminal fermentation with the liquid phase (Velle et al., 1997, 1998; Volden et al., 1998). This may therefore make free AA useful feed supplements even when fed in an unprotected form to high producing dairy cows. Alternatively, D,L-Met could also have stimulated microbial growth and efficiency from the additional rumen degradable methionine, which was approximately 135% more compared to MHA (Kajikawa, 2000). Even though ruminal bacteria can grow on ammonia nitrogen as a sole source of nitrogen, their growth efficiency is improved in the presence of AA, especially tryptophane, tyrosine, glutamate and methionine (Van Soest, 1994; Kajikawa, 2000).

The NEFA concentrations in all blood samples from both groups of cows were within ranges reported by other researchers as normal (Drackley et al., 1991, 1992; Smith et al., 1997). This is consistent with the urine ketone concentrations determined 14 days postcalving, which showed a low incidence of ketosis.

## 5. Conclusions

When D,L-Met was fed to supply a similar amount of methionine postruminally as provided by MHA, it performed similarly to MHA in promoting milk yield, contents of milk fat and protein, linear somatic cell count, animal body condition, and days to first service in dairy cows. Overall, D,L-Met performed as well as MHA when fed at levels aimed at supplying similar amounts of methionine postruminally as would be supplied by MHA fed at the recommended level.

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