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# Supplementing Limited Methionine Diets with Rumen-Protected Methionine, Betaine, and Choline in Early Lactation Holstein Cows

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## ABSTRACT

Eighty lactating Holstein cows from 21 to 91 d in milk were fed a corn silage-based total mixed ration (TMR) formulated with the Met content limited (42 g/d) to investigate the impact of supplementing rumen-protected (RP) forms of Met, betaine, and choline on performance and metabolism. One of 4 supplements was blended into the TMR to produce 4 dietary treatments: 1) control, 2) 20 g/d of RP-Met, 3) 45 g/d of RP-betaine, and 4) 40 g/d of RP-choline. Calcium salts of fatty acids were used to protect both RP-betaine and RP-choline supplements. A similar amount of Ca salts of fatty acids was included in both control and RP-Met supplements to provide equal amounts of fat to all treatments. Overall, no differences in intake, milk yield, or milk composition were observed in primiparous cows. Average dry matter intake, body weight, and body condition score were not different among treatments in multiparous cows. Milk yield was higher in multiparous cows fed RP-choline compared with the other treatments. Multiparous cows fed RP-choline had higher milk protein yield than cows fed control or RP-betaine but was not different from cows fed RP-Met. Multiparous cows fed RP-choline had higher milk fat yield than cows fed RP-Met but was not different from cows fed control or RP-betaine. There were no beneficial effects of RP-betaine supplementation to a Met-limited TMR.
**Key words:** choline, betaine, methionine, dairy

## INTRODUCTION

Methionine is frequently one of the most limiting amino acids in dairy rations, and Met metabolism is closely linked to that of betaine and choline. An improved understanding of the mechanisms that regulate these overlapping pathways is needed, because these compounds can be fed to lactating dairy cows in a way that potentially will improve lactation performance and reduce the incidence of ketosis and fatty liver.

Although Met is important in the dairy cow because it is required for milk protein synthesis, Met also is involved in many pathways including the synthesis of phospholipids, carnitine, creatine, and polyamines (Bequette et al., 1998). In addition, Met is the source of the methyl donor *S*-adenosyl methionine, the metabolite that provides methyl groups in a variety of reactions including the de novo synthesis of choline from phosphatidylethanolamine. When choline is oxidized irreversibly to betaine, betaine can provide methyl groups that recycle homocysteine to Met. Because of these metabolic relationships, dietary supply of either choline or betaine affects Met requirements, and Met supply can affect betaine and choline metabolism.

Because both choline (Erdman et al., 1984) and betaine (Mitchell et al., 1979) are susceptible to rapid ruminal degradation, the amounts available for absorption are limited. Therefore, dairy cows may benefit from rumen-protected (**RP**) supplementation of choline or betaine. Emmanuel and Kennelly (1984) reported that 28% of absorbable Met was used for choline synthesis in lactating goats. Therefore, in dairy cattle diets, if Met is limited then choline is likely limited as well, and a portion of the dietary Met requirement is used to provide choline.

Phosphatidylcholine is the predominant form of choline phospholipids and makes up more than 50% of phospholipids in mammalian cell membranes (Zeisel, 1992). It is also an essential component of very low density lipoproteins (**VLDL**) and cannot be substituted with other phospholipids (Zeisel, 1992). Choline deficiency reduces VLDL formation and results in fatty liver, because the export of triglycerides from the liver is limited (NRC, 1998).

Choline supplementation consistently increases VLDL secretion from the liver in rats (Zeisel, 1993), and Met supplementation increases VLDL synthesis in the liver of calves (Auboiron et al., 1994). Therefore, optimizing the dietary supply of Met, betaine, and choline could reduce the incidence of fatty liver in early lactation dairy cattle. Because clinical ketosis often is

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associated with fatty liver, it has been speculated that choline could play a role in ketosis prevention as well (Erdman, 1992).

Feeding RP forms of Met to early lactation dairy cattle has increased milk and milk protein yield (Illg et al., 1987; Donkin et al., 1989), as well as milk fat yield (Overton et al., 1996). Researchers also have reported that dairy cattle can produce more milk when fed supplemental RP-choline (Erdman and Sharma, 1991; Pinotti et al., 2003). Sharma and Erdman (1988) compared the effects of Met (45.6 g/d) and choline (30 g/d) abomasal infusions in midlactation multiparous (**MP**) Holstein cows both with and without infusions of 2-amino-2-methyl-1-propanol (**2AMP**), a chemical that inhibits de novo choline synthesis from Met. When 2AMP was infused, supplemental choline increased yield of milk, protein, and fat compared with Met, which suggested that blocking the ability of Met to supply choline limited milk production. When 2AMP was not infused, choline infusion resulted in higher milk fat yield compared with Met infusion, which indicated an observable requirement for choline in lactating dairy cattle that was independent of Met supply.

Very little research has investigated the use of RP-betaine in ruminants, and the research that has been reported focused on the ability of betaine to improve carcass traits and not lactation performance (Fernandez et al., 2000; Löest et al., 2002). Nevertheless, if milk yield in dairy cows is limited as a result of a methyl group deficiency, then supplying betaine should increase milk production.

The objective of this study was to evaluate the effects of supplementing RP forms of Met, betaine, or choline to a limited Met diet on the performance and metabolism of early lactation Holstein cows by parity.

## MATERIALS AND METHODS

### *Diets and Cow Management*

The Institutional Animal Care and Use Committee of North Carolina State University approved all animal procedures. Eighty Holstein cows from the Piedmont Research Station in Salisbury, North Carolina, were assigned randomly to 1 of 4 treatment groups within either primiparous (**PP**) or MP blocks. Each treatment group consisted of 8 PP and 12 MP cows. Four three-quartered MP cows were included in the study with 1 assigned to each of the 4 treatments. Cows were added to the study individually over approximately a 12-mo period at the time each calved. After calving, cows were trained to Calan feeding stations (American Calan Inc., Northwood, NH) and adjusted to their treatment diets. By 21 DIM, cows were adjusted to the feeding stations and consuming experimental diets fed as a TMR. To provide adequate adaptation to feeding stations and diets, data collection began at 28 DIM and continued through 91 DIM. Cows were housed in a free-stall barn, fed for ad libitum consumption, and daily feed allocations and orts were recorded for each cow. Overall, the orts percentage was 15% when averaged from all intake measurements taken throughout the study.

**Table 1.** Ingredient composition of dietary treatments (% of DM)

| Ingredient, % of DM | Basal TMR |
|---|---|
| Corn silage, unprocessed | 40.7 |
| Cottonseed hulls | 10.3 |
| Soybean hulls | 12.7 |
| Ground, dry, shelled corn | 7.2 |
| 48% soybean meal | 9.9 |
| Porcine blood meal, ring-dried | 1.9 |
| Citrus pulp | 10.4 |
| Cane molasses, dried | 0.48 |
| Sodium bicarbonate | 0.83 |
| Salt, plain white | 0.31 |
| Dicalcium phosphate | 0.48 |
| Calcitic limestone | 0.39 |
| Vitamin-trace mineral premix[1] | 0.51 |
| Dyna-mate[2] | 0.10 |
| Urea | 0.64 |
| Ca salts of fatty acids + treatment[3] | 3.1 |

[1]Vitamin-trace mineral premix contained the following: 21.5% Ca, 5.5% S, 3.87% Zn, 3.87% Mn, 1.18% Cu, 9,650 ppm Fe, 700 ppm I, 590 ppm Co, 250 ppm Se, 1,215,420 IU/kg of vitamin A, 304,545 IU/kg of vitamin $D_3$, and 3,646 IU/kg of vitamin E.

[2]22% S, 18% K, and 11% Mg (Mosaic, Riverview, FL).

[3]0.68 kg/d of Ca salts of fatty acids fed alone (Robt Morgan Inc., Paris, IL) or blended with rumen-protected (RP)-Met (Degussa, Allendale, NJ), RP-choline (Robt Morgan Inc.), or RP-betaine (Robt Morgan Inc.).

A corn silage-based TMR was formulated to meet the NRC (2001) recommendations for $NE_L$, MP, RDP, RUP, macrominerals, microminerals, and the vitamins A, D, and E (Table 1). In addition, the TMR was formulated to contain a limited amount of Met, but adequate Lys, so that this basal diet supplied approximately 163 g of Lys and 42 g of Met (Lys:Met ratio of 3.75:1) according to NRC (2001) (Table 2).

All diets contained approximately 58.3% DM, 17.6% CP, and 23.7% ADF (Table 3). One of 4 supplements was added to this Met-limited TMR to form the 4 experimental diets: 1) control with no added Met, betaine, or choline (control); 2) 20 g/d of RP-Met (**RP-MET**); 3) 45 g/d of RP-betaine (**RP-BET**); or 4) 40 g/d of RP-choline (**RP-CHOL**). Calcium salts of soy fatty acids were included as the primary fat source in the treatment diets, because both the betaine and choline supplements were protected with this fat source (Robt Morgan Inc., Paris, IL). Unlike the betaine and choline supplements, the RP-MET used was an encapsulated product (Degussa, Allendale, NJ) instead of fat-protected. Therefore, Ca salts of soy fatty acids were added so that all 4 supplements provided similar amounts of fat to the diet (Robt

**Table 2.** Predicted intestinal flow of Lys and Met according to NRC (2001) and the Mepron Dairy Ration Evaluator

| | Dietary treatment[1] | | | |
|---|---|---|---|---|
| Item | Control | RP-MET | RP-BET | RP-CHOL |
| NRC | | | | |
| Lys:Met ratio[2] | 3.88:1 | 2.96:1 | 3.88:1 | 3.88:1 |
| Lys,[3] g/d | 163 | 163 | 163 | 163 |
| Met,[3] g/d | 42 | 55 | 42 | 42 |
| Lys,[4] % of essential AA | 14.8 | 14.6 | 14.8 | 14.8 |
| Met,[4] % of essential AA | 3.8 | 4.9 | 3.8 | 3.8 |
| Mepron Dairy Ration Evaluator | | | | |
| Lys:Met ratio[5] | 3.75:1 | 2.89:1 | 3.75:1 | 3.75:1 |
| Lys,[6] g/d | 165 | 165 | 165 | 165 |
| Met,[6] g/d | 44 | 57 | 44 | 44 |
| Lys,[7] % of essential AA | 15.1 | 14.9 | 15.1 | 15.1 |
| Met,[7] % of essential AA | 4.0 | 5.1 | 4.0 | 4.0 |

[1]RP-MET = 20 g/d of rumen-protected (RP) Met; RP-BET = 40 g/d of RP-betaine; RP-CHOL = 45 g/d of RP-choline.

[2]Calculated from predicted flows of digestible Lys and Met to small intestine (g/d) according to NRC (2001).

[3]Predicted flow of digestible Lys and Met to small intestine (g/d) as calculated by NRC (2001).

[4]Calculated from NRC (2001) using predicted flow of digestible Lys, Met, and essential AA to small intestine (g/d).

[5]Calculated from predicted flows of Lys and Met according to the Mepron Dairy Ration Evaluator (Version 2.1, Degussa-Hüls Corp., Allendale, NJ).

[6]Predicted flow to intestine formulated according to the Mepron Dairy Ration Evaluator (Version 2.1, Degussa-Hüls Corp.).

[7]Calculated from the Mepron Dairy Ration Evaluator (Version 2.1, Degussa-Hüls Corp.) using the predicted flow of digestible Lys, Met, and essential AA to intestine (g/d).

Morgan Inc.). The level of RP-MET supplementation was chosen so that enough Met was provided to result in approximately a 3:1 postruminal Lys:Met ratio as recommended by Schwab (1996) in that treatment (Table 2). As a result, the RP-MET treatment contained adequate dietary Met, whereas the control, RP-BET, and RP-CHOL treatments all contained limited amounts of Met. Equal molar amounts of betaine and choline were provided by the RP-BET and RP-CHOL supplements, which supplied equal amounts of methyl groups. However, RP-MET provided substantially fewer methyl groups. Each of the 4 supplements was preblended with porcine blood meal, citrus pulp, dried molasses, sodium bicarbonate, salt, dicalcium phosphate, limestone, a vitamin-trace mineral premix, potassium magnesium sulfate, and urea (Table 1). The 4 premixes were blended with the other basal TMR ingredients to produce the 4 treatment TMR.

**Table 3.** Chemical composition of dietary treatments[1]

| Item[2] | Mean | SD |
|---|---|---|
| DM, % of diet | 58.3 | 2.6 |
| CP | 17.6 | 1.1 |
| Soluble CP | 5.4 | 5.4 |
| NDICP[3] | 2.56 | 0.67 |
| ADICP[4] | 1.00 | 0.20 |
| NDF | 36.0 | 1.7 |
| ADF | 23.7 | 1.5 |
| NFC[5] | 36.4 | 2.0 |
| Starch | 19.9 | 1.7 |
| $NE_L$, Mcal/kg[6] | 1.61 | 0.22 |
| Fat | 3.50 | 0.27 |
| Ash | 6.68 | 0.69 |
| Ca | 0.87 | 0.13 |
| P | 0.38 | 0.03 |
| Mg | 0.28 | 0.03 |
| K | 1.31 | 0.10 |

[1]All means are reported as a percentage of DM unless otherwise indicated.

[2]Analysis conducted with TMR samples (n = 54).

[3]NDICP = neutral detergent insoluble CP.

[4]ADICP = acid detergent insoluble CP.

[5]NFC = 100 − (NDF + CP + fat + ash).

[6]$NE_L$ = 0.866 − [0.007 × ADF (% of DM)].

### *Sample Collection and Analysis*

Four treatment TMR were sampled once a week and composited by month for analysis (n = 54). In addition, individual feed ingredients were sampled monthly and analyzed so that the TMR formulation could be adjusted if there was significant variation in the content of DM, CP, and ADF of ingredients throughout the study (Constable Laboratory, North Carolina Department of Agriculture, Raleigh). The TMR was reformulated during the experiment to account for differences in corn silage DM content, but this reformulation did not alter the

percentage of diet DM of individual ingredients. After collection, weekly TMR samples were frozen at –20°C until they were thawed and dried for 48 h in a 60°C oven. Then, dried weekly samples were ground through a Wiley mill fitted with a 1-mm screen (Arthur H. Thomas, Philadelphia, PA) and composited by month. The composited TMR samples were analyzed for DM, CP, NDF, ADF, protein fractions, and minerals by the Cumberland Valley Analytical Laboratory (Hagerstown, MD; Cumberland Valley Analytical Services, 2007). The ingredient and nutrient compositions of the treatment diets are reported in Table 1 and Table 3, respectively.

All cows were weighed and body condition scored weekly before the a.m. feeding. Body condition score was assessed according to the guidelines of Ferguson et al. (1994). Cows were milked twice daily at 0100 and 1300 h, and milk yields were recorded at each milking. Milk samples were composited once weekly from consecutive a.m. and p.m. milkings and frozen at –20°C until analysis. These composited samples were analyzed for milk fat, milk true protein, and MUN by the United Federation of DHIA Laboratory (Blacksburg, VA). Milk fat and true protein were analyzed according to AOAC (1990) procedures, whereas the Bentley ChemSpec 150 analyzer (Chaska, MN) was used to determine MUN concentrations by means of a modified Berthelot reaction (Chaney and Marbach, 1962).

Blood was collected from a coccygeal vessel before the a.m. feeding on 28, 49, 70, and 91 ± 5 DIM. Two samples were collected from each cow into vacutainers containing either EDTA or no additive. After collection, all samples were immediately placed on ice for transport to the laboratory. The EDTA-containing samples were centrifuged for 15 min at 2,500 × *g* at 4°C, and plasma was harvested and frozen until analysis. Vacutainers containing blood with no additive were kept on ice for at least 2 h to allow samples to clot and were centrifuged for 15 min at 2,500 × *g* at 4°C. Plasma was analyzed for NEFA using Wako reagent kits (Wako Chemicals, 1995). Total serum cholesterol, triglycerides, urea N, and BHBA were analyzed at the Texas Veterinary Medical Diagnostic Laboratory (Amarillo, TX). High-density lipoproteins (**HDL**) in serum were analyzed by the Michigan State University Diagnostic Center for Population and Animal Health (Lansing). Very low density lipoproteins were calculated from serum triglycerides so that VLDL (mg/dL) = triglycerides (mg/dL) ÷ 5 (Friedewald et al., 1972). Low-density lipoproteins (**LDL**) were calculated using the Friedewald equation where LDL (mg/dL) = cholesterol (mg/dL) – [HDL (mg/dL) + VLDL (mg/dL)] (Friedewald et al., 1972).

### *Statistical Analyses*

This experiment used a factorial arrangement of treatments, the factors being dietary treatment, parity (PP or MP), and time. Cow within treatment and parity provided replication with measurements over time on the same cow. Data were analyzed by repeated measures ANOVA as recommended by Littell et al. (1998) using the mixed procedure with the autoregressive (1) covariance structure (SAS Institute, 2004). Because the incidence of disorders of fat metabolism is greater in MP cows (Rasmussen et al., 1999), data were analyzed by parity. Parity was highly significant, and there was a tendency for the treatment × parity interaction to be significant ($P = 0.057$). As a result, the slice option in the LSMEANS statement was used to test for treatment effects within either PP or MP cows rather than the main effect means for treatment (SAS Institute, 2004). Least squares means for treatments were compared using the PDIFF option to carry out the least significant difference procedure with statistical significance declared at $P < 0.05$.

## RESULTS AND DISCUSSION

### *Feed Intake, BW, and BCS*

As expected, there was a parity effect on intake with MP cows (22.2 ± 1.4 kg/d) consuming more DM ($P < 0.01$) than PP cows (19.7 ± 1.4 kg/d). However, there were no treatment effects on average daily DMI within PP or MP cows (Tables 4 and 5).

There were no treatment differences in mean BW or mean BCS for PP or MP cows (Tables 4 and 5). There was a parity effect ($P < 0.01$) for BW with MP cows (591 ± 17 kg) weighing more than PP cows (481 ± 17 kg) as would be expected. Body condition scores indicated that the cows used in this study were not overconditioned and therefore at low risk for developing fatty liver.

### *Milk Yield and Composition*

In PP cows, there were no differences between treatments in yields of milk, milk true protein, and milk fat. There also were no treatment effects for PP cows in milk protein content and milk fat content. As expected, yields of milk, protein, and fat were lower in PP cows than in MP cows. Concentrations of both milk fat and milk protein were higher in PP cows (2.91 ± 0.07%; 2.68 ± 0.03%) than in MP cows (2.70 ± 0.07%; 2.50 ± 0.03%).

Multiparous cows fed RP-CHOL produced more milk than MP cows fed control, RP-MET, or RP-BET (Table 5). Multiparous cows fed RP-MET or RP-CHOL produced more milk protein (kg/d) than MP cows fed control. Increased milk protein production in cows fed RP-

**Table 4.** Daily milk yield, milk composition, intake, BW, and BCS as affected by dietary treatment for primiparous cows (n = 8 per treatment)

| | Dietary treatment[1] | | | | | |
|---|---|---|---|---|---|---|
| Item | Control | RP-MET | RP-BET | RP-CHOL | SEM | $P \leq$ |
| Milk | | | | | | |
| Yield, kg/d | 27.9 | 28.0 | 26.1 | 27.5 | 1.3 | 0.73 |
| True protein, % | 2.60 | 2.77 | 2.67 | 2.68 | 0.06 | 0.28 |
| True protein, kg/d | 0.73 | 0.76 | 0.70 | 0.74 | 0.04 | 0.72 |
| Fat, % | 2.97 | 2.71 | 3.03 | 2.93 | 0.15 | 0.46 |
| Fat, kg/d | 0.84 | 0.77 | 0.79 | 0.79 | 0.05 | 0.81 |
| MUN, mg/dL | 17.2 | 15.6 | 15.8 | 15.2 | 0.9 | 0.40 |
| DMI, kg/d | 19.7 | 20.0 | 18.8 | 20.2 | 1.4 | 0.90 |
| ECM,[2] kg/d | 25.6 | 24.8 | 23.9 | 24.9 | 1.2 | 0.81 |
| 3.5% FCM,[3] kg/d | 25.8 | 24.4 | 24.0 | 24.7 | 1.3 | 0.78 |
| ECM/DMI, kg/kg | 1.29 | 1.37 | 1.30 | 1.26 | 0.16 | 0.98 |
| BW, kg | 479 | 485 | 476 | 485 | 19 | 0.98 |
| BCS[4] | 2.18 | 2.25 | 2.16 | 2.14 | 0.13 | 0.93 |

[1]RP-MET = 20 g/d of rumen-protected (RP) Met; RP-BET = 40 g/d of RP-betaine; RP-CHOL = 45 g/d of RP-choline.

[2]ECM (kg/d) = [milk yield (kg/d) × 0.327] + [milk fat (kg/d) × 12.86] + [milk true protein (kg/d) × 7.65] (Dairy Records Management Systems, 2006).

[3]3.5% FCM (kg/d) = [milk yield (kg/d) × 0.4324] + [milk fat (kg/d) × 16.2162] (Dairy Records Management Systems, 2006).

[4]5-point scale where 1 = very thin to 5 = obese (Ferguson et al., 1994).

MET compared with cows fed control indicated that Met was limited in the control diet (Armentano et al., 1997). Multiparous cows fed RP-BET produced less milk protein (kg/d) than MP cows fed RP-CHOL but similar amounts to MP cows fed control or RP-MET. There were no differences in milk protein content between dietary treatments for MP cows. Multiparous cows fed RP-CHOL produced more milk fat (kg/d) than MP cows fed RP-MET, whereas MP cows fed control or RP-BET produced amounts of milk fat that were similar to that of MP cows fed either RP-CHOL or RP-MET. Within MP cows, there were no differences in milk fat content. Dietary treatments did not result in differences in MUN concentrations in PP or MP cows, which sug-

**Table 5.** Daily milk yield, milk composition, intake, BW, and BCS as affected by dietary treatment for multiparous cows (n = 12 per treatment)

| | Dietary treatment[1] | | | | | |
|---|---|---|---|---|---|---|
| Item | Control | RP-MET | RP-BET | RP-CHOL | SEM | $P \leq$ |
| Milk | | | | | | |
| Yield, kg/d | 37.7$^{b}$ | 39.8$^{b}$ | 38.6$^{b}$ | 44.1$^{a}$ | 1.2 | 0.01 |
| True protein, % | 2.47 | 2.61 | 2.44 | 2.49 | 0.06 | 0.18 |
| True protein, kg/d | 0.92$^{c}$ | 1.04$^{ab}$ | 0.96$^{bc}$ | 1.10$^{a}$ | 0.04 | 0.01 |
| Fat, % | 2.80 | 2.46 | 2.91 | 2.64 | 0.13 | 0.11 |
| Fat, kg/d | 1.03$^{ab}$ | 0.99$^{b}$ | 1.11$^{ab}$ | 1.16$^{a}$ | 0.05 | 0.05 |
| MUN, mg/dL | 17.0 | 16.0 | 17.4 | 17.2 | 0.8 | 0.58 |
| DMI, kg/d | 21.9 | 20.8 | 21.7 | 24.3 | 1.4 | 0.32 |
| ECM,[2] kg/d | 32.5$^{b}$ | 33.8$^{b}$ | 34.3$^{b}$ | 37.9$^{a}$ | 1.1 | 0.01 |
| 3.5% FCM,[3] kg/d | 32.8$^{b}$ | 33.3$^{b}$ | 34.9$^{b}$ | 38.0$^{a}$ | 1.1 | 0.01 |
| ECM/DMI, kg/kg | 1.56 | 1.80 | 1.67 | 1.68 | 0.15 | 0.73 |
| BW, kg | 574 | 606 | 577 | 607 | 15 | 0.26 |
| BCS[4] | 2.25 | 2.32 | 2.17 | 2.14 | 0.10 | 0.59 |

[a–c]Means within a row lacking a common superscript differ ($P < 0.05$).

[1]RP-MET = 20 g/d of rumen-protected (RP) Met; RP-BET = 40 g/d of RP-betaine; RP-CHOL = 45 g/d of RP-choline.

[2]ECM (kg/d) = [milk yield (kg/d) × 0.327] + [milk fat (kg/d) × 12.86] + [milk true protein (kg/d) × 7.65] (Dairy Records Management Systems, 2006).

[3]3.5% FCM (kg/d) = [milk yield (kg/d) × 0.4324] + [milk fat (kg/d) × 16.2162] (Dairy Records Management Systems, 2006).

[4]5-point scale where 1 = very thin to 5 = obese (Ferguson et al., 1994).

**Table 6.** Plasma and serum metabolites as affected by dietary treatment for primiparous cows (n = 8 per treatment)

| | Dietary treatment[1] | | | | | |
|---|---|---|---|---|---|---|
| Item | Control | RP-MET | RP-BET | RP-CHOL | SEM | $P \leq$ |
| SUN,[2] mg/dL | 15.8 | 14.2 | 14.2 | 13.1 | 1.0 | 0.31 |
| Plasma NEFA, mEq/L | 0.388 | 0.377 | 0.298 | 0.341 | 0.064 | 0.76 |
| Serum total cholesterol,[3] mg/dL | 171.4 | 155.8 | 182.5 | 167.1 | 11.4 | 0.43 |
| HDL, mg/dL | 111.7 | 101.0 | 101.4 | 106.0 | 4.7 | 0.34 |
| LDL,[4] mg/dL | 51.9 | 51.1 | 77.4 | 57.4 | 10.4 | 0.27 |
| VLDL,[5] mg/dL | 2.85 | 2.85 | 2.79 | 2.85 | 0.18 | 0.99 |
| Serum triglycerides, mg/dL | 14.2 | 14.3 | 14.0 | 14.3 | 0.9 | 0.99 |
| BHBA, μmol/L | 481 | 401 | 452 | 473 | 81 | 0.89 |
| Ketosis incidence[6] | 0 | 0 | 0 | 0 | NA[7] | NA |

[1]RP-MET = 20 g/d of rumen-protected (RP) Met; RP-BET = 40 g/d of RP-betaine; RP-CHOL = 45 g/d of RP-choline.

[2]SUN = serum urea N.

[3]HDL = high-density lipoproteins; LDL = low-density lipoproteins; VLDL = very low density lipoproteins.

[4]LDL (mg/dL) = cholesterol (mg/dL) − [HDL (mg/dL) + VLDL (mg/dL)] (Friedewald et al., 1972).

[5]VLDL (mg/dL) = triglycerides (mg/dL) ÷ 5 (Friedewald et al., 1972).

[6]Ketosis incidence is calculated as the number of cows from each treatment where BHBA > 1,400 μmol/L for any of the 4 sampling times (Duffield et al., 1997). Ketosis incidence data were not analyzed statistically, because the study was not designed to detect differences in the incidence of a disorder.

[7]NA = not applicable.

gest that N utilization efficiency was similar between treatments.

Fat-corrected milk was calculated so that 3.5% FCM (kg/d) = [milk (kg/d) × 0.432] + [fat (kg/d) × 16.216] (Dairy Records Management Systems, 2006). Yields of FCM were not different between dietary treatments within PP cows, but FCM yield was higher in MP cows fed RP-CHOL compared with those fed control, RP-MET, or RP-BET. Energy-corrected milk was calculated so that ECM = [milk (kg/d) × 0.327] + [fat (kg/d) × 12.86] + [protein (kg/d) × 7.65] (Dairy Records Management Systems, 2006). Similar to FCM, ECM yield was not different between dietary treatments within PP cows, but ECM yield was higher in MP cows fed RP-CHOL than in MP cows on all other treatments. Feed efficiency, reported as kilograms of ECM per kilogram of DMI, was not different as a result of dietary treatment within PP or MP cows.

### *Blood Metabolites*

There were no differences in plasma NEFA as a result of dietary treatment in either PP or MP cows (Tables 6 and 7). However, plasma NEFA was higher in MP cows (0.546 ± 0.026 mEq/L) than in PP cows (0.351 ± 0.032 mEq/L). Higher plasma NEFA in MP cows suggested that they were mobilizing more stored energy to support milk production and may account for differences in milk yield seen between parities in response to dietary treatments.

The concentrations of serum urea N were not different in response to either dietary treatment or parity, suggesting that overall N utilization was similar across parities and treatments. Concentrations of serum BHBA were not different in response to dietary treatment for PP or MP cows but were higher in MP cows (585 ± 33 μmol/L) than in PP cows (452 ± 40 μmol/L). Ketosis incidence was determined to be the number of cows from each treatment with BHBA > 1,400 μmol/L at any of the 4 sampling times. This calculation was based on the work of Duffield et al. (1997), who suggested 1,400 μmol/L as the level of BHBA that indicated that a cow was subclinically ketotic. Ketosis incidence data were not analyzed statistically, because the study was not designed to detect differences in the incidence of a disorder, but numerically no PP cows had a BHBA sample that indicated that they were subclinically ketotic. The lower BHBA concentrations in PP cows may indicate why PP and MP cows responded differently to dietary treatments.

Serum triglycerides were not different in response to dietary treatment for PP and MP cows (Table 6 and 7). Serum total cholesterol was not different in PP cows. Within MP cows, those fed RP-BET or RP-CHOL had higher serum total cholesterol than those fed RP-MET but were not different from those fed control. Multiparous cows (197.0 ± 4.6 mg/dL) had higher serum cholesterol than PP cows (169.1 ± 5.7 mg/dL). Serum HDL was not different as a result of dietary treatment for either parity. Serum LDL was higher in MP cows fed RP-BET than in cows fed control or RP-MET; however, serum LDL in MP cows fed RP-CHOL was not different from other treatments. There was no treatment effect for serum LDL in PP cows. Multiparous cows (95.4 ±

**Table 7.** Plasma and serum metabolites as affected by dietary treatment for multiparous cows (n = 12 per treatment)

| | Dietary treatments[1] | | | | | |
|---|---|---|---|---|---|---|
| Item | Control | RP-MET | RP-BET | RP-CHOL | SEM | $P \leq$ |
| SUN,[2] mg/d | 14.9 | 14.6 | 16.1 | 15.3 | 0.8 | 0.62 |
| Plasma NEFA, mEq/L | 0.574 | 0.554 | 0.561 | 0.496 | 0.052 | 0.72 |
| Serum total cholesterol,[3] mg/dL | 191.2[ab] | 177.9[b] | 209.4[a] | 209.6[a] | 9.3 | 0.05 |
| HDL, mg/dL | 103.7 | 93.2 | 100.8 | 102.0 | 3.8 | 0.22 |
| LDL,[4] mg/dL | 84.6[b] | 82.5[b] | 109.5[a] | 105.5[ab] | 8.5 | 0.05 |
| VLDL,[5] mg/dL | 2.98 | 2.81 | 2.64 | 2.86 | 0.15 | 0.45 |
| Serum triglycerides, mg/dL | 14.9 | 14.1 | 13.2 | 14.3 | 0.7 | 0.45 |
| BHBA, μmol/L | 716 | 502 | 571 | 552 | 65 | 0.13 |
| Ketosis incidence[6] | 4 | 2 | 3 | 1 | NA[7] | NA |

[a,b]Means within a row lacking a common superscript differ ($P < 0.05$).

[1]RP-MET = 20 g/d of rumen-protected (RP) Met; RP-BET = 40 g/d of RP-betaine; RP-CHOL = 45 g/d of RP-choline.

[2]SUN = serum urea N.

[3]HDL = high-density lipoproteins; LDL = low-density lipoproteins; VLDL = very low density lipoproteins.

[4]LDL (mg/dL) = cholesterol (mg/dL) − [HDL (mg/dL) + VLDL (mg/dL)] (Friedewald et al., 1972).

[5]VLDL (mg/dL) = triglycerides (mg/dL) ÷ 5 (Friedewald et al., 1972).

[6]Ketosis incidence is calculated as the number of cows from each treatment where BHBA > 1,400 μmol/L for any of the 4 sampling times (Duffield et al., 1997). Ketosis incidence data were not analyzed statistically, because the study was not designed to detect differences in the incidence of a disorder.

[7]NA = not applicable.

4.2 mg/dL) had higher serum LDL concentrations than PP cows (59.2 ± 5.2 mg/dL). There was no effect of dietary treatment on serum VLDL within either parity. Reports of blood lipid profiles in dairy cattle fed RP-choline are limited, and we are not aware of reports of lipid profiles in dairy cattle fed RP-betaine. Guretzky et al. (2006) reported that feeding RP-choline to periparturient cows did not alter lipid metabolism possibly because the cows were not overconditioned and not at a high risk of developing fatty liver. The cows utilized in the present study were not overconditioned, which may have contributed to limited effects of dietary treatments on lipoprotein and lipid profiles.

## CONCLUSIONS

Feeding RP-choline to MP cows that received a Met-limited diet improved milk yield and increased milk protein yield. In this study, supplementing RP-betaine was not beneficial. Therefore, it appears that there was no enhancement of Met production from homocysteine derived from betaine. This offers speculation that the effect of RP-choline could be due to an increased supply of phosphatidylcholine rather than the role of choline as a methyl donor. However, there are several other possibilities to the method of action including increasing phosphatidylcholine, supplying methyl groups, or providing Met.

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