EFFECTS OF MET HYDROXY ANALOG (MHA®) SUPPLEMENTATION OF DAIRY COW'S DIETS ON MILK YIELD AND MILK COMPOSITION

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Abstract

Twenty one Holstein Friesian crossbred (>87.5% Holstein Friesian) lactating dairy cows, averaging 103 ± 53 days in milk, 12.5 ± 3.0 kg of milk, 58 ± 19 mo old and 412 ± 56 kg body weight (BW), were blocked by parity first and then stratified random balanced for milking days, milk yield, age and body weight into three groups of 7 cows. The first group (control) received approximately 17.4 kgDM of total mixed ration (TMR) which comprised approximately 7, 6, and 30 kg of commercial concentrate, corn silage and fresh cut grass, respectively. The second group was fed with the basal diet as the control group and supplemented with 11 g/d of Met hydroxy analog (MHA®). The third group was fed the same way as the control group with 22 g/d of Met hydroxy analog (MHA®). Performance parameters showed that DM, CP, and NEL intakes, final body weight and live weight change were similar in all treatments. Milk yield and milk composition were unaffected, however, UFA was increased while SFA was reduced by MHA® supplementation.

Keywords: Met hydroxy analog, milk production, milk composition, milk fatty acids

Introduction

The strategy for meeting the metabolizable protein requirement of the high producing dairy cow is to first maximize microbial protein synthesis and flow, and then to meet any shortfall in metabolizable protein with bypass sources of protein and amino acids. Two amino acids that are most often limiting for the synthesis of milk and milk protein in high producing dairy cows are Lys and Met (Schwab *et al*., 1992; Rulquin *et al*., 1993; Pisulewski *et al*., 1996). Rumen-protected amino acids and analogs can be incorporated into the diet to target these specific amino acid deficiencies without contributing additional amino-N beyond the animal's requirements, but the success of their use depends on the

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confidence and accuracy of the estimated amino acid delivery. The dry calcium salt of D,L-2-hydroxy-4-(methylthio)-butanoic acid (HMB), more commonly known as Met hydroxy analog (MHA), has been the most extensively studied Met analog although it is no longer commercially available. The efficacy of the MHA to provide a source of Met depends on its resistance to microbial degradation in the rumen, its rapid escape from the rumen with the liquid phase of digesta, and its subsequent absorption and metabolism to Met within the tissues.

Increasing the duodenal amino acid supply may result in an improved pattern of amino acids for protein synthesis in tissues (Clark, 1975). Rumen-protected methionine added to diets containing soybean meal as the supplemental protein source has increased milk production (Schingoethe *et al*., 1988). The response to protected amino acid feeding reported in the literature is variable depending on the protein source in the basal diet. Inclusion of corn gluten meal as the major protein source in dairy cattle rations may effectively increase postruminal supply of most amino acids but may provide less than adequate lysine for maximal milk protein synthesis (Stern *et al*., 1983). Milk protein synthesis may be limited by the supply of precursors reaching the mammary gland, in particular the essential amino acids (Clark, 1975). If an essential amino acid is the key limiting substrate for milk protein synthesis and the amino acid transport systems are operating well below saturation in the mammary gland (Baumrucker, 1985), then increased delivery of a limiting amino acid should increase milk protein synthesis.

The most efficient way to increase the postruminal Met supply is to use "rumen-protected" Met. Numerous derivatives and analogs of Met (for a detailed list, see Schwab, 1995) have been tested for their resistance to degradation, with the Met hydroxy analog, or D, L-2-hydroxy-4-(methylthio)-butanoic acid (HMB), being the most studied. The use of RPMet in production studies with lactating dairy cows increased milk protein output by 5% (Robinson *et al*., 1995). Additionally, its main effects on milk production and composition were an increase in fat yield, especially when RPMet was given repeatedly at doses of 25 to 35 g/d to multiparous dairy cows in early lactation receiving an adequate protein supply in the diet, whereas protein yield was not modified and milk production was rarely increased by RPMet supple-mentation (St-Pierre and Sylvester, 2005). Recently, it has been demonstrated that the RPMet allows a significant Met supply to cows (Graulet *et al*., 2005) and an increase in their milk protein yield (Noftsger *et al*., 2005; St-Pierre and Sylvester, 2005). The objectiveof this study was to investigate the effects of feeding Met hydroxy analog (MHA®) onperformance of lactating dairy cows.

Materials and Methods

Animals and Treatments

Twenty one Holstein Friesian crossbred (>87.5% Holstein Friesian) lactating dairy cows, averaging 103 ± 53 days in milk, 12.5 ± 3.0 kg of milk, 58 ± 19 mo old and 412 ± 56 kg body weight (BW), were blocked by parity first and then stratified random balanced for milking days, milk yield, age and body weight into three groups of 7 cows. The first group (control) received approximately 17.4 kgDM of total mixed ration (TMR) which based on the requirement of the cows. TMR comprised approximately 7, 6, and 30 kg of commercial concentrate, corn silage and fresh cut grass (*Brachiaria ruziziensis,* 45 d cutting interval), respectively. The second group was fed with the basal diet the same as the control group and supplemented with 11 g/d of Met hydroxy analog (calcium salt of HMTBa (2-hydroxy-4 methylthio butanoic acid), MHA®) (Novus International Inc., USA). The third group was fed the same way as the control group but with 22 g/d of Met hydroxy analog (MHA®). Once ingested, 60% of the MHA® is utilized in the rumen where it stimulates microbial protein synthesis. The

remaining 40% leaves the rumen with the liquid phase of the digesta and is absorbed by diffusion along the digestive tract. Once absorbed, it is rapidly taken up by tissues where it is converted to L-methionine. MHA was supplemented by dress topping during TMR feeding after morning milking. All cows also had free access to clean water and were individually housed in a free-stall unit and individually fed according to treatments for three equal meals of TMR at 07.00, 10.00, and 16.00 h. Chemical and fatty acid compositions of TMR are given in Table 1 and Table 2. The experiment lasted for 35 days with the first 5 days was the adjustment period, followed by 6 periods of 5-day interval measurement period.

Measurements, Sample Collection, and Chemical Analysis

Feeds offered and left after eating of individual cow were weighed for two consecutive days of each period and samples were taken and dried at 60°C for 48 h. At the end of the experimental period, feed samples were composited and subsamples were taken for further chemical analysis. Samples were ground through 1 mm screen and subjected to proximate analysis. The crude protein content was determined by Kjeldahl analysis (AOAC, 1995). Ether extract was determined using petroleum ether in a Soxtec System (AOAC, 1995). Neutral detergent fiber and acid detergent fiber were determined using the method described by Van Soest *et al*. (1991), adapted for Fiber Analyzer. Chemical analysis was expressed on the basis of the final DM.

Cows were milked twice daily at 05.00 and 15.00 h and milk yields were recorded for each cow. Samples of milk (evening + morning) were collected at each milking for two consecutive days weekly and stored at 4°C with a preservative (bronopol tablet; D&F Control System, San Ramon, CA) until analyzed for fat, protein, lactose and solid not fat contents using a Milko-Scan S50 analyzer (Tecator, Denmark). All cows were weighed at the start and at the end of the experiment.

Milk Fatty Acid Analysis

Milk samples were collected from individual cow on day 25 of the experiment. For fatty acid analysis, an aliquot of milk (~30 mL) was centrifuged at 17,800 × 3 g for 30 min at 8°C, and then 300–350 mg of fat cake was removed. Lipid extraction was conducted following the procedures described by Hara and Radin (1978), using a volume of 18 ml of hexane and isopropanol (3:2, vol/vol)/g of fat cake. After vortexing, a sodium sulfate solution (6.7% $NaSO_4$ in distilled H_2O) was added at a volume of 12 ml/g of fat cake. The hexane layer was transferred to a tube containing 1 g of $NaSO_4$ and after 30 min, the hexane layer was removed and stored at –20°C until methylation.

Fatty acid methyl esters (FAME) were prepared by procedure described by Ostrowska *et al*. (2000). After methylation was completed, 10 ml of deionized water was added. The solution was transferred to a 40-ml centrifuged tube and 5 ml of hexane was added for FAME extraction. The solution was centrifuged at 2000g, at 10°C for 20 min and then the hexane layer was dried over sodium sulfate and was taken into vial for analysis by Gas Chromatography (GC) (Hewlett Packard GC system HP6890 A; Hewlett Packard, Avondale, PA) equipped with a 100 m × 0.25 mm fused silica capillary column (SP2560, Supelco Inc, Bellefonte, PA, USA). Injector and detector temperatures were 240°C. The column temperature was kept at 70°C for 4 min, then increased at 13°C/min to 175°C and held at 175°C for 27 min, then increased at 4°C/min to 215°C and held at 215°C for 31 min.

Feed Fatty Acid Analysis

TMR samples were collected from each of the treatment groups. They were then extracted for lipid (Folch, *et al*., 1957; and Metcalfe *et al*., 1996). Extracted lipids were further subjected to saponification and methylation following the procedures described by Ostrowska *et al*. (2000). Fatty acid methyl esters (FAME) were then subjected to fatty acid analysis by Gas Chromatography (GC)

Table 1. Chemical composition (% of DM) of TMR used in the experiment

Chemical composition	TMR
Dry matter	40.42
Crude protein	12.20
Crude fat	3.14
Ash	10.29
Crude fiber	25.00
Non-fiber carbohydrate	17.90
Neutral detergent fiber	62.12
Acid detergent fiber	35.40
Acid detergent lignin	6.04
Neutral detergent insoluble nitrogen	0.96
Acid detergent insoluble nitrogen	0.53
TDN1X (%)[1]	50.93
DEP (Mcal/kgDM)[3]	2.59
MEP (Mcal/kgDM)[4]	2.17
NELP (Mcal/kgDM)[5]	1.34

[1] TDN_{1X} (%) = tdNFC + tdCP + (tdFA × 2.25) + tdNDF – 7 (NRC, 2001)

[2] DE_{1X} (Mcal/kg) = [(tdNFC/100) × 4.2] + [(tdNDF/100) × 4.2]+[(tdCP/100) × 5.6] + [(FA/100) × 9.4] – 0.3

[3] DE_P (Mcal/kgDM) = DE_{1X} × Discount (NRC, 2001)

[4] ME_p = [1.01 × (DE_p) – 0.45] + [0.0046 × (EE – 3)] (NRC, 2001)

[5] NE_{LP} = ([0.703 × ME_p (Mcal/kg)] – 0.19) + ([(0.097 × ME_p + 0.19)/97] × [EE – 3]) (NRC, 2001)

Table 2. Fatty acid compositions of TMR (% of total fatty acid)

Fatty acid profile	TMR
C10:0	1.06
C12:0	16.66
C14:0	5.38
C16:0	17.44
C18:0	3.07
C18:1n9c	13.65
C18:2n6c	21.68
C18:3n3	13.52
C20:1	5.92
C20:5n3	1.15
C22:0	0.48

(Hewlett Packard GC system HP6890 A; Hewlett Packard, Avondale, PA) equipped with a 100 m × 0.25 mm fused silica capillary column (SP2560, Supelco Inc, Bellefonte, PA, USA).

Statistical Analysis

Measurements of intake, milk production and milk fatty acid composition were analyzed by ANOVA for a randomized complete block design (Steel and Torrie, 1980) using Statistical Analysis System (SAS, 1996).

Results and Discussion

Chemical compositions of TMR used in the present study are given in Table 1 and are in the ranges of commonly reported practice (NRC, 2001; Suksombat and Chullanandana, 2008). However, CP content of TMR seems to be lower than that recommended by NRC (2001) which should approximately be a minimum of 14% for diets of lactating dairy cows. In addition, NFC content was also lower while NDF and ADF are higher than that recommended by NRC (2001). The National Research Council (NRC) recommends that lactating cow rations should contain 30 to 40% NFC, 19 to 21% ADF and 25 to 28% NDF. Currently, many dairy nutritionists also evaluate the concentration of starch in the ration, with common recommendations ranging from a minimum of 21 to a maximum of 27 percent (NRC, 2001). The lower CP and NFC or higher NDF and ADF of TMR were due to mature fresh cut grasses containing high fiber and low CP and NFC added in TMR. TMR contained fatty acids in descending order; C18: 2n6c, C16:0, C12:0, C18:1n9c, and C18:3n3 of 21.68, 17.44, 16.66, 13.65, and 13.52% of total fatty acids, respectively (Table 2).

Dry matter (DM), crude protein (CP) and NE_L intakes, final body weight and body weight change were similar ($p > 0.05$) in all treatment groups (Table 3). Similar responses were reported in other studies (Rulquin and Delaby, 1977). On the other hand, in some studies, DM intake of cows receiving RPMet significantly increased or showed non-significant trends (Schwap *et al*., 1992; Vanhatalo *et al*., 1999; Trinacty *et al*., 2006). Non significant effect of MHA® supplementation on intake in the present study was probably due to the fact that cows produced low milk yield thus increased amino acid supply to duodenum had no effect on nutrient intake (Oldham, 1984).

There were no significant differences in milk, fat, protein, lactose, solid-not-fat (SNF) and total solid yields (Table 4). Milk compositions were also unaffected by supplementation of MHA® (Table 4). Milk yield in response to Met supplementation has not been consistent in the literature. Stage of lactation (Schwap *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 previous research works, milk and milk protein production were found to increase when high quality protein or mixtures of amino acids were infused into the abomasum of lactating dairy cows (Clark, 1975; Spires *et al*., 1975). Milk and milk protein yields have be were reported to increase in one study when hydroxymethylmethionine-calcium was fed to lactating cows (Kaufmann and Lupping, 1979). In other studies, feeding an analog of methionine [a-hydroxy-7-(methylmercapto) butyrate-calcium] either increased milk yield and fat production (Polan *et al*., 1970) or increased milk fat percentage (Bhargava *et al*., 1977). However, in other investigations this analog failed to improve these production parameters (Stokes *et al*., 1981). Lundquist *et al*. (1982) reviewed studies in which cows were fed with supplemental methionine or methionine hydroxy analog. Production of 4% fat-corrected milk tended to increase most when cows were fed with milk fat-depressing diets in early lactation. European researchers reported that feeding methionine embedded in a fat matrix to reduce its bacterial degradation in the rumen produced variable responses in milk yield (Kaufmann and Lupping, 1982). Other methods of protecting methionine have been investigated, but have not demonstrated a great potential for improving milk production or milk composition (Chalupa, 1975). However, in

Table 3. Effect of Met hydroxy analog (MHA®) supplementation on DM, CP, and NE_L intakes; live weight and live weight change

	Control	11 g MHA®/d	22 g MHA®/d	SEM	P-value
DM intake (kg/d)	13.85	13.52	13.49	0.14	0.50
CP intake (g/d)	1690	1651	1645	16.50	0.50
NE_L intake (Mcal/d)	18.51	18.07	18.02	0.18	0.50
Initial body weight (kg)	389	431	403	9.99	0.20
Final body weight (kg)	413	448	421	11.00	0.41
Body weight change (g/d)	824	500	600	90.49	0.34

SEM = standard error of the mean.

Table 4. Effect of Met hydroxy analog (MHA®) supplementation on milk yield

	Control	11 g MHA®/d	22 g MHA®/d	SEM	P-value
Milk yield, kg/d	11.60	11.74	10.88	0.48	0.73
3.5% FCM, kg/d	12.98	12.83	12.23	0.44	0.77
Milk fat					
%	4.22	4.14	4.29	0.13	0.89
g/d	492	486	467	16.39	0.83
Milk protein					
%	2.70	2.65	2.64	0.01	0.23
g/d	315	311	287	12.39	0.66
Milk lactose					
%	4.20	4.14	4.13	0.02	0.23
g/d	490	486	449	19.45	0.66
Milk solid non fat					
%	7.95	7.81	7.82	0.03	0.22
g/d	928	917	851	36.38	0.62
Milk total solid					
%	12.17	11.96	12.11	0.15	0.84
g/d	1420	1404	1318	50.43	0.70

SEM = standard error of the mean; 3.5% FCM = (0.432 × kg of milk) + (kg of fat × 16.23)

Table 5. Effect of Met hydroxy analog (MHA®) supplementation on milk fatty acids (% of total fatty acid)

	Control	11 g MHA®/d	22 g MHA®/d	SEM	P-value
C4:0	0.67[ba]	1.29	1.96[a]	0.18	0.04
C6:0	1.18	1.30	1.37	0.12	0.82
C8:0	0.93	0.82	0.86	0.05	0.71
C10:0	1.99	1.76	1.86	0.09	0.60
C11:0	0.32	0.31	0.29	0.02	0.70
C12:0	7.96	7.60	7.61	0.12	0.40
C13:0	0.31	0.32	0.29	0.01	0.63
C14:0	13.94	13.41	13.13	0.23	0.38
C14:1	1.94	2.14	1.77	0.08	0.21
C15:0	1.05	1.08	0.98	0.02	0.20
C16:0	33.88	31.31	32.56	0.81	0.45
C16:1	2.90	3.03	3.20	0.10	0.51
C17:1	0.23	0.26	0.24	0.04	0.97
C18:0	6.85	6.50	6.72	0.19	0.75
C18:1n9t	1.39	1.57	1.62	0.11	0.65
C18:1n9c	22.60[b]	25.53[a]	23.86[a]	0.39	0.03
C18:2n6c	1.01	1.00	1.03	0.03	0.96
C18:3n3	0.74[a]	0.15[b]	0.34[b]	0.08	0.03
C20:0	0.06	0.02	0.06	0.02	0.48
C20:1	0.18	0.21	0.15	0.02	0.37
C21:0	0.08[b]	0.40[a]	0.19[a]	0.05	0.04
Short chain FA	13.37	13.41	14.25	0.42	0.64
Medium chain FA	53.93	51.23	51.90	0.66	0.25
Long chain FA	32.93	35.39	33.98	0.52	0.18
Unsaturated FA	30.99[b]	33.90[a]	32.22[a]	0.43	0.04
Saturated FA	69.23[a]	66.13[b]	67.90[a]	0.44	0.04

SEM = standard error of the mean

[a,b,c] Means within row with different superscripts differ

dairy cows, HMB supplementation used to have an effect on rumen fermentations that induced an increase in the fat yield but extremely rarely in the protein yield (Bhargava *et al.*, 1977; Lundquist *et al.*, 1982; Hansen *et al.*, 1991) Thus, HMB did not seem to effectively replace absorbed Met for milk protein synthesis. The present study failed to find the different in milk yield and milk composition due to MHA® supplementation. This probably because the cows used in this study produce low milk yield, therefore, increase in Met supply to duodenum did not affect milk yield and milk composition.

Milk fatty acids, C4:0, C18:1n9c, C21:0 and UFA, were increased while C 8:3n3 and SFA were reduced by MHA® supplementation. Similar results were also reported (Pisulewski *et al.*, 1996; Třináctý *et al.*, 2006). Increases in UFA in milk due to MHA® supplementation in the present study suggested that Met may have impact on de novo synthesis of UFA in the mammary gland (Pisulewski *et al.*, 1996). Reducing the SFA content and increasing that of long-chain UFA have been associated with increased healthfulness of milk. In terms of human health, these changes may represent an improvement in the FA profile of milk because SFA have been reported to constitute the hypercholesterolemic portion of milk fat.

Conclusions

It can be concluded from the present study that supplementation of MHA® had no effect on feed intake, milk yield, milk composition and body weight change. However, it increased UFA and decreased SFA in dairy cows' milk.

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