Effects of dietary betaine on milk yield and milk composition of mid-lactation Holstein dairy cows

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ABSTRACT

Betaine, naturally found in plants and an oxidative product of choline, is converted to acetate in the rumen, which may be used for milk fat synthesis. The objective of this study was to determine the effect of supplemental dietary betaine on milk yield and milk composition. Eighteen Holstein dairy cows (126 ± 5 d in milk; mean $\pm$ SD) were randomly assigned to a sequence of treatments of rumen-unprotected betaine at 0, 25, 50, and 100 g/d added to a standard lactation ration in a 4×4 Latin square design. Animals were fed individually with feed intake and milk yield recorded daily. Body condition score and body weight were recorded on the last day of each period that lasted 16 d, with milk sampled on the last 2 d of each period. Milk composition was determined by a Dairy Herd Improvement Association laboratory and milk fatty acids were determined by gas chromatography. Data collected over the last 2 to 3 d were analyzed using the MIXED procedure in SAS (SAS Institute Inc., Cary, NC). Milk yield (mean $\pm$ SEM) was increased by betaine when fed at 100 g/d ($22.4, 22.5, 22.8, 24.1 \pm 1.19$ kg/d for 0, 25, 50, and 100 g of betaine/d, respectively). No effect of dietary betaine was detected on dry matter intake, feed efficiency, body weight, or body condition score. Percentages of milk fat, lactose, solids-not-fat, and somatic cell count were not altered; however, protein concentration was decreased by betaine supplementation as compared with the control (3.35, 3.28, 3.27, and $3.28 \pm 0.07\%$ for 0, 25, 50, and 100 g of betaine/d, respectively). Daily yields of milk protein, fat, lactose, energy-corrected milk, and 3.5% fat-corrected milk did not differ with betaine supplementation. Overall, inclusion of dietary betaine at 100 g/d increased milk yield, whereas all levels of betaine supplementation decreased milk protein percent and slightly altered milk fatty acid profile. Further studies are needed to determine the

ruminal fermentation characteristics and the optimum rate of supplemental betaine for dairy cows.

Key words: betaine, milk yield, milk fatty acid

INTRODUCTION

Betaine, naturally present in wheat and sugar beets, is an oxidative product of choline and a trimethylated derivative of glycine (de Zwart et al., 2003). Commercially available betaine is a coproduct of the sugar beet industry (Lever and Slow, 2010) and is extracted from molasses by water-based chromatographic separation and crystallization (Craig, 2004). Betaine functions as an osmolyte to maintain cell function and volume, a methyl donor to increase methionine and decrease homocysteine concentrations, and is fermented to acetate in the rumen (Mitchell et al., 1979).

Supplemental rumen-unprotected betaine fed at 4 g/kg of the diet has been demonstrated to increase milk yield, milk fat content, and the concentration of the FA synthesized de novo in lactating dairy goats (Fernández et al., 2004a,b, 2009a). Fernández et al. (2009b), however, detected no changes in milk yield of lactating dairy goats fed betaine at 4 g/kg of the diet, although an increase in the concentration of medium-chain FA was observed. Recently, Wang et al. (2010) reported increased yields of milk, milk fat, ECM, 4.0% FCM, and milk fat percentage in lactating dairy cows fed rumen-unprotected betaine at 100 g/d. However, Davidson et al. (2008) reported no alteration in milk yield and components when feeding 100 g/d rumen-protected betaine to lactating dairy cows. Thus, the response of milk production and composition to dietary betaine is equivocal in goats and limited in cows. Therefore, the objective of this study was to determine the effect of supplemental dietary betaine on milk yield and milk composition in mid-lactation dairy cows.

MATERIALS AND METHODS

Animals and Treatments

Eighteen Holstein dairy cows (126 ± 5 DIM; mean $\pm$ SD) weighing 623 (± 16 ; mean $\pm$ SD) kg were blocked

Received August 8, 2011.

Accepted August 2, 2012.

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by parity and randomly assigned to a sequence of treatments in a 4 × 4 Latin square design with 4 periods and 4 treatments of rumen-unprotected betaine (betaine 97% purity; Amalgamated Sugar Co., Nampa, ID) at 0, 25, 50, and 100 g/d top-dressed on a lactation ration (Table 1) formulated to meet the nutrient requirements of a cow producing 22 kg of milk/d (NRC, 2001). Betaine was mixed with dried distillers grain to equal a total amount of 200 g. Each period lasted 16 d with a washout day in between each period and milk sampled on the last 2 d of each period. Milk composition (fat, protein, and lactose, determined by infrared spectroscopy with a Bentley 2000 mid-infrared milk analyzer; Bentley Instruments Inc., Chaska, MN) and SCC (determined by flow cytometry with a Somacount 300 instrument; Bentley Instruments Inc.) were analyzed by a certified DHIA laboratory (Burlington, WA). Energy-corrected milk yield was calculated as (milk yield, kg/d × 0.327) + (milk fat, kg/d × 12.86) + (milk protein, kg/d × 7.65) and 3.5% FCM was calculated as (milk yield, kg/d × 0.4324) + (milk fat, kg/d × 16.2162) according to Davidson et al. (2008). Cows were housed in a freestall

barn and fed individually using Calan gates (American Calan Inc., Northwood, NH) with water available at all times. Feed intake and orts were measured daily. Cows were milked twice daily at 0500 and 1700 h, and BW and BCS were measured on d 16 of each period. Cows were weighed after milking and before feeding, and body condition scored while in lockups using a score of 1 to 5, where a score of 1 indicates emaciation and a score of 5 indicates obesity (Wildman et al., 1982). Two trained individuals scored cows every period and scores were averaged. All animal procedures were approved by the University of Idaho (Moscow) Animal Care and Use Committee (protocol 2009-38).

Sampling

Milk samples were obtained twice daily on d 15 and 16 of each period. Milk samples were kept refrigerated and transported to the laboratory after the morning and evening milking. Milk samples from each day were pooled proportionately by yield. Total mixed ration samples were obtained daily and stored at −20°C. Daily TMR samples were composited into weekly samples and then composited into 3-wk samples. The nutrient composition of TMR and betaine samples were determined by Dairy One Cooperative Inc. (Ithaca, NY) via near-infrared (NIR) analysis, whereas the mineral composition of these samples was determined via wet chemistry (Table 1).

Milk, Betaine, and TMR FA Analysis

Milk and betaine lipids were extracted using 2:1 chloroform:methanol (Clark et al., 1982) and methylated using base-catalyzed transesterification (Christie, 1982). Ground freeze-dried TMR and betaine samples were methylated in a 2-step procedure using methanolic HCl and sodium methoxide according to Kramer et al. (1997).

The FA composition was determined using a gas chromatograph (Agilent 7890a series with autoinjector; Agilent Technologies Inc., Santa Clara, CA) fitted with a flame ionization detector and a 100-m × 0.25-mm (with 0.2-μm film) capillary column coated with (88%-cyanopropyl)-methylarylpolsiloxane (Agilent Technologies Inc.). The carrier gas was hydrogen at a column flow rate of 2 mL/min and hydrogen flow to the detector was 40 mL/min with a detector temperature of 280°C. After sample injection, the oven temperature was 120°C for 1 min and then increased to 175°C at a rate of 10°C/min and held for 10 min, and then increased to 210°C and held for 5 min, and then increased to 230°C at a rate of 5°C/min and held for 5 min. To identify FA, retention times were compared with a

Table 1. Feed ingredients and analyzed nutrient composition¹ of the basal ration and analyzed nutrient composition of betaine²

Item	Amount	SEM
Ingredient, % of DM		
Alfalfa hay	13.7	
Triticale silage	23.0	
Rolled barley	16.5	
Ground corn	15.4	
Canola meal	16.5	
Distillers grain	12.2	
Vitamin-mineral premix ³	2.4	
Sodium bicarbonate	0.5	
Nutrient composition		
DM, %	50.9	0.76
CP, % of DM	18.0	0.15
Soluble protein, % of CP	30.0	1.53
ADF, % of DM	22.3	1.92
NDF, % of DM	35.5	2.04
NFC, % of DM	34.4	1.89
Crude fat, % of DM	4.3	0.21
Ash, % of DM	7.9	0.16
TDN, % of DM	68.7	1.33
Calcium, % of DM	0.77	0.04
Phosphorus, % of DM	0.56	0.05
Magnesium, % of DM	0.33	0.03
Potassium, % of DM	1.67	0.14
NE _L , Mcal/kg	1.61	0.03

¹Nutrient analysis [near-infrared (NIR) and wet chemistry analysis; Dairy One Cooperative Inc., Ithaca, NY] as performed on 3 composited samples (3-wk composites) from all periods.

²Betaine (95.1% of DM) used contained (on DM basis) CP (75%), soluble protein (100% of CP), ADF (0.3%), NDF (0.4%), NFC (24.4%), starch (1.1%), crude fat (0.2%), TDN (91%), Ca (0.003%), K (0.03%), and NE_L (2.2 Mcal/kg).

³Vitamin-mineral premix contained Ca (17.5%), P (0.17%), S (0.3%), Mg (3.1%), Fe (0.08%), Zn (0.28%), Mn (0.17%), Se (0.001%), vitamin A (275 kIU/kg), vitamin D (77 kIU/kg), and vitamin E (1,320 IU/kg).

butter oil standard with certified values (CRM 164; European Community Bureau of Reference, Brussels, Belgium) and a Supelco 37 FA methyl ester (FAME) standard mixture (Sigma-Aldrich, St. Louis, MO).

Statistical Analysis

Data were analyzed in a 4×4 Latin square design assuming 4 treatments 0, 25, 50, and 100 g of betaine and 4 periods. In each period, a total of 18 cows were used, leading to 2 squares with 4 cows and 2 squares with 5 cows. All statistical computations were conducted in MIXED procedures of SAS (version 9.2; SAS Institute Inc., Cary, NC). Treatment means were compared using pairwise comparisons with a Tukey adjustment to control the experimentwise error rate. Significance was declared at $P < 0.05$.

RESULTS AND DISCUSSION

The experiment was completed with no incidences of clinical disease.

Betaine FA Composition

The NIR analysis yielded 0.16% crude fat in betaine. However, betaine samples yielded close to 0% lipid when extracted with 2:1 chloroform:methanol and when analyzed by GC, no FA were detected.

Milk Yield

An increase in milk yield (22.4 to 24.1 kg/d) was observed for cows consuming 100 g of dietary betaine/d (Table 2) as compared with 0, 25, and 50 g of betaine/d (overall betaine effect: $P = 0.02$). This is in agreement with several studies that demonstrated an increase in

milk yield in lactating dairy goats (1.47 to 2.0 kg/d; Fernández et al., 2004b; 1.32 to 1.48 kg/d; Fernández et al., 2004a) fed 10 g of rumen-unprotected betaine/d and an increase in milk yield in lactating dairy cows (26.0 to 26.7 kg/d; Wang et al., 2010) with supplementation of 100 g of rumen-unprotected betaine/d. In other studies, however, milk yield was not increased by rumen-unprotected betaine supplementation in lactating dairy goats (Fernández et al., 2009a,b) or cows fed rumen-protected betaine (Davidson et al., 2008). When betaine is supplemented, rumen fermentation decreases betaine concentrations, with an increase in acetate concentrations (Mitchell et al., 1979). An increase in acetate was shown to increase the rate at which milk is synthesized in some studies (Rook and Balch, 1961; Rook et al., 1965; Purdie et al., 2008) but not in all (Rook, 1979; Lough et al., 1983). The results reported here indicate that milk yield was increased with supplementation of 100 g of betaine/d without detectable changes in protein or lactose secretion, possibly due to the magnitude of the increase.

Milk Composition

Milk Protein Percentage and Milk Protein Yield. Milk protein percentage was decreased (overall betaine effect: $P = 0.005$) by betaine supplementation (Table 2). In previous experiments however, dietary betaine had no detectable effect on milk protein percentage of lactating dairy cows (Davidson et al., 2008; Wang et al., 2010) or goats (Fernández et al., 2004b).

Milk protein yield was not affected ($P = 0.34$) by betaine supplementation. Previous findings indicate that milk protein yield was not affected by feeding of rumen-protected betaine (Davidson et al., 2008) or rumen-unprotected betaine (Wang et al., 2010) supplementation. Overall, milk protein yield was not affected

Table 2. Dry matter intake and milk component yields and percentages from mid-lactating Holstein cows fed varying levels of dietary betaine

Item	Betaine, g/d				SEM	Treatment <i>P</i> -value
	0	25	50	100		
DMI, kg/d	19.0	19.0	18.2	20.2	1.08	0.18
Milk production, kg/d						
Milk yield	22.4 ^a	22.5 ^a	22.8 ^a	24.1 ^b	1.19	0.02
Milk fat	0.86	0.82	0.78	0.85	0.06	0.37
Milk protein	0.75	0.75	0.75	0.79	0.04	0.34
Milk lactose	1.04	1.05	1.07	1.12	0.07	0.13
Milk composition, %						
Milk protein	3.35 ^a	3.28 ^b	3.27 ^b	3.28 ^b	0.07	0.005
Milk fat	3.77	3.61	3.43	3.56	0.15	0.30
Milk lactose	4.54	4.58	4.58	4.61	0.10	0.42
SCC ($\times 1,000$ /mL)	398	318	388	296	181	0.36

^{a,b}Means within a row that do not share a common letter are significantly different ($P < 0.05$).

by betaine supplementation, although milk protein percent decreased.

Milk Fat Percentage and Milk Fat Yield. Milk fat percentage did not differ ($P = 0.30$) among treatments by betaine supplementation. Studies demonstrated that milk fat content remained unchanged with rumen-protected (Davidson et al., 2008) or rumen-unprotected betaine (Fernández et al., 2004a, 2009b) supplementation in lactating dairy cows and goats, respectively. In contrast, milk fat content increased as a result of betaine supplementation in lactating dairy goats (Fernández et al., 2004b, 2009a) and early- to mid-lactation dairy cows (Wang et al., 2010). Within the rumen, betaine is converted to acetate by microorganisms (Mitchell et al., 1979) and acetate is directly correlated with milk fat (Bauman and Grinari, 2003).

No difference was detected in milk fat yield ($P = 0.37$) with betaine supplementation. Similarly, no difference was detected in the yields (means $\pm$ SEM) of 3.5% FCM (24.0, 23.2, 22.5, and 24.0 ± 1.48 kg/d for 0, 25, 50, and 100 g of betaine/d, respectively; $P = 0.34$) with betaine supplementation as well as the yields (means $\pm$ SEM) of ECM (24.2, 23.5, 22.5, and 24.4 ± 1.43 kg/d for 0, 25, 50, and 100 g of betaine/d, respectively; $P = 0.35$).

Milk SCC. Somatic cells play a protective role against infectious disease in the bovine mammary gland. No differences were observed for milk SCC ($P = 0.36$) relative to betaine supplementation. These results are similar to those reported previously (Fernández et al., 2004a,b, 2009a). No clinical infections were detected during the experimental period.

Milk FA

SFA. No differences were observed for SFA content of milk individually or in total ($P = 0.89$ for the sum of C4:0 to C20:0) as well as the sum of the FA synthesized de novo, including C4:0 to C16:0 ($P = 0.86$) with betaine supplementation (Table 3). Fernández et al. (2004a) reported similar results in lactating dairy goats, demonstrating that the sum of C4:0 to C20:0 was not different as a result of betaine supplementation, although individual FA, including C8:0, C10:0, and C12:0, increased with betaine supplementation. Furthermore, Fernández et al. (2004b) reported an increase in C8:0, C10:0, C12:0, and C17:0 as well as an increase in the sum of C6:0 to C20:0 as a result of betaine supplementation in lactating dairy goats. In another study, the sum of the SFA was not altered with betaine supplementation; however, C12:0, C14:0, and C15:0 increased with betaine supplementation, whereas C16:0 decreased in lactating dairy goats (Fernández et al., 2009b). Furthermore, the effects of dietary betaine

on the sum of the FA synthesized de novo have not been previously reported.

MUFA. No effect of betaine supplementation was detected on the sum of MUFA *trans* C10:1, C14:1, C15:1, C16:1, C17:1, C18:1 isomers, and *cis*-9 C18:1 ($P = 0.93$) with betaine supplementation (Table 3), although a difference was detected in the individual FA C15:1 ($P = 0.04$). Similarly, Fernández et al. (2009b) reported that betaine did not affect the sum of MUFA including C10:1 to C17:1, although C15:1 was not included in the sum as in the present study. In another study, Fernández et al. (2004b) demonstrated increases in milk C10:1 and C18:1 as well as the sum of C10:1, C16:1, and C18:1. Fernández et al. (2004a) did not detect a difference in C18:1 as a result of betaine supplementation. Further, Jensen (2002) reported a concentration of milk C15:1 at 0.3%, similar to the current experiment.

PUFA. In the present study, a decrease was observed in the sum of PUFA, including *cis*-9,*cis*-12 C18:2, *cis*-6,*cis*-9,*cis*-12 C18:3, *cis*-9,*cis*-12,*cis*-15 C18:3, *cis*-9,*trans*-11 C18:2, *cis*-11,*cis*-14 C20:2, and *cis*-11,*cis*-14,*cis*-17 C20:3 ($P = 0.02$), with all levels of betaine supplementation (Table 3), although the individual PUFA were not altered in response to betaine supplementation. Fernández et al. (2009b) reported that betaine supplementation did not change the sum or individual PUFA, including *trans* C18:2, *cis*-9,*cis*-12 C18:2, *cis*-9,*cis*-12,*cis*-15 C18:3, *cis*-9,*cis*-11 C18:2, and *cis*-5,*cis*-8,*cis*-11,*cis*-14 C20:4, in milk. Furthermore, the sum of the PUFA, including C18:2 (linoleic) and C18:3 (linolenic), did not change with betaine supplementation (Fernández et al., 2004a,b), although individually, C18:3 (linolenic) increased in lactating dairy goats (Fernández et al., 2004b).

DMI

Dry matter intake did not differ ($P = 0.19$) among treatments by betaine supplementation. In agreement, previous studies reported that DMI was unaffected by rumen-protected betaine (Davidson et al., 2008) and rumen-unprotected betaine (Wang et al., 2010) supplementations in lactating dairy cows. However, Löest et al. (2002) observed an increase in DMI in steers when rumen-unprotected betaine was top-dressed on the upper one-fourth of the ration, which might have increased palatability of the ration and stimulated intake.

Feed Efficiency

Feed efficiency, including milk production divided by DMI (1.29, 1.28, 1.33, and 1.26 ± 0.07 kg/d for 0, 25, 50, and 100 g of betaine/d respectively; means

Table 3. Milk FA (weight percentage of total identified FA) from mid-lactating Holstein cows fed varying rates of dietary betaine

FA	Betaine, g/d				SEM	Treatment <i>P</i> -value
	0	25	50	100		
C4:0	2.00	2.04	1.98	2.05	0.09	0.94
C6:0	1.77	1.71	1.69	1.70	0.03	0.17
C8:0	1.14	1.14	1.08	1.10	0.03	0.40
C10:0	2.63	2.47	2.46	2.52	0.08	0.26
C12:0	3.17	2.99	2.97	2.97	0.11	0.30
C13:0	0.13	0.13	0.12	0.13	0.01	0.39
C14:0	10.61	10.27	10.21	10.39	0.20	0.40
C15:0	1.17	1.15	1.15	1.19	0.03	0.72
C16:0	29.64	30.72	31.20	30.41	0.86	0.43
C17:0	0.56	0.57	0.54	0.56	0.02	0.79
C18:0	10.59	10.38	10.43	10.51	0.51	0.94
C20:0	0.18	0.15	0.16	0.15	0.01	0.22
C10:1	0.36	0.35	0.34	0.34	0.02	0.40
C14:1	1.43	1.47	1.41	1.46	0.10	0.68
C15:1	0.33 ^a	0.32 ^a	0.30 ^b	0.32 ^a	0.01	0.04
C16:1	1.71	1.75	1.85	1.77	0.13	0.72
C17:1	0.26	0.26	0.26	0.27	0.01	0.96
<i>trans</i> C18:1 isomers ¹	2.79	2.87	2.78	2.72	0.11	0.42
<i>cis</i> -9 C18:1	24.63	24.70	24.46	24.85	0.56	0.92
<i>cis</i> -9, <i>cis</i> -12 C18:2	2.87	2.68	2.67	2.65	0.10	0.11
<i>cis</i> -6, <i>cis</i> -9, <i>cis</i> -12 C18:3	0.11	0.10	0.11	0.11	0.004	0.16
<i>cis</i> -9, <i>cis</i> -12, <i>cis</i> -15 C18:3	0.40	0.36	0.40	0.38	0.02	0.10
<i>cis</i> -9, <i>trans</i> -11 C18:2	0.87	0.87	0.85	0.84	0.04	0.81
<i>cis</i> -11, <i>cis</i> -14 C20:2	0.33	0.24	0.23	0.28	0.04	0.25
<i>cis</i> -11, <i>cis</i> -14, <i>cis</i> -17 C20:3	0.31	0.26	0.27	0.29	0.02	0.17
Unsaturated:saturated ratio ²	0.58	0.57	0.57	0.57	0.02	0.90
Σ PUFA ³	4.90 ^a	4.51 ^b	4.53 ^b	4.60 ^b	0.14	0.02
Σ SFA ⁴	63.50	63.69	63.98	63.66	0.68	0.89
Σ Unsaturated ⁵	36.40	36.23	35.92	36.27	0.68	0.89
Σ MUFA ⁶	31.51	31.71	31.39	31.72	0.61	0.93
Σ de novo ⁷	50.87	51.32	51.59	51.14	0.83	0.86

^{a,b}Means within a row that do not share a common letter are significantly different ($P < 0.05$).

¹*trans* C18:1 isomers = the sum of *trans* C18:1 isomers including *trans*-6 to *trans*-11.

²Unsaturated:saturated ratio = the sum of the unsaturated FA divided by the sum of the SFA.

³Σ PUFA = the sum of *cis*-9,*cis*-12 C18:2 – *cis*-11,*cis*-14,*cis*-17 C20:3.

⁴Σ SFA = sum of C4:0 to C20:0.

⁵Σ Unsaturated = the sum of MUFA and PUFA.

⁶Σ MUFA = the sum of *cis*-9 C10:1 to C18:1.

⁷Σ de novo = the sum of C4:0, C6:0, C10:0, C12:0, C14:0, and C16:0.

± SEM; $P = 0.80$), FCM divided DMI (1.45, 1.38, 1.40, and 1.27 ± 0.09 kg/d for 0, 25, 50, and 100 g of betaine/d, respectively; means ± SEM; $P = 0.47$), and ECM divided by DMI (1.46, 1.40, 1.43, and 1.29 ± 0.09 kg/d for 0, 25, 50, and 100 g of betaine/d, respectively; means ± SEM; $P = 0.52$), were not affected by betaine supplementation. These results are similar to those of previous studies where feed efficiency was unaffected by rumen-protected betaine (Davidson et al., 2008) and rumen-unprotected betaine supplementation (Wang et al., 2010).

BCS and BW

Body condition score (mean ± SEM) was not affected (2.58, 2.64, 2.59, and 2.63 ± 0.05 for 0, 25, 50, and

100 g of betaine/d, respectively; $P = 0.28$) by betaine supplementation. Body weight (mean ± SEM) was not affected (601, 605, 596, and 608 ± 16.1 kg/d for 0, 25, 50, and 100 g of betaine/d, respectively; $P = 0.08$) by betaine supplementation either. Effects of feeding rumen-unprotected betaine have not been previously reported regarding BCS and BW.

CONCLUSIONS

Dietary betaine supplementation increased milk yield, milk fat percentage, and the FA synthesized de novo in some studies. Overall, the present study confirms that 100 g of dietary rumen-unprotected betaine/d increased milk yield and all levels of betaine supplementation slightly altered the milk FA profile and decreased milk

protein percentage. No change, however, was detected in yields of milk components. Furthermore, dietary betaine did not alter milk fat or lactose percentage, ECM, 3.5% FCM, BW, or BCS in the present study. Further studies are needed to determine the ruminal fermentation characteristics and optimum rate of supplemental betaine for dairy cows and its effect on rumen fermentation.

ACKNOWLEDGMENTS

Funding for this project was, in part, awarded by The Amalgamated Sugar Company, (Nampa, ID) to M. Chahine. Funding was also provided by the Idaho Agricultural Experimental Station (Moscow). The authors acknowledge J. Peak, S. L. Shields, D. L. Sevier, and J. K. Kinch (all of the University of Idaho, Moscow) for technical assistance with this project.

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