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Lipid Metabolite Profiles and Milk Production for Holstein and Jersey Cows Fed Rumen-Protected Choline During the Periparturient Period[1]

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ABSTRACT

Choline is important for assembly of very low density lipoproteins to export triglyceride from liver; however, studies to assess the effect of rumen-protected choline (RPC) supplementation on blood lipid metabolites in periparturient dairy cows have not been conducted. Thirty-two multiparous Holstein and 10 multiparous Jersey cows were randomly assigned to control or RPC treatments. A close-up diet was fed from approximately 3 wk before parturition through parturition, followed by a lactation diet from parturition through 49 d postpartum. For RPC, diets were topdressed once daily with 60 g of a RPC product (25% choline as choline chloride) from 21 d before expected parturition through 21 d postpartum. Treatment did not affect dry matter intake either prepartum (12.0 vs. 12.1 kg/d for RPC and control, respectively) or during the first 3 wk postpartum (14.8 vs. 15.7 kg/d, respectively). Daily yields of 3.5% fat-corrected milk (39.4 vs. 37.4 kg/d), fat (1.46 vs. 1.38 kg/d), and protein (1.09 vs. 1.05 kg/d) did not differ statistically by treatment (RPC vs. control, respectively). Jersey cows in the control group had lower concentrations of nonesterified fatty acids and β-hydroxybutyrate in plasma during d 1 to 10 postpartum than did other breed and treatment combinations. Cows fed RPC tended to have greater serum triglycerides prepartum (17.0 vs. 14.7 mg/dL) and lower plasma phospholipid at parturition (65.2 vs. 78.1 mg/dL) than control cows. Treatment did not affect cholesterol and phospholipid at other time points, but concentrations followed patterns of dry matter intake pre- and postpartum. Cows were in moderate body condition score (mean = 3.3) at the start of the study and did not lose excessive condition by 3 wk postpartum (mean body condition score loss = 0.5); therefore, cows might not have been at great risk for hepatic lipid accumulation. Additionally, calculated Met balance was negative postpartum; supplemental RPC might not have spared enough Met to produce a physiological benefit. More research is needed to determine how choline affects prevention or alleviation of fatty liver syndrome and to confirm potential differences between Holstein and Jersey cows.

Key words: rumen-protected choline, transition period, lipid metabolism

INTRODUCTION

Choline is a vitamin-like compound whose metabolism interacts very closely with Met and vitamin B_{12} metabolism. Feedstuffs for dairy cattle contain free choline and phosphatidylcholine (Sharma and Erdman, 1989); however, because the choline and phosphatidylcholine content of plants is relatively small and ruminal degradation of choline and phosphatidylcholine is extensive (Sharma and Erdman, 1989), intestinal supply is not enough to meet tissue requirements. In rodents, 15 to 40% of the daily choline requirement is met through biosynthesis from Met (Zeisel, 1981). Infusion of radioactively labeled Met and choline into the blood of lactating goats revealed that 6% of the choline pool in the body is derived from Met and 28% of the Met pool is used for choline synthesis (Emmanuel and Kennelly, 1984). De novo synthesis of choline presumably is equally important for lactating dairy cows. Although choline is not normally deficient, physiological states such as pregnancy and lactation greatly increase the demand for choline (Zeisel, 1990, 2000). Methionine and lysine are the 2 most limiting AA for milk production in dairy cattle (NRC, 2001). Therefore, supplemental choline could spare a portion of Met needed to meet daily choline needs, which would leave a larger supply of Met for milk production.

Choline is a precursor for very low density lipoprotein (**VLDL**) assembly in the liver. Both the number of VLDL particles in the Golgi fraction of the cell and the number of nascent particles in the lumen of the endoplasmic reticulum were reduced when phosphati-

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dylcholine synthesis was inhibited in rat hepatocytes (Vance and Vermeulen, 1998). Media deficient in choline impaired secretion of VLDL from rat hepatocytes but resulted in no change in the rate of disappearance of radioactivity from apoproteins B, E, or C (Yao and Vance, 1988). Supplementation of media with Met or choline stimulated the synthesis of phophatidylcholine in hepatocytes to rates of those not choline deficient. Therefore, because phosphatidylcholine is critical for VLDL formation, physiological conditions that increase the need to mobilize hepatic triglyceride (**TG**) from the liver also may increase the requirement for choline.

During the periparturient period, DMI is depressed, energy balance becomes negative, and stored TG in adipose tissue is mobilized, resulting in increased NEFA in blood. These NEFA are taken up by the mammary gland for milk synthesis, are oxidized by the liver, or are esterified to TG in the liver (Drackley, 1999). As in nonruminant species, esterified TG can be exported from the liver as VLDL, but the rate of this process is limited in ruminants compared with other species (Grummer, 1993). Because phosphatidylcholine is required for VLDL assembly, the lack of sufficient dietary choline supply, coupled with the increased demand for Met for milk synthesis, could render choline a limiting substrate for VLDL synthesis. This conditional deficiency of choline would further slow the rate of TG export from liver, which could contribute to the development of fatty liver and limit milk production.

Rumen-protected choline (**RPC**) products have been fed to periparturient dairy cows to increase the supply of choline to the small intestine with the goal of increasing milk or component yields or alleviating the development of fatty liver syndrome (Hartwell et al., 2000; Piepenbrink and Overton, 2003; Pinotti et al., 2003; Overton and Waldron, 2004). Profiles of NEFA, TG, cholesterol, and phospholipids in blood are altered in cows having naturally occurring fatty liver compared with normal cows (Reid et al., 1983; Holtenius, 1989; Mazur et al., 1989). With the exception of NEFA and BHBA, however, no data are available on the effect of RPC on blood lipid profiles in periparturient cows. Therefore, the objective of this study was to characterize blood profiles of lipids and other metabolites and to determine production responses to RPC supplementation during the periparturient period.

MATERIALS AND METHODS

All procedures were conducted under protocols approved by the University of Illinois Institutional Animal Care and Use Committee. At 25 d before expected parturition, 32 Holstein and 10 Jersey cows entering their second or greater lactation were moved into individual tie stalls and fed a close-up diet (Table 1) balanced to meet or exceed NRC (2001) recommendations at ad libitum intake. On d 21 before expected parturition, cows were randomly assigned by expected calving date to either be supplemented with RPC (Reashure, Balchem Encapsulates, Slate Hill, NY) from d –21 rela-

Table 1. Ingredient and nutrient composition of the basal close-up diet fed from d-21 through d 0 relative to parturition and of the basal lactation diet fed from 1 through 49 DIM

	Diet[1]	
Item	Close-up	Lactation
	(% of DM)	
Ingredient		
Corn silage	38.15	28.13
Alfalfa silage	6.55	20.10
Alfalfa hay	10.06	—
Cottonseed	—	9.65
Corn grain, ground	10.06	26.05
Soybean meal, 48% CP	5.07	5.14
Soybean meal, expeller[2]	—	6.03
Soy hulls	20.81	1.45
Vitamin and mineral mix[3]	0.38	0.20
Salt	—	0.20
Dicalcium phosphate	0.42	0.52
Sodium bicarbonate	—	0.96
Limestone	—	1.41
Magnesium oxide	—	0.12
Vitamin E[4]	—	0.04
Anionic supplement[5]	8.12	—
Urea	0.38	—
Nutrient content[6]		
CP	15.5 ± 0.16	17.2 ± 0.48
ADF	25.2 ± 0.42	20.6 ± 0.67
NDF	38.9 ± 1.98	31.0 ± 1.45
Ether extract	3.2	4.9
NFC	38.3	41.3
NE_L, Mcal/kg	1.64 ± 0.022	1.75 ± 0.031
Ca	1.02 ± 0.049	1.33 ± 0.078
P	0.36 ± 0.027	0.48 ± 0.040
Mg	0.43 ± 0.025	0.30 ± 0.024
K	1.33 ± 0.050	1.51 ± 0.079
Na	0.11 ± 0.151	0.36 ± 0.052
S	0.18 ± 0.016	0.18 ± 0.017
DCAD, mEq/kg	–54	308

[1]For choline-treated cows, 60 g of rumen-protected choline (Reashure, Balchem Encapsulates, Slate Hill, NY) were top-dressed onto the diet at the a.m. feeding from 21 d prior to expected parturition through 21 DIM. Reashure contained a minimum of 250,000 mg of choline as choline chloride/kg.

[2]SoyPLUS (West Central Soy, Ralston, IA).

[3]Mix contained a minimum of 5.0% Mg, 10.0% S, 7.5% K, 2.0% Fe, 3.0% Zn, 3.0% Mn, 5,000 mg of Cu/kg, 250 mg of I/kg, 40 mg of Co/kg, 150 mg of Se/kg, 2,200,000 IU of vitamin A/kg, 660,000 IU of vitamin D_3/kg, and 22,000 IU of vitamin E/kg.

[4]Contained 44,000 IU/kg.

[5]SoyChlor (West Central Soy, Ralston, IA).

[6]Nutrient content based on 4-wk composites of feed samples ± SD (n = 7 for close-up diet; n = 8 for lactation diet). Values for ether extract, NFC, and DCAD were estimated by the NRC (2001) model using mean composition data.

tive to expected parturition through parturition or to consume the basal close-up diet without supplementation (control). The RPC was supplemented as a top-dress of 60 g/d applied to the TMR at the a.m. feeding and supplied 15 g of dietary choline/d as choline chloride. From parturition through 49 DIM, all cows consumed a lactation diet (Table 1) balanced to meet or exceed NRC (2001) requirements for cows in early lactation at ad libitum intake. Cows in the RPC group continued to have RPC top-dressed through 21 DIM.

Both pre- and postpartum diets were mixed daily and fed as a TMR. All cows were fed individually 2 times daily with approximately one-half of their daily ration offered at 1100 h and the other half offered at 1700 h. Amount of feed fed and refused was recorded daily from –22 through +49 d relative to calving to calculate daily DMI. Cows were housed in tie stalls and were allowed to exercise daily between 0700 and 0900 h in an outside lot. At 10 d before expected parturition, cows were moved to individual maternity pens until parturition. Feed was offered in individual feed bunks inside each maternity pen on the same schedule as cows in tie stalls, and exercise was allowed daily between 0900 and 1000 h in an outside lot. After parturition, cows were returned to tie stalls. Cows were milked twice daily at 0300 and 1500 h, and milk yields were recorded. When cows were ≥10 DIM, consecutive a.m. and p.m. milk samples were taken weekly and composited in proportion to milk yield. Milk samples were preserved (800 Broad Spectrum Microtabs II, D & F Control Systems, Inc., San Ramon, CA) and analyzed for contents of fat, protein, lactose, urea N, and SCC by mid-infrared procedures (AOAC, 1995) in a commercial laboratory (Dairy Laboratory Services, Dubuque, IA).

Body weight was recorded once weekly for all cows after the morning milking and before the morning feeding. Additionally, cows were weighed immediately after parturition, and calf birth weight was recorded. Body condition score (Wildman et al., 1982) was assessed independently by 3 individuals once weekly, and the 3 scores were averaged for each cow. Individual feed ingredients were sampled weekly, and DM content (AOAC, 1995) was determined for each component. Diets were adjusted for DM content of ingredients on a weekly basis. Weekly samples were frozen at –20°C and then composited monthly for analyses of DM, CP, NDF, ADF, Ca, P, K, and Mg by wet chemistry techniques at a commercial laboratory (Dairy One, Ithaca, NY). For energy calculations, Dairy One used the Ohio State summative energy equation to predict TDN at maintenance and the NRC (2001) equations to calculate NE_L at 3× maintenance; the Van Soest variable discount system was used for forages.

Blood was sampled from the coccygeal vein or artery at 0900 h (after orts were removed and before the a.m. feeding) on d –22, –21, –19, –16, and –13; daily from d –10 through 10; and d 13, 16, 19, 21, 28, 35, 42, and 49 relative to parturition. Blood was collected into evacuated serum tubes (SST, Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) containing clot activator and into evacuated plasma tubes (Becton Dickinson Vacutainer Systems) containing K_3 EDTA. Plasma tubes were immediately placed on ice after collection for transport to the laboratory. All tubes were centrifuged at 1,300 × *g* for 10 min to obtain serum and plasma, which were aliquotted and stored at –20°C until further analysis.

The concentration of NEFA was determined in plasma using a commercially available enzymatic-colorimetric kit (NEFA C, Wako Chemicals USA, Inc., Richmond, VA) with modifications reported by Johnson and Peters (1993). Phospholipids in plasma were measured using a commercial kit (Phospholipids B, Wako Chemicals USA, Inc.). In this procedure, choline is liberated by phospholipase D and then oxidized to betaine by choline oxidase with simultaneous production of hydrogen peroxide, which, in the presence of peroxidase, couples 4-aminoantipyrine and phenol to yield a chromagen that is detected spectrophotometrically at 505 nm. The concentration of TG was determined in serum using a commercially available kit (Stanbio Triglyceride LiquiColor Procedure No. 2200, Stanbio Laboratory, Boerne, TX). In this procedure, lipase produces free glycerol that is phosphorylated by glycerol kinase. The resulting glycerol-3-phosphate is oxidized by glycerylphosphate oxidase and hydrogen peroxide, which, in the presence of peroxidase, reacts with 4-aminoantipyrine and 4-chlorophenol to produce a quinoneimine that is quantified at 500 nm. Concentrations of glucose (Peterson and Young, 1968), BHBA (Williamson and Mellanby, 1974), urea N (Crocker, 1967), total protein (Weichselbaum, 1945), and cholesterol (Allain et al., 1974) in plasma were measured with an auto-analyzer using enzymatic kits (Glucose/HK kit, Roche Diagnostics Corp., Indianapolis, IN; BHBA kit number 310-A, Sigma Chemical Co., St. Louis, MO; urea N kit number 535, Sigma Chemical Co.; total protein kit, Roche Diagnostics, Inc.; cholesterol/HP kit, Roche Diagnostics, Inc.). For determination of NEFA, BHBA, and glucose concentration, all available time points corresponding to planned sample days around calving (d –10 to 10) were used; however, from the daily samples taken around calving, only samples from d –10, – 5, 0, 5, and 10 were used to determine TG, phospholipid, urea N, total protein, and cholesterol concentrations.

Table 2. NRC (2001) model inputs and estimates used to predict MP balance and Met as a percentage of MP for the close-up and lactation diets fed to Holstein and Jersey cows

	Close-up diet[1]		Lactation diet[2]	
Variable	Holstein	Jersey	Holstein	Jersey
Inputs				
DMI, kg	12.9	12.2	17.0	13.4
BW, kg	715	506	630	463
BCS	3.4	3.3	2.8	2.9
Day of gestation	269	269	—	—
DIM	—	—	11	11
Milk, kg/d	—	—	35.6	25.7
Fat, %	—	—	4.06	4.72
Protein, %	—	—	3.03	3.61
Lactose, %	—	—	4.86	4.79
Estimates				
NE_L requirement, Mcal/d	13.9	10.2	36.7	29.6
NE_L supply, Mcal/d	21.3	21.8	28.8	22.8
NE_L balance, Mcal/d	7.4	11.5	–7.8	–6.8
MP requirement, g/d	851	678	2,237	1,887
MP supply, g/d	1,237	1,301	1,881	1,494
MP balance, g/d	386	623	–356	–393
Lys, % of MP	6.80	6.70	6.61	6.64
Met, % of MP	1.84	1.91	1.85	1.86

[1]Close-up diet fed from d 23 through d 0 relative to parturition.

[2]Lactation diet fed from parturition through 49 DIM.

Energy balance was calculated both pre- and postpartum for each cow using equations from NRC (2001). Intake of NE_L was calculated by multiplying the daily DMI by NE_L density in the diet determined using the monthly composites of individual feed ingredients as just described. Maintenance NE_L (Mcal/d) was calculated as $BW^{0.75} \times 0.080$. Pregnancy requirements for NE_L (Mcal/d) were calculated as $[(0.00318 \times \text{day of gestation} - 0.0352) \times (\text{calf birth weight}/45)]/0.218$. Requirements of NE_L for milk production were calculated as $(0.0929 \times \text{fat percentage}) + (0.0547 \times \text{protein percentage}) + (0.0395 \times \text{lactose percentage})$.

The NRC (2001) model was used to predict balances of RDP and RUP and to estimate the Met content of diets as a percentage of MP. Data for cows, including breed, age, lactation number, DMI, BW, BCS, milk production, and milk composition, and weather data during the study were used in the model for predictions (Table 2). Analyzed values for individual feed ingredients (alfalfa silage, alfalfa hay, cottonseed, corn silage, expeller soybean meal, vitamin and mineral mix) were used to adjust the default values in the model. For all other feedstuffs, the default values were used for predictions. Although estimates for Met content could have been under- or overpredicted by the model, they are useful for biological interpretation because of the interaction of Met with choline metabolism.

Data were analyzed as a randomized design using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC) with the following model:

$$y_{ijkl} = \mu + W_i + B_j + WB_{ij} + T_k + WT_{ik} + BT_{jk} + WBT_{ijk} + C_{(ijk)l}$$

where y_{ijkl} = an observation from week i or day relative to calving, breed j, treatment k, and cow l; μ = the grand mean; W_i = effect of week i (or day); B_j = effect of breed j; WB_{ij} = effect of the week (or day) by breed interaction; T_k = effect of treatment k; WT_{ik} = effect of the week (or day) by treatment interaction; BT_{jk} = effect of the breed by treatment interaction; WBT_{ijk} = effect of the week (or day) by breed by treatment interaction; and $C_{(ijk)l}$ = random experimental error from cow l nested within week i (or day), breed j, and treatment k.

The REPEATED statement was used for variables measured over weeks (BW, BCS, DMI, milk yield, and milk components) or days (blood metabolites). For data from the day of parturition and variables not measured over time (BW change and BCS change), the MIXED procedure of SAS was used without the REPEATED statement and week or day relative to calving; all associated interactions were removed from the model. The random error term used for all mixed models was cow within breed and treatment, and the covariance structure yielding the lowest Akaike's information criterion was used (Littell et al., 1998). Degrees of freedom were estimated by using the Satterthwaite option in the model statement. When significant interactions with treatment occurred, linear contrast statements were

Table 3. Incidence of twinning and health problems in Holstein and Jersey cows fed either a control lactation diet or the control diet top-dressed with 60 g of rumen-protected choline (RPC) from 21 d prepartum through 21 d postpartum

	Treatment			Breed[1]	
Variable	Control	RPC	P^1	Holstein	Jersey
Twinning	0	3	0.07	3	0
Days carried calf	274	278	0.20	—	—
Milk fever[2]	1	0	0.31	0	1
Retained placenta[3]	1	5	0.08	6	1
Metritis[2]	2	3	0.63	5	0
Displaced abomasum	0	0	—	0	0
Ketonuria[4]	6	5	0.73	10	1
Mastitis[5]	4	6	0.47	6	4
Foot/leg problems[6]	4	3	0.68	6	1
Total	18	22	0.83	33	8
Cows with >1 health problem	5	7	0.49	11	1

[1]*P* values are reported for treatment comparisons. Breed data are reported for descriptive purposes.

[2]Diagnosed and treat by a veterinarian.

[3]Fetal membranes retained >48 h postpartum.

[4]Defined as having a positive urine ketone test (trace to strong).

[5]Clincal cases of mastitis diagnosed and treated by farm staff and/or veterinarians.

[6]Included hairy heel warts (n = 2), foot rot (n = 1), interdigital dermatitis (n = 1), abcess (n = 1), hock bruise (n = 1), and laminitis (n = 1).

constructed to explore them. Health and twinning data were analyzed with the FREQ procedure in SAS and were interpreted using the Fisher's Exact Test probabilities. Tendencies or trends in all data were declared at $0.05 < P \leq 0.15$.

Parity and previous mature-equivalent yields of milk, fat, and protein were balanced ($P > 0.37$) between treatment groups (parity = 2.8 ± 1.11; milk = 10,710 ± 2,129 kg; fat = 421 ± 62 kg; protein = 340 ± 51 kg). Before statistical analysis, daily measurements for DMI and milk yield were condensed to weekly means. Although the design of the experiment allowed for covariate adjustment of means, breed effects and interactions of breed with treatment or day relative to parturition were significant for BW, milk yield, milk components, and blood metabolites ($P < 0.05$). Therefore, to make conclusions about breed effects, covariate adjustment was not used. To avoid problems with fitting covariance structure, pre- and postpartum data and data from the day of parturition were analyzed separately. Because the main objective of this study was to determine whether RPC affected blood metabolites or production variables, postpartum data were initially analyzed including all 7 wk of the study. However, when significant treatment effects or interactions occurred, or when important biological trends were evident in the data postpartum, data were evaluated using predetermined periods of interest. These periods were the postpartum treatment period (wk 1 to 3) and the post-treatment period (wk 4 to 7) and addressed the question of whether there was an effect of RPC only during supplementation or if the effect carried beyond the supplementation period.

RESULTS AND DISCUSSION

The close-up diet fed before parturition was predicted by the NRC (2001) model to supply adequate amounts of MP to both breeds (Table 2). During the first 21 DIM, the NRC (2001) model predicted that both breeds consuming the lactation diet had a negative MP balance, although the Lys and Met content as a percentage of MP was similar to the amount provided in the close-up diet (approximately 7.0 and 1.85%, respectively). The desired Lys and Met supply to the duodenum to maximize milk protein content and yield is 7.2 and 2.4% of MP, respectively (NRC, 2001). The predicted Lys supply to the intestine was near predicted requirements; however, predicted Met supply was deficient by 6.5 g/d. Given this state of negative Met balance, supplemental choline would presumably spare some quantity of Met used for de novo choline synthesis and contribute a physiological benefit to these cows. Others have addressed the problem of low duodenal Met supply by, for example, adding corn gluten meal to the diet (Piepenbrink and Overton, 2003).

The incidence of twinning tended ($P = 0.07$) to be greater for cows in the RPC group compared with the control group (Table 3), which likely contributed to the tendency ($P = 0.08$) for more cows in the RPC group to have a retained placenta. Twinning occurred only in Holsteins and did not affect gestation length ($P =$

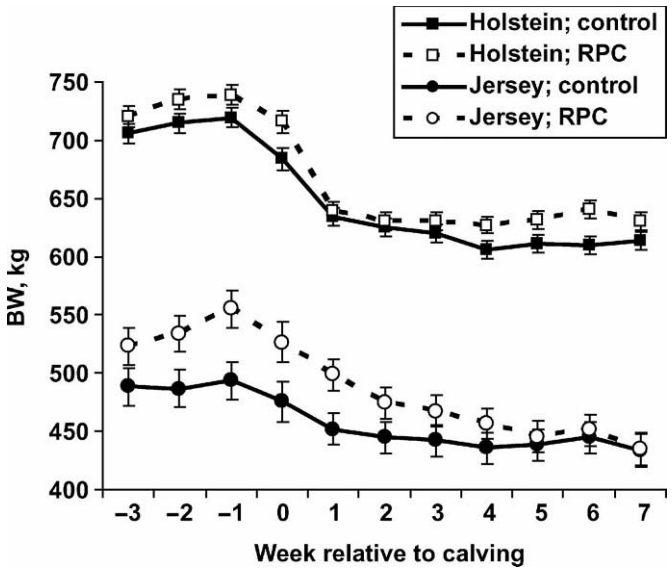

Figure 1. Least squares means and associated SE for weekly BW of Holstein and Jersey cows fed either a control diet or the control diet top-dressed with 60 g of rumen-protected choline (RPC) from 21 d prepartum through 21 d postpartum. Prepartum: week and breed, $P < 0.001$; treatment, interaction of breed by week, interaction of treatment by week, and interaction of treatment by breed by week, $P > 0.18$. Parturition: breed, $P < 0.001$; treatment and interaction of breed by treatment, $P > 0.15$. Postpartum (wk 1 to 7): week and breed, $P < 0.001$; interaction of breed by treatment by week, $P = 0.002$; treatment, interaction of breed by week, interaction of treatment by week, and interaction of breed by week, $P > 0.15$. Postpartum (wk 1 to 3): interaction of treatment by week by breed (linear), $P = 0.07$; week and breed, $P < 0.0001$; other effects, $P > 0.18$.

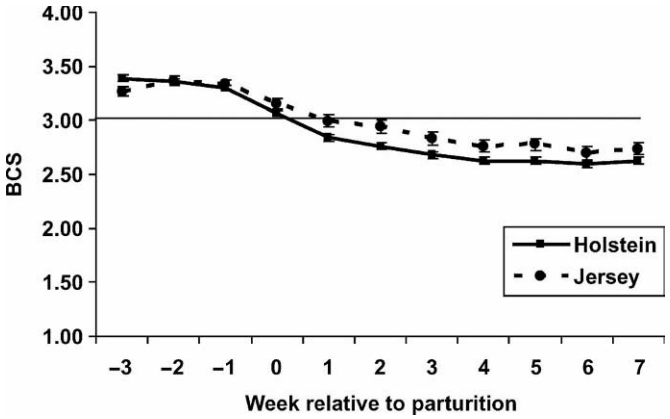

Figure 2. Least squares means and associated SE of weekly BCS (5-point scale) for Holstein and Jersey cows fed either a control diet or the control diet top-dressed with 60 g of rumen-protected choline from 21 d prepartum through 21 d postpartum. Prepartum: all effects and interactions, $P > 0.45$. Parturition: all effects and interactions, $P > 0.46$. Postpartum: week, $P < 0.001$; breed, $P = 0.06$; treatment, two-, and three-way interactions of main effects, $P > 0.25$.

0.20) between treatments (Table 3). The tendencies for increased twinning and associated retained placentas would not be related to supplemental RPC because number of fetuses was established long before initiating RPC supplementation. Deletion of cows that calved with twins from the data set did not change interpretation of treatment effects (data not shown). Other health problems occurred predominantly postpartum and incidence was not different between treatments ($P \geq 0.31$). Consequently, it is unlikely that health problems biased treatment effects. Although important for biological interpretation of subsequent production data, caution should be used when interpreting these health data as treatment effects because animal numbers were small. Treatment did not affect calf birth weight (data not shown; $P = 0.26$).

Week relative to parturition and breed affected BW pre- and postpartum (Figure 1), and week relative to parturition affected BCS pre- and postpartum (Figure 2). No treatment effects or interactions were observed prepartum ($P > 0.15$). The greatest change in BW occurred in the first 3 wk after parturition, which tended to be affected by treatment ($P = 0.12$). This change was most likely attributed to mobilization of body stores around parturition. Cows of both breeds were in intermediate BCS (Wildman et al., 1982) at the beginning of the study, which suggested that they were not at great risk for mobilization of large amounts of body lipid (Gearhart et al., 1990). Jerseys tended ($P = 0.06$) to have greater mean BCS postpartum than Holsteins (2.9 vs. 2.7), although the magnitude of this difference may not be biologically significant. An interaction of breed by treatment by week affected BW postpartum. This interaction resulted from a difference in the rate of BW loss between Jersey cows fed RPC and control Holstein cows observed during wk 1 to 7 and only tended ($P = 0.07$) to be significant during the choline supplementation period (the first 3 wk postpartum). Treatment did not affect total BW gained or lost from –3 wk to parturition ($P = 0.48$) or during the first 7 wk of lactation ($P = 0.16$). Similarly, treatment did not influence total BCS gained or lost from –3 wk to parturition ($P = 0.70$), parturition to +3 wk postpartum ($P = 0.44$), or over the first 7 wk of lactation ($P = 0.62$).

Hartwell et al. (2000) observed that periparturient cows fed 12 g of protected choline/d had greater BW loss than did cows fed 0 or 6 g/d; however, total BCS loss was not affected by treatment. Piepenbrink and Overton (2003) reported no effect of RPC on BW or BCS when supplemented at 19 g/d, although cows lost approximately one BCS unit. Supplementation of cows with a protected choline product during early lactation also had no effect on BW (Erdman and Sharma, 1991).

Week relative to parturition affected DMI as a percentage of BW prepartum (Figure 3). Jerseys consumed more DM as a percentage of BW prepartum

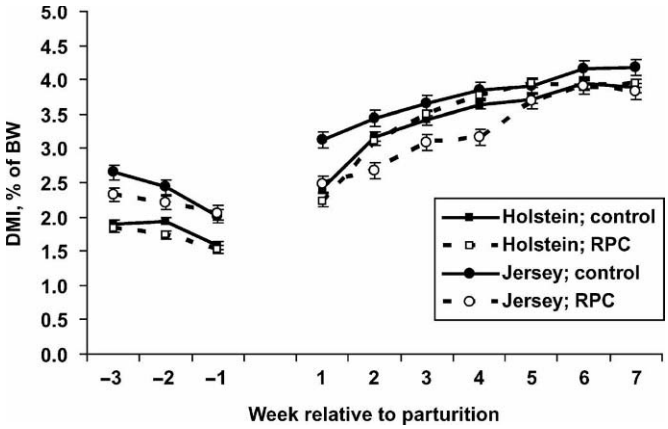

Figure 3. Least squares means and associated SE for weekly DMI as a percentage of BW for Holstein and Jersey cows fed either a control diet or the control diet top-dressed with 60 g of rumen-protected choline (RPC) from 21 d prepartum through 21 d postpartum. Prepartum: week, $P < 0.001$; breed, $P = 0.003$; treatment, two-, and three-way interactions of main effects, $P > 0.18$. Postpartum (wk 1 to 7): interaction of breed by week, $P = 0.003$; treatment, breed, week, and other two- and three-way interactions of main effects, $P > 0.18$. Postpartum (wk 1 to 3): treatment, $P = 0.06$; week and interaction of breed by week, $P < 0.001$; interaction of breed by treatment, $P = 0.11$; other main effects and interactions, $P > 0.49$.

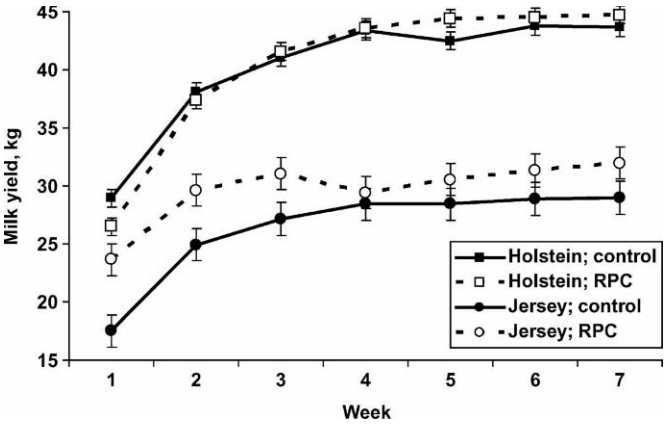

Figure 4. Least squares means and associated SE for weekly milk yield for Holstein and Jersey cows fed either a control diet or the control diet top-dressed with 60 g of rumen-protected choline (RPC) from 21 d prepartum through 21 d postpartum. Week 1 to 7: breed and week, $P < 0.001$; interaction of breed by week, $P < 0.01$; treatment and other two- and three-way interactions, $P > 0.26$. Week 1 to 3: breed, week, and interaction of breed by week, $P < 0.001$; interaction of breed by week by treatment, $P = 0.11$; other effects, $P > 0.23$.

than did Holsteins ($P = 0.003$). The main effects of treatment and the two- or three-way interactions were not significant prepartum ($P > 0.18$). Postpartum DMI (Table 4) was not affected by treatment, although breed by week interactions were significant. The breed by week interaction also was significant for DMI as a percentage of BW (Figure 3), as Jerseys consumed more DM than Holsteins during the first 3 wk of lactation ($P < 0.001$). For DMI as a percentage of BW during the first 3 wk of lactation (Figure 3), the effects of treatment ($P = 0.06$) and the treatment by week interaction ($P = 0.11$) approached significance, indicating that relative DMI tended to be lower for cows fed RPC. The effects of treatment, breed, and week and other two- and three-way interactions, however, were not significant ($P > 0.18$) over the first 7 wk of lactation. Others have observed no effect of protected choline on DMI in transition or lactating cows (Erdman and Sharma, 1991; Hartwell et al., 2000; Piepenbrink and Overton, 2003).

Treatment did not affect milk yield (Table 4; $P > 0.40$). Similarly, 3.5% FCM yield was not influenced by treatment ($P = 0.36$). A breed by week interaction affected milk yield over the 7 wk of lactation studied (Figure 4). This interaction was present primarily dur-

Table 4. Least squares means for DMI and milk yield for Holstein and Jersey cows fed either a control lactation diet or the control diet top-dressed with 60 g of rumen-protected choline (RPC) from 21 d prepartum through 21 d postpartum

	Treatment			*P*				
Variable	Control	RPC	SEM	Breed	Treatment (Trt)	Breed × Trt	Week	Breed × week
DMI, kg/d								
wk 1 to 3	15.7	14.8	0.71	0.001	0.41	0.47	<0.001	<0.001
wk 1 to 7	18.3	17.8	0.65	<0.001	0.61	0.23	<0.001	<0.001
Milk yield,[1] kg/d								
wk 1 to 3	29.6	31.6	1.69	<0.001	0.40	0.23	<0.001	<0.001
wk 1 to 7	33.3	35.0	1.45	<0.001	0.40	0.45	<0.001	<0.001
3.5% FCM yield,[2] kg/d								
wk 2 and 3	45.1	48.1	3.46	0.003	0.40	0.33	0.26	0.23
wk 2 to 7	45.6	47.9	1.91	<0.001	0.39	0.61	0.78	0.21

[1]An interaction of breed by treatment by week tended to affect milk yield during the first 3 wk of lactation ($P = 0.11$).

[2]Fat-corrected milk = 0.4324 × (milk yield) + 16.2162 × (fat yield). Milk components were not sampled until cows were ≥10 DIM; therefore, FCM was not calculated for the first week.

Table 5. Least squares means for milk components during the first 7 wk of lactation for Holstein and Jersey cows fed either a control lactation diet or the control diet top-dressed with 60 g of rumen-protected choline (RPC) from 21 d prepartum through 21 d postpartum

	Treatment			*P*				
Milk component	Control	RPC	SEM	Breed	Treatment (Trt)	Breed × Trt	Week	Breed × week
Fat								
%	4.09	4.09	0.085	<0.001	0.99	0.63	<0.001	0.30
kg/d	1.38	1.46	0.060	<0.01	0.34	0.72	0.45	0.77
Protein								
%	3.12	3.04	0.056	<0.001	0.34	0.19	<0.001	0.36
kg/d	1.05	1.09	0.043	<0.001	0.60	0.68	0.18	0.05
Lactose								
%	4.91	4.81	0.035	0.67	0.07	0.29	<0.001	0.57
kg/d	1.71	1.75	0.074	<0.001	0.66	0.59	<0.001	0.02
TS								
%	13.03	12.85	0.106	<0.001	0.23	0.13	<0.0001	0.50
kg/d	4.46	4.63	0.178	<0.001	0.50	0.63	0.68	0.13
SCC	2.9	3.2	0.44	0.04	0.55	0.92	0.01	0.33
MUN, mg/dL	14.4	13.7	0.59	0.22	0.44	0.75	0.11	0.14

ing the first 3 wk of lactation when RPC was supplemented. As expected, Jerseys had lower milk yield than Holsteins, and week affected milk yield during the first 7 wk of lactation. Although treatment and other two- and three-way interactions were not significant ($P > 0.26$) for the entire 7 wk study, differences tended to exist among treatments and breeds during the first 3 wk of lactation (interaction of treatment by week by breed, $P = 0.11$). Each breed had a different rate of milk yield increase in the first 3 wk of lactation. Jersey cows in the RPC group produced more milk than controls, which most influenced this interaction and resulted in the nonsignificant 2-kg/d advantage in milk yield over the first 7 wk of lactation for the RPC group. Similarly, RPC cows had a numerical 3.0-kg/d increase in FCM yield over controls in early lactation (wk 2 and 3) and a 2.3-kg/d increase in FCM yield over the 7-wk experiment.

Over the experimental period, yields of fat, protein, lactose, and solids as well as urea N content and somatic cell score were not affected by treatment (Table 5; $P > 0.34$). Compared with Holsteins, Jerseys had higher contents of fat (4.49 vs. 3.69%) and protein (3.33 vs. 2.82%) in milk, although Holsteins produced more fat (1.6 vs. 1.3 kg/d) and protein (1.2 vs. 1.0 kg/d) over the study period. Lactose percentage tended to be greater in control cows ($P = 0.07$) than in RPC cows, although total lactose produced was not different between groups ($P = 0.66$). The tendency for a greater percentage of lactose was not significant during the RPC supplementation period ($P = 0.22$). Somatic cell score was higher in Jerseys than in Holsteins and decreased as lactation progressed.

Responses in milk yield to feeding RPC products during the transition period or early lactation in general have been positive (Overton and Waldron, 2004), although responses have varied considerably. Responses might have been influenced by diet characteristics such as CP or percentage of forage in the diet. Supplementation of protected choline in diets formulated to be below NRC (1989) recommendations for CP resulted in a quadratic increase in 3.5% FCM yield for cows in early lactation (Erdman and Sharma, 1991). A second experiment by these researchers also revealed that 3.5% FCM yield response to choline was greater when a 13% CP diet was fed compared with a 16.5% CP diet. The effect of supplemental choline at up to 12 g/d depended on the RUP content of the diet for cows fed diets containing 4.0 or 6.2% RUP (Hartwell et al., 2000). Supplementation of the 4.0% RUP diet with 12 g of choline/d increased milk yield by 7.1 kg/d compared with the same choline supplementation level in the diet containing 6.2% RUP. A tendency for higher fat yield and 3.5% FCM yield was observed for cows supplemented with protected choline from 21 d prepartum through 63 DIM when Met was not limiting in the diet (Piepenbrink and Overton, 2003). Supplementation with a rumen-protected product to deliver 20 g of choline/d to a diet with >70% of DM as forage from 21 d prior to parturition through 30 DIM increased milk yield, 3.5% FCM yield, and fat percentage compared with control cows (Pinotti et al., 2003).

From these studies, it seems that dietary characteristics have a role in the milk yield response that is observed when RPC is fed. In the present study, CP content of the diet exceeded NRC (2001) recommendations, although MP flow to the small intestine was estimated to be deficient by the NRC (2001) model (Table 2). As nearly one-third of the Met pool is used to make choline de novo in ruminants (Emmanuel and

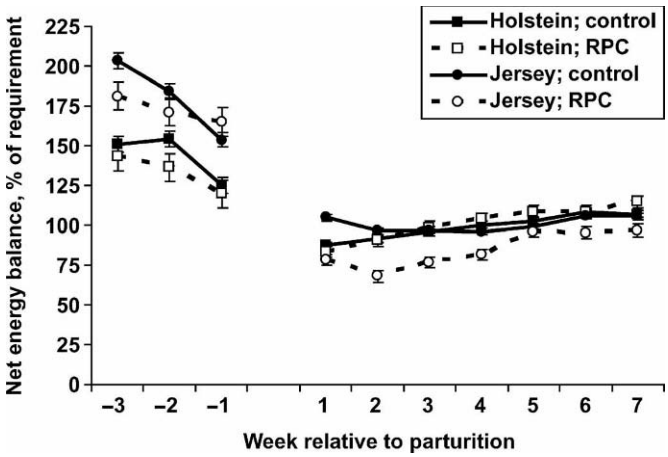

Figure 5. Least squares means and associated SE of net energy balance as a percentage of requirement for Holstein and Jersey cows fed either a control lactation diet or the control diet top-dressed with 60 g of rumen-protected choline (RPC) from 21 d prepartum through 21 d postpartum. Prepartum: breed, $P = 0.01$; week, $P < 0.001$; interaction of treatment by week, $P = 0.13$; all other effects and interactions, $P > 0.36$. Postpartum (wk 1 to 7): week, $P < 0.001$; treatment, $P = 0.10$; breed, $P = 0.08$; interaction of breed by treatment and interaction of week by treatment, $P = 0.03$; interaction of breed by week, $P = 0.01$. Postpartum (wk 1 to 3): interaction of breed by week, $P < 0.001$; treatment, week, and interaction of breed by treatment, $P = 0.02$; breed and other interactions, $P > 0.22$.

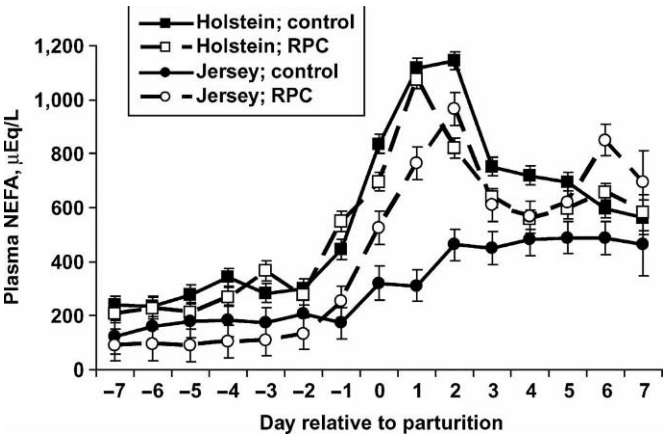

Figure 6. Least squares means and associated SE for plasma NEFA concentration from 7 d prepartum through 7 d postpartum for Holstein and Jersey cows fed either a control diet or the control diet top-dressed with 60 g of rumen-protected choline (RPC) from 21 d prepartum through 21 d postpartum. Prepartum: day, $P = 0.002$; treatment, breed, and two- and three-way interactions, $P > 0.21$. Parturition: breed, $P = 0.07$. Postpartum: day, $P = 0.002$; interaction of breed by treatment, $P = 0.04$; interaction of day by breed, $P = 0.01$; treatment, interaction of treatment by day, and interaction of treatment by breed by day, $P > 0.32$.

Kennelly, 1984), the importance of Met for synthesis of choline should not be ignored. If the synthesis of choline from Met is inhibited, yields of milk, milk components, and FCM are depressed compared with cows fed supplemental choline (Sharma and Erdman, 1988). In rodents deficient in choline, the activity of the liver enzyme phosphatidylethanolamine-N-methyltransferase is greatly upregulated, resulting in a greater use of S-adenosylmethionine for choline synthesis (Zeisel, 1981, 1990). In the present study, perhaps a portion of the absorbed Met was obligatorily being used for choline synthesis. Therefore, despite the fact that choline was supplemented, it might not have been sufficient to spare a quantity of Met needed to benefit milk production.

Predicted net energy balance is shown as a percentage of requirements in Figure 5. Cows in both treatments moved toward negative energy balance prepartum, were in negative energy balance postpartum, and approached positive energy balance by wk 4 of lactation. Cows in the RPC group tended ($P = 0.10$) to experience greater negative energy balance postpartum compared with control cows (–2.23 vs. –0.18 Mcal/d); Jersey cows in the RPC group greatly influenced this effect (interaction of breed by treatment, $P = 0.03$). Over the first 3 wk of lactation, cows fed RPC were in greater negative energy balance than controls (87.1 vs. 91.3% of requirement, $P = 0.02$; –5.80 vs. –1.64 Mcal/d, $P = 0.02$); however, this difference was again largely driven by Jerseys (interaction of breed by treatment, $P = 0.02$). Holsteins returned to positive energy balance postpartum more quickly than did Jerseys (interaction of breed by week, $P = 0.01$) over the 7-wk period postpartum. The difference in energy balance observed for Jersey cows in the RPC group may be explained by a lack of difference between DMI and the tendency for greater milk production by these cows during the first 3 wk of lactation when RPC was supplemented. With the exception of the Jerseys in the control group, the lack of a treatment difference or interactions with treatment of BCS suggested that both breeds mobilized similar amounts of energy to meet their energy requirements.

Supplemental RPC did not affect NEFA ($P > 0.21$) or BHBA ($P > 0.31$) concentrations around parturition (Figures 6 and 7). Except for Jersey cows in the control group, plasma NEFA spiked within the first 3 d postpartum (interaction of breed by treatment, $P = 0.04$). Plasma BHBA followed a similar pattern (interaction of breed by treatment, $P = 0.05$). With the exception of the control Jersey cows, plasma NEFA was affected by day relative to parturition pre- and postpartum. An interaction of day by breed affected plasma NEFA postpartum, which again resulted from Jersey cows in the control group. These cows were in more positive energy balance and produced less milk than those in the RPC group, which may explain the lower NEFA and BHBA postpartum. Because of this atypical re-

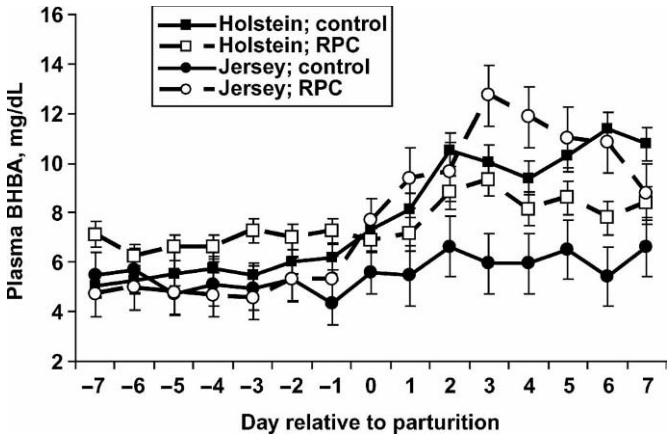

Figure 7. Least squares means and associated SE for plasma BHBA concentration from 7 d prepartum to 7 d postpartum for Holstein and Jersey cows fed either a control diet or the control diet top-dressed with 60 g of rumen-protected choline (RPC) from 21 d prepartum through 21 d postpartum. Prepartum: day, $P = 0.05$; treatment, breed, and two- and three-way interactions, $P > 0.31$. Parturition: treatment, breed, interactions, $P > 0.39$. Postpartum: interaction of breed by treatment, $P = 0.05$; treatment, breed, and two- and three-way interactions, $P > 0.34$.

sponse at calving, data were examined for outlying data points; however, none were identified.

Except for control Jersey cows, the spike in NEFA observed at parturition was typical for cows in the transition period, and concentrations were of the magnitude at which fatty infiltration of the liver might be expected to occur (Grummer, 1993; Grum et al., 1996). Hartwell et al. (2000) observed no effect of RPC on plasma NEFA during the transition period, whereas Pinotti et al. (2003) observed lower NEFA at parturition for cows receiving choline. Although treatment did not lower NEFA or BHBA, Piepenbrink and Overton (2003) reported increased liver glycogen and numerically lower liver TG accumulation for cows supplemented with RPC. Liver slices from cows fed RPC had greater capacity to convert propionate to glucose and a lower rate of conversion of palmitate to esterified products, but these responses were not large enough to achieve statistical significance (Piepenbrink and Overton, 2003). It seems reasonable to conclude from these data that, although the amount of NEFA (or BHBA) in circulation may be unaltered by RPC supplementation, enhanced VLDL assembly and transport could still occur in hepatocytes. In our experiment, however, these results may be confounded by the negative Met balance of the diet so that a considerable portion of the supplemental choline was used to spare Met rather than to enhance VLDL assembly and transport.

Supplemental RPC had no effect on concentrations of glucose, urea N, or total protein in plasma (Table 6). Jersey cows, which were in greater negative energy balance postpartum than Holsteins, tended ($P = 0.06$) to have lower plasma glucose postpartum. The concentration of TG in serum tended ($P = 0.08$) to be elevated in RPC cows compared with control cows prepartum, which might be indicative of greater TG export from the liver because DMI was not different between groups (Mazur et al., 1989); however, this effect was not present at parturition ($P > 0.73$). Interestingly, Jerseys had higher serum TG than did Holsteins postpartum. This effect was influenced by Jerseys in the RPC group that tended to have greater TG postpartum (interaction of breed by treatment, $P = 0.07$).

Cows in the RPC group had lower plasma phospholipids than controls at parturition (Figure 8); however, an interaction of breed by treatment ($P = 0.03$) also was present. This interaction resulted from Jerseys in the control group having greater plasma phospholipid concentration at parturition compared with the RPC group, whereas Holsteins in both treatment groups had similar plasma phospholipid concentrations. In a pattern that closely resembled DMI, plasma phospholipid concentration decreased as parturition approached and then increased after parturition. Jerseys had greater plasma phospholipid concentrations before parturition than did Holsteins; however, this difference was not present after parturition ($P = 0.60$). The RPC group had lower phospholipid concentrations than did controls postpartum, the concentration was similar between groups during the first 3 wk of lactation, and the RPC group had lower phospholipid concentrations during wk 4 through 7 of lactation (interaction of treatment by day, $P = 0.01$).

The pattern observed for serum cholesterol concentration in blood pre- and postpartum was strikingly similar to that observed for phospholipid (Figure 9), and it too appeared to follow the pattern of DMI. Association of patterns of change in phospholipids and cholesterol with changes in DMI may not be surprising given that most circulating TG, and hence TG-rich lipoproteins, are of intestinal origin (Emery et al., 1992).

Alterations in lipid metabolite profiles, such as lower concentrations of cholesterol, albumin, and globulin (Reid et al., 1983); lower cholesterol and phospholipid (Holtenius, 1989); or lower TG, cholesterol, and apoprotein A-1 (Mazur et al., 1989) have been observed in blood from cows with fatty infiltration of the liver. Research also has demonstrated that dietary choline supplementation does not result in increased blood TG or phospholipid concentrations (Erdman et al., 1984; Atkins et al., 1988; Erdman and Sharma, 1991; Sharma and Erdman, 2003). Therefore, an alteration of lipid metabolites in cows fed RPC could indicate

Table 6. Least squares means for concentrations of glucose, urea N, and total protein in plasma and triglyceride (TG) in serum for Holstein and Jersey cows fed either a control lactation diet or the control diet top-dressed with 60 g of rumen-protected choline (RPC) from 21 d prepartum through 21 d postpartum

	Treatment			*P*				
Metabolite and stage[1]	Control	RPC	SEM	Breed	Treatment (Trt)	Breed × Trt	Day	Breed × day
Glucose, mg/dL								
Prepartum	68.9	66.8	2.18	0.20	0.51	0.42	0.33	0.54
Parturition	76.3	74.2	5.93	0.37	0.77	0.30	—	—
Postpartum	56.8	55.4	1.31	0.06	0.45	0.52	0.20	0.69
Urea N, mg/dL								
Prepartum	14.0	13.4	0.43	0.07	0.33	0.05	0.32	0.22
Parturition	12.6	14.4	1.00	0.69	0.22	0.73	—	—
Postpartum	15.3	15.5	0.47	0.53	0.85	0.24	0.09	0.07
Total protein, g/dL								
Prepartum	7.4	7.3	0.16	0.94	0.55	0.33	0.01	0.65
Parturition	6.9	6.8	0.19	0.92	0.73	0.56	—	—
Postpartum	7.5	7.7	0.11	0.21	0.22	0.70	<0.01	0.06
TG, mg/dL								
Prepartum	14.7	17.0	0.90	0.21	0.08	0.11	0.01	0.58
Parturition	17.1	17.3	2.33	0.76	0.95	0.47	—	—
Postpartum	13.6	13.9	0.68	0.04	0.73	0.07	0.03	0.26

[1]Prepartum = mean concentration for 3 wk prior to parturition, parturition = sample within 0 to 24 h postpartum, and postpartum = mean concentration for 7 wk postpartum.

a physiological benefit with regard to prevention or alleviation of fatty liver. In the present study, however, none of the lipid components were altered by RPC supplementation compared with controls. The higher phospholipid concentration observed for control Jersey cows compared with Jersey cows fed RPC would support results observed for NEFA, BHBA, and energy balance, as these cows would have been least likely to experience fatty infiltration of liver. However, for all cows, the blood concentrations of TG, cholesterol, and phospholipid observed were higher than values for cows with fatty liver in the studies discussed previously; therefore, neither treatment group likely was at risk for fatty liver. More research is needed regarding lipid metabolite profiles in response to dietary RPC to determine whether they can accurately predict effects on prevention or alleviation of fatty liver syndrome.

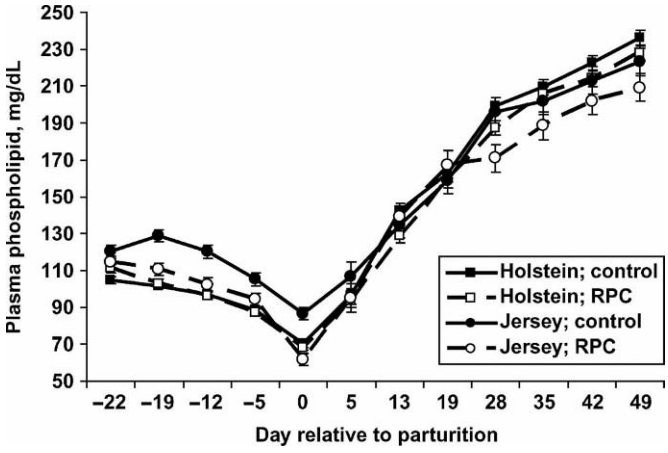

Figure 8. Least squares means and associated SE for plasma phospholipid concentration pre- and postpartum for Holstein and Jersey cows fed a control diet or the control diet top-dressed with 60 g of rumen-protected choline (RPC) from 21 d prepartum through 21 DIM. Prepartum: day, $P < 0.001$; breed, $P = 0.001$; interaction of breed by treatment, $P = 0.13$; other two- and three-way interactions, $P > 0.22$. Parturition: treatment, $P = 0.02$; interaction of breed by treatment, $P = 0.03$; breed, $P = 0.33$. Postpartum: day, $P < 0.001$; interaction of treatment by day, $P = 0.01$; interaction of breed by treatment by week, $P = 0.13$; treatment, breed, and other two-way interactions, $P > 0.36$.

CONCLUSIONS

In this study, blood lipid metabolites did not reveal any alterations in lipid metabolism as a result of feeding RPC during the transition period. Cows were not overconditioned to begin the study (mean BCS = 3.3) and lost only 0.5 BCS unit through the first 3 wk postpartum. Had cows been overconditioned and, therefore, at greater risk for hepatic accumulation of TG, perhaps evidence for the ability of RPC to alter blood lipid profiles would have been present. Differences between breeds were not expected a priori, but Jersey cows seemed to respond differently to RPC supplementation than Holsteins. However, because energy balance and NEFA profiles after parturition for the control Jerseys were atypical, these data are not compelling. Potential differences in response to RPC and its relationship to Met status between Jerseys and Holsteins deserve additional research.

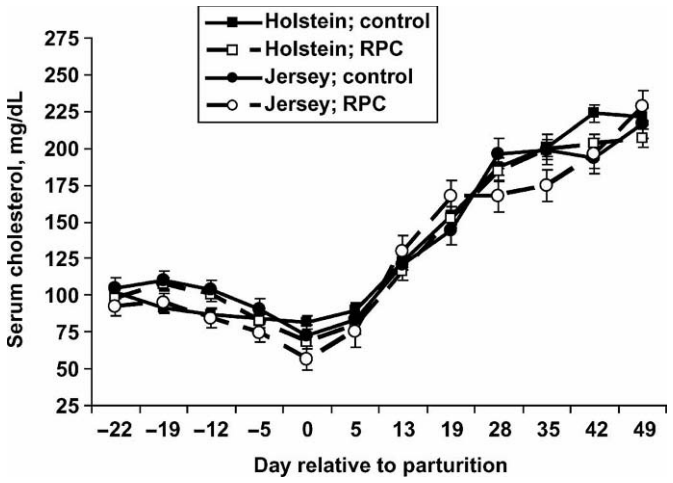

Figure 9. Least squares means and associated SE for serum cholesterol concentration pre- and postpartum for Holstein and Jersey cows fed a control diet or the control diet top-dressed with 60 g of rumen-protected choline (RPC) from 21 d prepartum through 21 DIM. Prepartum: day, $P = 0.06$; breed, treatment, and two- and three-way interactions, $P > 0.19$. Parturition: all effects and interactions, $P > 0.23$. Postpartum: day, $P < 0.001$; treatment, breed, and two- and three-way interactions, $P > 0.35$.

The 2.3-kg increase in FCM yield observed for cows supplemented with RPC was smaller than responses observed by other researchers and was not statistically significant. Because demands for Met for milk production are high and nearly one-third of the Met pool is needed for de novo synthesis of choline, dietary supply of Met to the small intestine may be important when deciding whether to supplement RPC. Benefits from RPC supplementation seem most likely for cows that are more susceptible to fatty liver and for diets that have low passage of Met to the small intestine. In the present study, however, even though the predicted percentage of Met in MP from the diets was less than NRC (2001) recommendations, large responses to RPC were not observed.

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