Effects of supplementation with ruminally protected choline on performance of multiparous Holstein cows did not depend upon prepartum caloric intake

M. G. Zenobi, R. Gardinal,¹ J. E. Zuniga,² A. L. G. Dias,³ C. D. Nelson, J. P. Driver, B. A. Barton,⁴
J. E. P. Santos, and C. R. Staples⁵

Department of Animal Sciences, University of Florida, Gainesville 32611

ABSTRACT

Objectives were to evaluate the effect of prepartum energy intake on performance of dairy cows supplemented with or without ruminally protected choline (RPC; 0 or 17.3 g/d of choline chloride; 0 or 60 g/d of ReaShure, Balchem Corp., New Hampton, NY). At 47 ± 6 d before the expected calving date, 93 multiparous Holstein cows were assigned randomly to 1 of 4 dietary treatments in a 2×2 factorial arrangement. Cows were fed energy to excess [EXE; 1.63 Mcal of net energy for lactation/kg of dry matter (DM)] or to maintenance (MNE; 1.40 Mcal of net energy for lactation/kg of DM) in ad libitum amounts throughout the nonlactating period. The RPC was top-dressed for 17 ± 4.6 d prepartum through 21 d postpartum (PP). After calving, cows were fed the same methionine-balanced diet, apart from RPC supplementation, through 15 wk PP. Liver was biopsied at -14 , 7, 14, and 21 d relative to parturition. Cows fed EXE or MNE diets, respectively, consumed 40 or 10% more Mcal/d than required at 15 d before parturition. Cows fed the MNE compared with the EXE diet prepartum consumed 1.2 kg/d more DM postpartum but did not produce more milk (41.6 vs. 43.1 kg/d). Thus, PP cows fed the EXE diet prepartum were in greater mean negative energy balance, tended to have greater mean concentrations of circulating insulin, fatty acids, and β -hydroxybutyrate, and had greater triacylglycerol in liver tissue (8.3 vs. 10.7% of DM) compared with cows fed the MNE diet prepartum. Cows fed RPC in transition tended to produce more milk (43.5 vs. 41.3 kg/d) and energy-corrected milk (44.2 vs. 42.0 kg/d) without increasing DM intake (23.8

vs. 23.2 kg/d) during the first 15 wk PP, and tended to produce more milk over the first 40 wk PP (37.1 vs. 35.0 kg/d). Energy balance of cows fed RPC was more negative at wk 2, 3, and 6 PP, but mean circulating concentrations of fatty acids and β -hydroxybutyrate did not differ from those of cows not fed RPC. Despite differences in energy balance at 2 and 3 wk PP, mean concentration of hepatic triacylglycerol did not differ between RPC treatments. Feeding RPC reduced the daily prevalence of subclinical hypocalcemia from 25.5 to 10.5%, as defined by concentrations of total Ca of <8.0 mg/dL in serum in the first 7 d PP. Pregnancy at first artificial insemination tended to be greater for cows fed RPC (41.3 vs. 23.6%), but the proportion of pregnant cows did not differ by 40 wk PP. Heifers born from singleton calvings from cows fed RPC tended to experience greater daily gain between birth and 50 wk of age than heifers from cows not supplemented with RPC. Feeding RPC for approximately 38 d during the transition period tended to increase yield of milk for 40 wk regardless of amount of energy consumed during the pregnant, nonlactating period.

Key words: choline, wheat straw, fatty liver, hypocalcemia

INTRODUCTION

For decades, farmers and scientists have been aware of the dangers of calving dairy cows with excess subcutaneous fat, also known as overconditioned cows, on their subsequent performance (Morrow, 1976). Negative consequences of overconditioned cows at calving have included reduced DMI, fatty liver, less milk production, increased metabolic problems, and poor reproductive performance (Drackley, 1999). The presence of overconditioned cows (BCS >3.75) on farms is likely less of a problem today than in the latter part of the 20th century. However, excessive intake of calories by nonlactating cows can lead to accumulation of body fat that is hidden from view (omental, mesenteric, and prerenal fat; Drackley et al., 2014), such that BCS was unchanged, although Drackley et al. (2014) did not

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¹Current address: Department of Animal Nutrition and Production, University of Sao Paulo, 13635-900, Pirassununga, Brazil.

²Current address: San Isidro, Perez Zeledón, San José, Costa Rica.

³Current address: Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, T6G 2P5, Canada.

⁴Current address: 52 Sunrise Park Rd, Balchem Corp., New Hampton, NY.

⁵Corresponding author: chasstap@ufl.edu

report carcass fat that included subcutaneous fat. This may cause managers to be content that their cows will not experience the negative effects of overconditioning on postpartum performance because BCS is within industry recommendations. Although it remains to be determined whether greater accumulation of visceral fat around parturition may have subsequent negative effects on performance, overconsumption of calories during the entire dry period compared with feeding to maintenance led to increased concentrations of plasma fatty acids (Janovick et al., 2011; Graugnard et al., 2013; Mann et al., 2015) and BHB (Janovick et al., 2011; Mann et al., 2015), and increased concentration of triacylglycerol (**TAG**) in liver (Janovick et al., 2011; Graugnard et al., 2013; Mann et al., 2015). If cows are overfed calories only during the last 3 wk of gestation (close-up period), a common current practice, negative results can occur postpartum, including greater loss of BW (Zhang et al., 2015) and a more rapid increase in hepatic TAG (Ji et al., 2012).

Choline is classified as a quasivitamin because it does not fit the classical definition of a vitamin in that it is not identified as being needed as an enzyme cofactor, it is required in gram rather than in milligram quantities by nonruminants, and, unlike B vitamins, it becomes an integral part of the structural component of tissues [e.g., phosphatidylcholine (**PC**) in cell wall membranes; Zeisel, 1991]. Choline is a major lipotropic compound in mammals in that it promotes the export of fat from the liver as part of very low density lipoproteins. Indeed, lactating dairy cows supplemented with ruminally protected choline (**RPC**) chloride around the time of parturition have experienced a reduction in concentration of hepatic TAG (Santos and Lima, 2009; Zom et al., 2011; Elek et al., 2013). This lipotropic effect of choline has been suggested as the main mechanism to help explain the frequent improvement in yield of milk by cows supplemented with RPC around parturition (Shahsavari et al., 2016).

Overfeeding energy during the entire dry period should increase hepatic concentrations of TAG. Therefore, the aim of the present experiment was to evaluate the effect of supplementation with RPC on performance of multiparous cows that have been fed calories to meet or greatly exceed requirements during the entire dry period. Our hypothesis was supplementation of RPC in the periparturient period would preferentially benefit those multiparous dairy cows that were more prone to develop fatty liver postpartum; that is, those overconsuming calories in the prepartum period. An interaction of prepartum caloric intake by RPC supplementation was expected. A second aim was to evaluate the effect of energy density of the prepartum diet and RPC sup-

plementation to dams in late lactation on the growth rate of their female offspring from birth to yearling age.

MATERIALS AND METHODS

Cows and Dietary Treatments

The experiment was conducted at the University of Florida dairy farm from November 2014 to September 2015. All procedures involving cows in the experiment were carried out according to the University of Florida's Institutional Animal Care and Use Committee.

At 47 ± 6 d before the expected calving date, pregnant, nonlactating Holstein cows with at least 1 previous lactation ($n = 109$) were enrolled in the experiment. Selection criteria included apparently healthy cows with no history of chronic disease before enrollment and a 305-d mature equivalent milk yield greater than 6,800 kg. For cows that completed the study ($n = 93$), the mean $\pm$ standard deviation for BW and BCS (Elanco Animal Health, 2009) were 735 ± 90 kg and 3.54 ± 0.33 , respectively, at the time of enrollment. Cows were assigned randomly to 1 of 4 treatments in a 2×2 factorial arrangement. One factor was RPC fed at 0 (**−RPC**) or 17.3 g/d of choline chloride (**+RPC**) per cow from 21 d before expected calving date through 21 d postpartum. The choline chloride was fed as part of 60 g/d of ReaShure (Balchem Corp., New Hampton, NY). The RPC was mixed with ground corn and dried molasses in a 30:56:14 ratio (as-is basis) and top-dressed (200 g/d) onto the TMR fed to individual cows once daily. Cows assigned to the **−RPC** treatment received 200 g/d of a top-dressing of ground corn and dried molasses in an 80:20 ratio (as-is basis). The second factor was prepartum diets formulated to 1.63 (excess energy; **EXE**) or 1.40 (maintenance energy; **MNE**) Mcal of NE_L /kg of dietary DM fed in ad libitum amounts (Table 1). Therefore, the 4 treatments were EXE intake without or with RPC and MNE intake without or with RPC. Adjusting the proportions of corn silage, triticale silage, and chopped wheat straw in the 2 prepartum diets largely accounted for the differences in NE_L concentration (Table 1). The major difference between the far-off and close-up diets was the inclusion of an acidogenic product (Biochlor; Arm and Hammer Animal Nutrition, Princeton, NJ) in the close-up diets to create diets with negative DCAD values (Table 2).

Feeding Management and Chemical Analyses

After calving, cows were fed the same diet (Tables 1 and 2), apart from RPC supplementation, through 15 wk PP. From dry off to 25 d before expected calv-

ing date, cows were grouped separately, according to energy treatment, in outdoor pens with free access to feed, shade, and water. Starting at 24 d before expected calving date, cows were relocated and grouped into 1 outdoor pen equipped with shade and fans, as well as sprinklers over the feeding area. Each cow was assigned randomly and trained (24 to 22 d before calving) to an individual feeding gate (Calan Broadbent feeding system, American Calan Inc., Northwood, NH). Within 6 h after calving, cows were moved to an open-sided freestall barn with sand-bedded stalls with fans mounted above the beds and a water sprinkler line in the feed

bunk area for cooling of cows. Cows were assigned and trained to an individual feeding gate (American Calan Inc.). Cows fully learned to open their assigned Calan gate within 1 d postpartum.

Wheat straw was purchased in 35-kg bales (Quality Hay Sales Inc., Willison, FL), stored in a closed container on farm, and chopped to a particle length of ≤ 5 cm as needed for the TMR. Methionine was formulated to be approximately 2.3% of MP according to NRC (2001) in both prepartum and postpartum diets. All diets were fed as a TMR once daily at 0930 h during the prepartum period and twice daily at 0730 and 1100

Table 1. Ingredient composition of diets fed during the far-off, close-up, and lactating periods

Ingredient, % of DM	Far-off diet ¹		Close-up diet ²		Lactating diet
	EXE ³	MNE ⁴	EXE	MNE	
Corn silage	58.3	22.1	58.3	23.3	45.2
Wheat straw	8.3	40.4	8.3	37.5	—
Triticale silage	—	6.2	—	5.8	—
Brewers grains, wet	16.7	16.7	16.7	16.7	—
Citrus pulp, dried	—	—	6.9	3.3	6.0
Canola meal, pelleted	6.2	10.8	2.1	7.4	—
Mineral-vitamin premix ⁵	3.8	3.8	—	—	—
Mineral-vitamin premix ⁶	—	—	4.2	4.2	—
Biochlor ⁷	—	—	3.5	1.8	—
Corn, ground	—	—	—	—	14.0
Soybean hulls	6.7	—	—	—	8.0
Soybean meal	—	—	—	—	15.2
Whole cottonseed	—	—	—	—	8.0
Mineral-vitamin premix ⁸	—	—	—	—	3.6

¹Diets differing in energy were fed from 47 ± 6 to 17.0 ± 4.6 d before calving.

²Diets differing in energy were fed from 17 ± 4.6 d before calving until the day of calving.

³EXE = excess energy. Diet formulated to supply energy in great excess of cow's requirement for maintenance and pregnancy (NRC, 2001).

⁴MNE = maintenance energy. Diet formulated to supply energy in slight excess of cow's requirement for maintenance and pregnancy (NRC, 2001).

⁵Contained (DM basis) 39.5% wheat bran, 20.8% calcium carbonate, 20.8% sodium chloride, 14.6% magnesium oxide, 2.8% vitamins A, D, and E, 2.0% ClariFly Livestock Premix 0.67% (Central Garden & Pet Co., Schaumburg, IL), 0.6% Sel-Plex 2000 (Alltech Biotechnology, Nicholasville, KY), 0.52% Rumensin 90 (Elanco Animal Health, Greenfield, IN), 0.36% IntelliBond Vital 4 (Micronutrients USA LLC, Indianapolis, IN), and 0.002% EDDI 80%. Each kilogram contained 3.6% CP, 8.4% Ca, 0.4% P, 8.0% Mg, 0.5% K, 0.07% S, 8.2% Na, 12.7% Cl, 98 mg of Fe, 965 mg of Zn, 204 mg of Cu, 785 mg of Mn, 13 mg of Se, 11 mg of Co, 18 mg of I, 227 kIU of vitamin A, 76 kIU of vitamin D, 2,267 IU of vitamin E, 1,034 mg of monensin, and 187 mg of diflubenzuron.

⁶Contained (DM basis) 62.7% corn gluten feed, 15.7% magnesium sulfate $\times 7$ H₂O, 8.9% calcium carbonate, 6.7% magnesium oxide, 2.2% sodium chloride, 2.0% ClariFly Livestock Premix 0.67% (Central Garden & Pet Co.), 0.67% vitamin E 500,000 kIU/kg, 0.45% Sel-Plex 2000 (Alltech Biotechnology), 0.38% Rumensin 90 (Elanco Animal Health), 0.27% IntelliBond Vital 4 (Micronutrients USA LLC), 0.036% vitamin A concentrate 650,000 kIU/kg, 0.013% vitamin D concentrate 500,000 kIU/kg, and 0.002% EDDI 80%. Each kilogram contained 13.5% CP, 3.7% Ca, 0.9% P, 5.5% Mg, 0.9% K, 2.3% S, 1.0% Na, 1.6% Cl, 151 mg of Fe, 724 mg of Zn, 165 mg of Cu, 543 mg of Mn, 9 mg of Se, 4 mg of Co, 16 mg of I, 233 kIU of vitamin A, 67 kIU of vitamin D, 3,362 IU of vitamin E, 748 mg of monensin, and 139 mg of diflubenzuron.

⁷Arm & Hammer Animal Nutrition, Princeton, NJ.

⁸Contained (DM basis) 29.9% sodium bicarbonate, 19.9% LysAAMet (Perdue Venture Milling, Salisbury, MD), 14.3% DCAD Plus (Arm & Hammer Animal Nutrition), 13.2% calcium carbonate, 7.7% magnesium oxide, 6.6% dicalcium phosphate, 6.6% sodium chloride, 1.0% vitamins A, D, and E, ClariFly Livestock Premix 0.67% (Central Garden & Pet Co.), 0.39% Sel-Plex 2000 (Alltech Biotechnology), 0.22% IntelliBond Vital 4 (Micronutrients USA LLC), 0.22% Rumensin 90 (Elanco Animal Health), and 0.002% EDDI 80%. Each kilogram contained 18.2% CP, 6.5% Ca, 1.3% P, 4.2% Mg, 7.0% K, 10.6% Na, 4.0% Cl, 0.2% S, 1,053 mg of Fe, 566 mg of Zn, 132 mg of Cu, 470 mg of Mn, 7.8 mg of Se, 3.9 mg of Co, 13.0 mg of I, 157 kIU of vitamin A, 34 kIU of vitamin D, 1,145 IU of vitamin E, 441 mg of monensin, and 66 mg of diflubenzuron.

Table 2. Chemical composition of diets (mean $\pm$ SD) fed during the far-off, close-up, and lactating periods

Component	Far-off diet ¹		Close-up diet ²		Lactating diet
	EXE ³	MNE ⁴	EXE	MNE	
NE _L , ⁵ Mcal/kg	1.63 $\pm$ 0.03	1.42 $\pm$ 0.03	1.63 $\pm$ 0.03	1.40 $\pm$ 0.02	1.68 $\pm$ 0.03
CP, % of DM	13.7 $\pm$ 0.4	13.6 $\pm$ 0.2	14.0 $\pm$ 0.6	13.5 $\pm$ 0.3	16.0 $\pm$ 1.2
MP, ⁶ g/d	1,095	881	1,168	951	2,796
Methionine, % of MP	2.28	2.18	2.40	2.30	2.30
Lysine:methionine	2.96	3.01	2.90	2.90	3.10
NDF, % of DM	46.6 $\pm$ 3.3	57.6 $\pm$ 1.6	43.2 $\pm$ 3.5	55.7 $\pm$ 1.7	37.1 $\pm$ 2.6
ADF, % of DM	25.4 $\pm$ 2.5	33.6 $\pm$ 1.7	26.9 $\pm$ 2.5	36.2 $\pm$ 1.7	24.0 $\pm$ 1.3
Starch, % of DM	22.8 $\pm$ 3.8	11.8 $\pm$ 1.4	19.6 $\pm$ 3.9	8.91 $\pm$ 1.5	21.9 $\pm$ 1.9
NFC, % of DM	30.2 $\pm$ 3.6	19.0 $\pm$ 1.4	33.7 $\pm$ 3.8	21.2 $\pm$ 1.4	36.7 $\pm$ 3.1
Ca, % of DM	0.62 $\pm$ 0.03	0.65 $\pm$ 0.01	0.54 $\pm$ 0.04	0.59 $\pm$ 0.03	0.58 $\pm$ 0.04
P, % of DM	0.35 $\pm$ 0.02	0.37 $\pm$ 0.01	0.35 $\pm$ 0.01	0.35 $\pm$ 0.01	0.33 $\pm$ 0.01
K, % of DM	0.95 $\pm$ 0.07	1.08 $\pm$ 0.09	0.90 $\pm$ 0.07	1.06 $\pm$ 0.09	1.42 $\pm$ 0.06
Na, % of DM	0.33 $\pm$ 0.01	0.34 $\pm$ 0.01	0.10 $\pm$ 0.02	0.09 $\pm$ 0.01	0.35 $\pm$ 0.04
S, % of DM	0.18 $\pm$ 0.01	0.21 $\pm$ 0.001	0.34 $\pm$ 0.04	0.33 $\pm$ 0.04	0.18 $\pm$ 0.01
Mg, % of DM	0.45 $\pm$ 0.01	0.47 $\pm$ 0.001	0.39 $\pm$ 0.05	0.43 $\pm$ 0.05	0.35 $\pm$ 0.02
Cl, % of DM	0.72 $\pm$ 0.05	0.82 $\pm$ 0.06	0.49 $\pm$ 0.07	0.45 $\pm$ 0.12	0.39 $\pm$ 0.06
Fe, mg/kg of DM	164 $\pm$ 17	154 $\pm$ 15	148 $\pm$ 17	185 $\pm$ 12	247 $\pm$ 60
Cu, mg/kg of DM	18 $\pm$ 0.5	16 $\pm$ 0.3	12 $\pm$ 0.9	15 $\pm$ 1.8	13 $\pm$ 3.0
Mn, mg/kg of DM	65 $\pm$ 13.2	67 $\pm$ 5.5	48 $\pm$ 13.0	57 $\pm$ 5.0	52 $\pm$ 8.3
Zn, mg/kg of DM	94 $\pm$ 9.3	83 $\pm$ 3.7	71 $\pm$ 10.0	75 $\pm$ 11.0	72 $\pm$ 9.3
DCAD, mEq/100 g of DM	6.9 $\pm$ 2.6	5.8 $\pm$ 1.2	-7.7 $\pm$ 5.0	-3.4 $\pm$ 1.6	29.1 $\pm$ 3.8

¹Diets differing in energy were fed from 47 $\pm$ 6 d to 17.0 $\pm$ 4.7 d before calving.

²Diets differing in energy were fed from 17.0 $\pm$ 4.6 d before calving until the day of calving.

³EXE = excess energy. Diet formulated to supply energy in great excess of cow's requirement for maintenance and pregnancy (NRC, 2001).

⁴MNE = maintenance energy. Diet formulated to supply energy in slight excess of cow's requirement for maintenance and pregnancy (NRC, 2001).

⁵Calculated using NRC (2001) equations.

⁶Calculated for actual DMI using NRC (2001) software.

h during the postpartum period. The amount of TMR offered to individual cows was adjusted daily to result in 2 to 5% refusals. Refusals were weighed once daily before the first morning feeding. For nonlactating and lactating cows, the TMR was pushed toward cows to allow easy consumption every 30 min or as needed during the first 2 h after feedings, every 2 h up to 1800 h, and, lastly, after cows were milked at night.

Nonforage feedstuffs (except wet brewer's grains) were mixed in 1,800-kg batches and stored in metal bins to serve as concentrate mixes. Wheat straw and wet feeds were sampled every 3 d, whereas concentrate mixtures were sampled weekly, dried at 55°C for 48 h in a forced-air oven, and dry weight was recorded. Dried individual feed samples were ground to pass a 1-mm screen of a Wiley mill (Thomas Scientific, Swedesboro, NJ). Dried, ground feed samples were composited monthly and analyzed using wet chemistry procedures (AOAC methods; Dairyland Laboratories Inc., Arcadia, WI). The daily DMI of individual cows were calculated based on DM of individual feeds measured at 105°C. The forage-to-concentrate ratios of the TMR (as-fed basis) fed were adjusted every 3 d using the rolling average of the DM values (105°C) of the wet feeds and concentrate mixes.

Cow and Calf Measurements

Average daily gain was determined for female calves using BW at birth, weaning (8 wk), and 50 wk of age. Calves were weighed within 2 h of birth and before feeding of colostrum (>50 g/L of IgG). A complete grain starter was offered in ad libitum amounts during the first 8 wk of life. The weaning process involved reducing milk consumption from 3 L twice daily to 3 L once daily at 6 wk of age, removal of milk from the diet at 7 wk of age, and movement of heifers from single to group housing on pasture at 61.1 $\pm$ 3.3 d of age. Body weights were measured on the day of moving to group housing. Upon weaning, heifers were offered only starter for 2 wk. A silage-based TMR in ad libitum amounts was offered thereafter through 50 wk of age. Body weight was measured in the morning before feeding of TMR at 350.0 $\pm$ 2.2 d of age.

Colostrum yield was recorded automatically (AfFlo milk meters, S.A.E. Afikim, Israel) as the first milking postpartum and a representative sample was collected and tested for Brix units using a digital refractometer (MA871; Milwaukee Instruments Inc., Rocky Mount, NC). Brix units (%) were converted to IgG (g/L) based on the equation reported by Biemann et al. (2010).

Yield of milk was recorded automatically (AfiFlo milk meters) at 1000 and 2200 h. Samples of milk were collected once weekly in 2 sequential milkings for measurements of fat, true protein, lactose, and SCC. Bronopol-B-14 (Advanced Instruments, Norwood, MA) was used as a preservative and samples were sent within a day of collection to Southeast Milk dairy laboratory (Bellevue, FL) for analysis of butterfat, true protein, lactose, and solids using a Bentley 2000 infrared component analyzer (Bentley Instruments Inc., Chaska, MN). Concentration of somatic cells was determined using a Bentley 500 Somacount flow cytometer (Bentley Instruments Inc.). Milk yield from each sampling was used to calculate the final concentration of milk components. Production of 3.5% FCM and ECM (kg/d) was calculated using the following equations:

$$3.5\% \text{ FCM} = (0.4324 \times \text{milk, kg/d}) \\ + (16.218 \times \text{milk fat, kg/d}), \text{ and}$$

$$\text{ECM} = (0.3246 \times \text{milk, kg/d}) + (12.86 \times \text{milk fat, kg/d}) + (7.04 \times \text{milk protein, kg/d}) \text{ (Orth, 1992)}.$$

During the prepartum period, BW and BCS were taken using increments of 0.25 units (Elanco Animal Health, 2009) by the same 2 trained individuals at dry off and the day of transfer to Calan gates. During the postpartum period cows were weighed twice daily on a walk-through scale (AfiWeigh, S.A.E. Afikim) located in the exit lane of the milking parlor. Body condition was scored at calving and 2, 4, 7, 10, and 15 wk postpartum as described previously. Energy balance (**EB**) was calculated according to NRC (2001).

Blood was collected at 12 and 7 d before expected calving and at 0, 1, 3, 7, 14, 21, and 35 d postpartum by puncture of the coccygeal vessels into evacuated tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ) containing lithium heparin and placed on ice. Within 30 min of collection, cold samples were centrifuged at $1,125 \times g$ for 15 min at room temperature, plasma was harvested, and multiple aliquots of 1.5 mL were frozen at -30°C until analysis.

Samples collected at -12 , -7 , 0, 7, 14, 21, and 35 d relative to calving were analyzed for BHB, fatty acids, plasma urea N, glucose, and insulin to assess energy status of the cows. Each run included a common control sample. A Technicon Autoanalyzer (Technicon Instruments Corp., Chauncey, NY) was used to measure concentrations of plasma glucose (Bran and Luebbe Industrial Method 339-19; Gochman and Schmitz, 1972) and urea N (Bran and Luebbe Industrial Method 339-01; Marsh et al., 1965)] in duplicate. Inter- and

intra-assay coefficients of variation were 1.9 and 0.7% for glucose and 2.2 and 1.0% for plasma urea N, respectively. Concentrations of fatty acids (NEFA-C kit; Wako Diagnostics Inc., Richmond, VA; as modified by Johnson and Peters, 1993) and BHB (Wako Autokit 3-HB; Wako Diagnostics Inc.) were determined in duplicate. Inter- and intra-assay coefficients of variation were 4.4 and 4.7% and 10.8 and 7.8% for fatty acids and BHB, respectively. Concentrations of insulin were analyzed using an ELISA kit (Mercodia Bovine Insulin ELISA, Mercodia Inc., Uppsala, Sweden); inter- and intra-assay coefficients of variation were 9.2 and 16.4%, respectively. Total Ca (**tCa**) was determined on plasma of blood collected at 0, 1, 3, and 7 DIM using an atomic absorption spectrophotometer (AAAnalyst 200, Perkin-Elmer Inc., Waltham, MA) with the flame technique and acetylene gas; inter- and intra-assay coefficients of variation were 1.2 and 0.5%, respectively.

Samples of liver tissue (0.5 to 1.5 g wet weight) were collected at -9.0 ± 4.4 , 7, 14, and 21 DIM using a stainless steel percutaneous liver biopsy tool (Aries Surgical, Davis, CA). An area between the 11th and 13th rib was scanned by ultrasound (Aloka SSD-500V equipped with a 3.5-MHz convex transducer, Aloka Co. Ltd., Tokyo, Japan) to determine the optimal intercostal location for liver tissue collection. After rinsing with sterile saline, liver was sliced into 3 sections, transferred into 3 cryovials, snap-frozen in liquid N, and stored at -80°C until analysis for TAG in triplicate. For analysis, samples were thawed on ice, blotted dry, and approximately 0.1 g of tissue was homogenized in 1 mL of saline in triplicate using an Ultra-Turrax T25 homogenizer (Rose Scientific Ltd., Edmonton, AB, Canada). After addition of 2 mL of 1:1 saline:methanol, samples were split into 3 aliquots. Dry matter was determined from 0.5 mL of homogenized liver sample by drying for 48 h in a 55°C forced-air oven. A second aliquot was analyzed for TAG (Folch et al., 1957) using a colorimetric assay (Foster and Dunn, 1973). A third aliquot was used to measure DNA content of the sample (Labarca and Paigen, 1980).

Definition and Diagnosis of Dystocia, Retained Fetal Membranes, Metritis, and Other Diseases

Dystocia was defined as calving assistance required for >15 min. Retained fetal membranes was declared when a cow failed to expel the fetal membranes within 24 h after parturition. The criteria used to identify cows for a health assessment throughout the experiment included inappetence at the morning feeding, milk yield deviation $>15\%$ compared with the previous day, and intervals between meals of >4 h during daylight hours.

Because the TMR for each cow was pushed up into a mound every hour, the disturbance of that mound was evidence of a meal. A complete physical examination, including rectal temperature and urine sample, was performed at 4, 7, and 12 DIM for diagnosis of disease; cows with a rectal temperature $\geq 39.5^{\circ}\text{C}$ were considered febrile. Diagnosis of metritis was based on a fetid watery discharge from the vagina and an enlarged uterus irrespective of rectal temperature. Fever concurrent with metritis was indicative of puerperal metritis. Milk fever was diagnosed based on clinical signs of recumbency or inability to rise. Subclinical hypocalcemia (SCH) was defined as plasma tCa concentrations of $< 8.0 \text{ mg/dL}$ (Reinhardt et al., 2011) at 0 (day of parturition), 1, 3, or 7 DIM. Hyperketonemia was defined as concentrations of plasma BHB $\geq 1.2 \text{ mM}$ for at least 1 d from 1 to 21 DIM (Oetzel, 2004). Cows diagnosed as having moderate ($\sim 0.4 \text{ mM}$) or large ketone concentrations ($> 0.8 \text{ mM}$) in urine were considered to have clinical ketosis (URS-1K, Teco Diagnostics, Anaheim, CA). In addition to 4, 7, and 12 DIM, urine was sampled if a cow was suspected of having ketosis. Diagnosis of clinical ketosis resulted in treatment with 500 mL of propylene glycol or intravenous infusion of 250 mL of 50% dextrose solution and 10 to 20 mL of a vitamin B complex, concurrent with an oral drench (Van Beek Natural Sci, Orange City, IA). Cows with moderate or severe ketosis for 3 d received an additional treatment of 10 mL of dexamethasone i.v. or i.m on d 3 and veterinary consult. Mastitis was defined as visible abnormalities in the milk or udder with or without systemic symptoms. Other disorders or diseases diagnosed included digestive problems (indigestion and displaced abomasum) and respiratory disease. Cows were removed from the experiment when failing to recover from a clinical disorder after treatment or surgery. Six, 2, 2, and 6 cows were removed from the experiment representing treatments EXE -RPC, EXE +RPC, MNE -RPC, and MNE +RPC, respectively (Table 3) and were not included in the statistical analyses.

Vaginal discharge was collected at 21 ± 1 and 40 ± 1 DIM using the Metrichick device (Kiwikit Ltd., Wales, New Zealand) and scored according to Sheldon et al. (2006). Cows having a vaginal discharge score > 2 were classified as having purulent vaginal discharge (Dubuc et al., 2010). Additionally, subclinical endometritis was assessed at 40 ± 1 DIM using the cytobrush technique (Lima et al., 2013). After sample collection, the cytobrush was rolled onto a slide and later stained using the Diff-Quick stain kit (IMEB, San Marcos, CA). Two technicians blinded to treatments read the slides. Two hundred cells were counted on each slide using a microscope at $400\times$ magnification to determine the proportion of PMNL relative to the total leukocytes and

endometrial cells. Cows with a proportion of PMNL $> 5\%$ were classified as having subclinical endometritis (Gilbert et al., 2005).

Reproductive Management and Reproductive Responses

Cows were subjected to synchronization of the estrous cycle and ovulation using a double Ovsynch protocol (Ribeiro et al., 2012). Estrous cyclicity was evaluated at 26 ± 3 and 40 ± 3 DIM by ultrasonographic examination of the ovaries using a portable ultrasound scanner equipped with a 7.5-MHz transrectal probe (Easi-Scan, BCF Technology, Rochester, MN). Cows without a corpus luteum $> 15 \text{ mm}$ during both examinations were considered anovular. Pregnancy per AI (P/AI) was diagnosed by ultrasonography on d 60 after insemination. Days in milk were recorded for first AI, P/AI at first AI, and P/AI to all AI performed during the first 210 DIM.

Statistical Analyses

Experiment was a randomized, complete block design with a 2×2 factorial arrangement of treatments. Cow was the experimental unit and considered random. Block was considered random. At the time of treatment assignment, prepartum cows were blocked by BCS and mature equivalent 305-d milk yield and, within each block, assigned randomly to 1 of the 4 treatments weekly. Pre- and postpartum data were analyzed separately using the MIXED procedure of SAS version 9.4 (SAS/STAT, SAS Institute Inc., Cary, NC). The REPEATED statement was used for dependent variables measured over time. Model included the fixed effects of energy intake prepartum (EXE vs. MNE), RPC intake (without vs. with), interaction between energy intake prepartum and RPC intake, day or week of measurement, and all 2- and 3-way interactions. Cow was nested within treatment and was the error term for testing the effects of treatment. The covariance structure with the lowest Akaike's information criterion was selected for each variable and spatial power was the covariance structure of choice when intervals between times of sampling were not spaced evenly. When an interaction with time was detected as significant, treatment means at time points of measure were partitioned using the SLICE command of SAS.

For data collected at single time points only, the MIXED procedure of SAS was used without the REPEATED statement. The Kenward-Roger method was used to calculate the approximate denominator degrees of freedom for the F tests in the statistical models. Continuous data were tested for the distribution of

the residuals after fitting the statistical models using Shapiro-Wilk and homogeneity of variance by plotting residuals against predicted values. Non-normally distributed data were subjected to Box-Cox transfor-

mation using the TRANS-REG procedure of SAS to achieve normality before analyses. The least squares means were back-transformed and standard errors of the mean were calculated as outlined by Jørgensen and

Table 3. Reasons for removal of cows from the experiment ($n = 16$) and incidence of diseases in the early postpartum period in multiparous Holstein cows ($n = 93$) fed prepartum diets formulated for caloric intake that greatly exceeded (EXE) or met requirement (MNE) of net energy and supplemented with (+) or without (-) rumen-protected choline (RPC) during the perinatal period

Measure	EXE		MNE		SEM	Contrasts and P -value ¹		
	-RPC	+RPC	-RPC	+RPC		E	RPC	E $\times$ RPC
Cows completing experiment, no.	22	25	25	21				
Cows removed from experiment, ² no.								
Chronic digestive upset	1	—	—	1		—	—	—
Displaced abomasum	1	2	—	2		—	—	—
Hardware disease	1	—	1	—		—	—	—
Spinal cord trauma	—	—	—	1		—	—	—
Toxic mastitis	1	—	—	1		—	—	—
Pericarditis prepartum	1	—	—	—		—	—	—
Failure to use Calan gate ³	1	—	1	1		—	—	—
Incidence, %								
Dystocia ⁴	0	4	0	0	—	—	—	—
Twins	4.5	4.0	24.0	4.8	—	—	—	—
Stillbirths	13.6	8.0	8.0	0.0	—	—	—	—
Fever ⁵	27.3	8.0	24.0	19.1	8.0	0.60	0.37	0.49
Uterine disease ⁶								
RFM	20.7	11.2	26.9	17.3	8.4	0.44	0.24	0.88
Metritis	19.4	17.2	21.4	11.1	8.5	0.73	0.41	0.58
Puerperal metritis	4.0	3.6	15.2	8.5	5.3	0.18	0.67	0.75
Endometritis-21 d	35.2	26.6	30.9	16.7	9.9	0.42	0.22	0.69
Endometritis-40 d	16.3	14.2	10.4	3.8	6.5	0.19	0.40	0.53
PVD	35.4	31.0	31.2	17.2	9.9	0.32	0.32	0.55
Endometritis subclin	4	8	0	0	—	—	—	—
Hyperketonemia ⁷								
≥ 1.20 to 2.89 mM	18.1	34.9	15.0	18.3	8.9	0.31	0.30	0.55
≥ 2.90 mM	4.5	20.0	0.0	0.0	—	—	—	—
Ketosis based on urine ⁸	15.8	18.1	2.8	17.7	7.6	0.20	0.15	0.22
Displaced abomasum	0.0	0.0	4.0	0.0	—	—	—	—
Mastitis								
Incidence ⁹	31.8	28.0	20.0	28.6	9.2	0.64	0.81	0.62
Prevalence ¹⁰	9.1	16.0	8.0	4.7	9.4	0.35	0.95	0.45
First case, ¹¹ DIM	60.1	50.1	50.4	42.0	11.9	0.46	0.45	0.95
Clinical milk fever	0.0	4.0	0.0	0.0	—	—	—	—
Respiratory disease	0.0	8.0	0.0	0.0	—	—	—	—
Digestive upsets	45.0	28.0	36.0	23.8	9.7	0.62	0.38	0.88
Morbidity ¹²	49.9	47.9	40.0	38.2	10.4	0.35	0.85	1.00

¹E = EXE vs. MNE; C = -RPC vs. +RPC; E $\times$ RPC = interaction.

²Failure to recover after prolonged treatment or from surgery.

³American Calan, Northwood, NH.

⁴Assisted calving if cows failed to progress toward delivery after 60 min from last observation.

⁵Rectal temperature $\geq 39.5^\circ\text{C}$ in the first 12 DIM.

⁶RFM (retained fetal membranes): retention >24 h postcalving. Metritis: watery, fetid uterine discharge regardless of fever within 12 DIM. Puerperal metritis: metritis concurrent with fever within 12 DIM. Endometritis-21 and -40: vaginal discharge score of >2 using metricheck tool (Sheldon et al., 2006) at 21 and 40 DIM. Cows having vaginal discharge score >2 were classified as having purulent vaginal discharge (PVD). Endometritis subclin: subclinical endometritis diagnosed as stained cells in uterine flush being $>5\%$ PMNL at 40 ± 1 DIM using the cytobrush technique.

⁷Plasma concentrations of BHB ≥ 1.20 mM in the first 21 DIM were considered evidence of subclinical ketosis; plasma concentrations of BHB ≥ 2.90 mM were considered evidence of clinical ketosis.

⁸Urine having moderate (~ 40 mg/dL) or large ketone concentrations (>80 mg/dL) and treated by veterinarians with oral propylene glycol or intravenous dextrose.

⁹(Number of diagnosed cows/number of cows per treatment) $\times 100\%$.

¹⁰(Number of 5-d antibiotic treatments for mastitis/number of cows per treatment) $\times 100\%$.

¹¹Only cows diagnosed with mastitis were considered.

¹²Incidence of any clinical disease (retained fetal membranes, mastitis, metritis, ketosis, respiratory disease, digestive problems, or hypocalcemia).

Pedersen (1998). Binary responses were analyzed by logistic regression using the GLIMMIX procedure of SAS. The model included the fixed effects of energy intake prepartum (EXE vs. MNE), RPC intake (with vs. without), and the interaction between energy intake prepartum and RPC intake. For repeated measurements within the same experimental unit, such as daily prevalence of SCH, the model also included the fixed effects of time, interaction of treatment and time, and the random effect of cow nested within treatment. Time to event, such as interval to pregnancy by 210 DIM, was analyzed with Cox's proportional hazard regression model using the PHREG procedure of SAS using a model that included the effects of energy intake prepartum (MNE vs. EXE), RPC intake (with vs. without), and the interaction between energy intake prepartum and RPC intake. Statistical significance was considered at $P \leq 0.05$ and tendency was considered at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

At 47 ± 6 d relative to expected calving date, cows assigned to the 4 dietary treatments did not differ in (mean $\pm$ SD) previous lactation number (1.9 ± 0.3), previous 305-d mature-equivalent milk yield ($12,115 \pm 315$ kg), BCS (3.55 ± 0.07), or BW (736 ± 20 kg). Sixteen of the 109 cows enrolled in the experiment were removed for the reasons provided in Table 3. The number of cows removed because of displaced abomasum or digestive problems that could be related to the energy density of the prepartum diets was similar ($n = 4$ and 3) for EXE and MNE treatments, respectively. By chance, 6 cows assigned to the MNE +RPC treatment delivered twins compared with 1 set of twins born to cows assigned to the other 3 treatments. None of the cows having twins experienced dystocia and completed the experiment. Diagnosis was more common for retained fetal membranes (5 of 9), clinical endometritis at 21 DIM (6 of 9), metritis between 4 and 12 DIM (6 of 9), and strong ketosis (3 of 9) compared with cows giving birth to single calves. Nevertheless, the incidence of these health disorders did not differ among treatments and were numerically similar (Table 3). Many cows delivering single calves ($n = 13$) were diagnosed with both metritis within the first 12 DIM and clinical endometritis at 21 DIM; all 22 of these cows were included in the final analyses.

Feeding different prepartum diets had no influence on any measure of morbidity postpartum. Although important, caution should be used when interpreting these health data, as animal numbers were few and thus power was low. Janovick et al. (2011) reported greater incidence of ketosis and displaced abomasum in

cows ($n = 47$) fed at 167 versus 112% of NE_L requirement for the entire dry period. Cows in their experiment fed the high-energy diet prepartum produced 5.6 kg/d more milk but only consumed 0.6 kg/d more DM compared with cows fed to maintenance during wk 2 to 3 of lactation, thus incidence of ketosis would likely increase. Mann et al. (2015) also reported fewer cases of postpartum hyperketonemia ($BHB \geq 1.2$ mM) for prepartum cows fed 112 compared with cows fed 126 and 152% of NE_L requirement.

Interactions between prepartum dietary energy intake and RPC supplementation were detected rarely; thus, our hypothesis was rejected. Therefore, results are presented and discussed under the heading of each main treatment separately.

Prepartum Energy Intake (EXE vs. MNE)

During the far-off period, mean intakes of DM and NE_L were 12.9 versus 10.9 kg/d and 21.0 versus 15.5 Mcal of NE_L /d for the EXE and MNE treatments, respectively. Individual cow DMI could not be analyzed during this period because cows were group-housed according to energy diet.

Parturition occurred earlier than expected on the basis of expected calving date, so only the last 15 d of gestation were used to analyze and report DMI prepartum. Mean DMI (kg/d or % of BW) did not differ because of the energy density of the prepartum diets (Table 4). Likewise, in a review article by Grummer et al. (2004), DMI was similar in cows fed diets averaging 42 and 54% NDF, averaging 1.68 and 1.64% of BW, respectively. Diets in the current experiment had similar NDF content to the values reported by Grummer et al. (2004), 43 and 56% NDF (Table 2), but DMI was less than they reported, averaging 1.52% of BW. This likely occurred because DMI was measured during the last 15 d of gestation and not 21 d as reported by Grummer et al. (2004). Although mean DMI was not affected, caloric intake differed as planned. Inclusion of straw at 37.5% of dietary DM successfully restricted consumption of calories during the dry period compared with cows fed the EXE diet. As a result of greater caloric intake, mean daily EB was greater ($P < 0.01$) by cows fed EXE compared with those fed MNE diets (1.24 vs. 1.00% of daily NE_L requirement; Table 4). During the last 15 d of gestation, intake of NE_L by cows fed EXE decreased from 140 to 102% of NE_L requirement, whereas that of cows fed MNE decreased from 109 to 90% of NE_L requirement (energy by time interaction, $P < 0.01$; Figure 1). On average, cows fed MNE were in negative EB the last 6 d of gestation (means of -0.6 and -1.7 Mcal/d for d 6 to 4 and 3 to 1, respectively). The DMI (kg/d and as % of BW) followed the same response as

Figure 1. Effect of prepartum dietary energy density [maintenance or excess (1.40 or 1.63 Mcal of NE_L/kg of feed DM)] on daily caloric intake expressed as a percentage of NE_L requirement (NRC, 2001). Effects of dietary energy ($P < 0.01$) and dietary energy $\times$ time interaction were detected ($P < 0.01$). Error bars represent SEM.

NE_L intake (energy by time interaction, $P < 0.05$; Table 4). Cows of both treatments consumed DM at 1.3% of BW at calving, which agrees with the review article of Grummer et al. (2004). The rate of decrease in DMI was 0.28 compared with 0.15 kg/d during the last 15 d of gestation for cows fed EXE and MNE, respectively. Likewise, the rate of decrease in DMI in the last days of gestation was greater in multiparous Holstein cows consuming excess calories throughout the dry period (Winkelman et al., 2008; Janovick and Drackley, 2010) compared with cows fed to maintenance. A more rapid decrease in DMI with approaching parturition has been associated with greater hepatic concentration of TAG at 1 DIM (Grummer et al., 2004).

Concentrations of some blood metabolites were influenced by caloric density of prepartum diet. Compared with cows fed MNE, cows fed EXE tended ($P = 0.07$) to have lower mean concentrations of plasma fatty acids (252 vs. 296 μ M) and had greater mean concentrations of plasma glucose (66.3 vs. 63.5 mg/dL) and insulin (0.42 vs. 0.33 ng/mL; Table 4) during the prepartum period. Mean plasma concentrations of BHB prepartum were unaffected by treatments. Janovick et al. (2011) reported the same effects of prepartum feeding of high energy-dense diets on plasma concentrations of these metabolites and insulin when cows were fed excess rather than maintenance amounts of energy for the entire dry period. Mean concentration of plasma urea N prepartum also was lower in cows fed excess calories (7.7 vs. 9.0 mg/dL), which is in agreement with Graugnard et al. (2012). This response pattern of plasma compounds fits with a scenario of differential intake of calories. Ruminal bacteria of cows consuming greater amounts of fermentable OM (i.e., EXE) would produce more propionate, a gluconeogenic precursor for the liver, resulting in greater circulating concentrations

Table 4. Prepartum responses of BW, BCS, DMI, energy balance (EB), and blood metabolites of multiparous Holstein cows fed prepartum diets formulated for caloric intake that greatly exceeded (EXE) or met requirement (MNE) of net energy and supplemented with (+) or without (–) rumen-protected choline (RPC) during the perinatal period

Variable	EXE		MNE		Contrasts and <i>P</i> -values ¹								
	–RPC	+RPC	–RPC	+RPC	SEM	E	RPC	E × RPC	Time (T)	E × T	RPC × T	E × RPC × T	
BW at dry off, kg	727	742	723	752	18.8	0.88	0.24	0.69	—	—	—	—	
BW, kg	727	728	710	734	17.0	0.72	0.43	0.49	<0.01	0.11	0.09	0.45	
BCS at dry off	3.52	3.52	3.54	3.60	0.07	0.28	0.45	0.50	—	—	—	—	
BCS loss ² (kg)	–0.07	0.00	–0.14	–0.10	0.04	0.03	0.11	0.58	—	—	—	—	
DMI, kg/d	10.6	11.5	10.4	10.5	0.40	0.14	0.26	0.28	<0.01	0.03	0.13	0.27	
DMI, % of BW	1.49	1.62	1.51	1.47	0.07	0.33	0.49	0.20	<0.01	<0.01	0.05	0.13	
EB, % of requirement	120	129	100	101	4.80	<0.01	0.27	0.41	<0.01	<0.01	0.17	0.41	
Blood plasma													
Fatty acids, ³ μM	241(269)	263(332)	276(304)	316(367)	24.5	0.07	0.20	0.80	0.75	0.74	0.69	0.26	
BHB, mM	0.27	0.31	0.28	0.27	0.03	0.38	0.39	0.28	0.87	0.66	0.72	0.76	
Glucose, mg/dL	65.7	67.0	64.5	62.5	1.05	0.01	0.74	0.11	0.20	0.02	0.93	0.58	
Insulin, ng/mL	0.41	0.43	0.33	0.33	0.04	0.02	0.79	0.78	0.34	0.19	0.12	0.61	
Urea N, mg/dL	8.1	7.4	9.4	8.7	0.40	<0.01	0.05	0.95	<0.01	0.07	0.06	0.41	

¹E = EXE vs. MNE; RPC = –RPC vs. +RPC; E $\times$ RPC = interaction of energy and RPC diets.

²Loss between dry-off and calving.

³Log-transformed; LSM in parenthesis are nontransformed.

of glucose and insulin. Cows consuming more energy would rely less on body reserves for energy (fewer fatty acids mobilized from adipose tissue). As supporting evidence, *in vitro* lipolytic rates by adipose tissue were lower from overfed versus restricted-fed dairy cows 1 wk before calving (Rukkwamsuk et al., 1998). Reduced concentrations of circulating urea N in plasma could have resulted from more efficient use of dietary N by ruminal microbes given an increased supply of fermentable OM, resulting in less conversion of ammonia to urea by the liver despite greater intake of CP (Table 2).

Cows were dried off at a mean BW of 734 and 737 kg and a mean BCS of 3.52 and 3.57 for EXE and MNE diets, respectively (Table 4). Body weight was not measured just before calving, so changes in BW during the dry period were not calculated. Cows lost approximately 0.08 units of BCS during the 7-wk nonlactating period (time, $P < 0.01$, Table 4), which agrees with the 0.1 to 0.2 BCS units lost during the dry period by cows fed 2 diets of similar energy density as the current experiment (Graugnard et al., 2013). Loss of body condition was slightly less ($P = 0.03$) for cows fed EXE compared with MNE (-0.03 vs. -0.12 BCS; Table 4). Others reported that BCS was unaffected by the amount of calories consumed by pregnant cows for 6 to 7 wk in the dry period (Grum et al., 1996; Winkelman et al., 2008). Drackley et al. (2014) failed to detect differences in BCS or carcass weight between nonlactating, nonpregnant cows consuming 15.3 vs. 25.8 Mcal of NE_L per day for 56 d before slaughter. Nevertheless, amounts of omental, mesenteric, and perirenal adipose tissues, all part of the visceral fat, were greater (57.2 vs. 31.8 kg) in cows consuming the most megacalories, an additional accumulation of 25.4 kg of fat that may negatively influence postpartum performance. It is important to mention that responses in BW and BCS to dietary energy density have been variable. Others reported gains of BW and BCS during the dry period when pregnant cows were consuming diets with increased energy density that result in caloric intakes much above requirements (Boisclair et al., 1986; Janovick and Drackley, 2010). Differences in BW and BCS reported among experiments may have resulted from differing amounts of physical activities by the nonlactating cows. Cows enrolled in the experiments of Boisclair et al. (1986) and Janovick and Drackley (2010) were housed in tiestalls, whereas cows in the current study were kept on outside dirt lots provided with shade structures.

After calving, mean DMI was lower ($P < 0.01$) for cows fed EXE than cows fed MNE diets (22.9 vs. 24.1 kg/d and 3.49 vs. 3.71% of BW; Table 5). Intake of DM as a percent of BW was lower or tended to be lower by cows fed EXE from 2 to 14 wk postpartum

(dietary energy by time interaction, $P < 0.01$; Figure 2A). Previous research failed to detect changes in DMI postpartum by feeding ad libitum amounts of higher versus lower energy-dense diets using high-fiber forages during the entire dry period (Janovick and Drackley, 2010; Mann et al., 2015) or for the last 21 to 24 d of pregnancy (Ji et al., 2012; Zhang et al., 2015). However, Kunz et al. (1985) and Agenäs et al. (2003) reported a slower increase in DMI after calving when prepartum cows consumed calories in excess rather than in maintenance amounts. Greater postpartal DMI in cows fed MNE diets could have resulted from (1) greater distension, and thus a greater volume of the ruminal compartment by the increased prepartum consumption of wheat straw (a slowly degradable, high-NDF feed ingredient) that carried over to the postpartum period, because gut distention is a major controlling factor of intake immediately postpartum (Allen et al., 2009); (2) a less drastic shift in EB between the prepartum and postpartum periods for cows fed MNE prepartum, allowing a more rapid and capable response by homeorhetic mechanisms postpartum; and (3) a lower circulating concentration of insulin pre- and postpartum.

Mean yields of milk, 3.5% FCM, and ECM did not differ between EXE and MNE treatment groups through 15 (Table 5; Figure 3A) and 40 wk of lactation (Table 5 and Figure 4A). Production of 3.5% FCM and ECM were not different during the first 2 wk of lactation, but cows fed MNE appeared to consistently produce more milk from 5 to 15 wk postpartum (energy by time interaction, $P = 0.01$; Figure 3A). Mean conversion of DMI to yields of ECM and 3.5% FCM ranged from 1.84 to 1.97 kg/kg across diets (Table 5). Efficiencies of conversion of DMI to ECM and FCM were greater during the first 4 wk postpartum by cows fed EXE (energy by time interaction, $P < 0.01$; Figure 5A), likely due to lower DMI and use of adipose and muscle to support milk production. Regardless of whether prepartum intake of calories during the entire dry period was reduced through feed restriction (Kunz et al., 1985; Boisclair et al., 1986; Rukkwamsuk et al., 1998; Winkelman et al., 2008) or by ad libitum feeding of high-NDF diets (Graugnard et al., 2013; Mann et al., 2015), production of milk was not different compared with cows grossly overfed calories. Mean values for milk fat, true protein, SCC, and lactose (kg/d or %) were not affected by energy density of the prepartum diet (Table 5).

Mean EB after calving was more ($P = 0.03$) negative for cows fed EXE (-1.6 vs. -0.1 Mcal/d; Table 5). The difference in EB between the 2 dietary energy treatment groups occurred primarily during the first 5 wk postpartum, with a tendency for a more negative EB detected at 10 and 12 wk postpartum for cows fed EXE compared with those fed MNE (energy by week

Table 5. Postpartum performance of multiparous cows fed prepartum diets formulated for caloric intake that greatly exceeded (EXE) or met requirement (MNE) of net energy and supplemented with (+) or without (–) rumen-protected choline (RPC) during the perinatal period

Variable	EXE		MNE		Contrasts and <i>P</i> -value ¹								
	–RPC	+RPC	–RPC	+RPC	SEM	E	RPC	E × RPC	Time (T)	E × T	RPC × T	E × RPC × T	
DMI, kg/d	22.6	23.2	23.8	24.3	0.40	<0.01	0.14	0.90	<0.01	0.14	0.42	1.00	
DMI, % of BW	3.45	3.53	3.74	3.68	0.07	<0.01	0.91	0.35	<0.01	<0.01	0.50	0.73	
Milk, kg/d for 15 wk	39.9	43.5	42.8	43.5	1.20	0.25	0.09	0.23	<0.01	0.15	0.80	0.23	
Milk, kg/d for 40 wk	33.8	37.5	36.1	36.6	1.20	0.53	0.09	0.18	<0.01	0.59	0.74	0.33	
Milk fat, kg/d	1.54	1.64	1.57	1.64	0.05	0.75	0.09	0.76	<0.01	0.06	0.07	0.17	
Milk protein, kg/d	1.17	1.25	1.25	1.29	0.03	0.09	0.07	0.56	<0.01	0.51	0.73	0.19	
ECM, kg/d	41.1	44.1	43.0	44.3	1.20	0.38	0.08	0.49	<0.01	0.01	0.21	0.78	
3.5% FCM, kg/d	42.2	45.4	43.9	45.4	1.30	0.51	0.07	0.52	<0.01	0.01	0.18	0.58	
Efficiency													
ECM/DMI	1.85	1.96	1.83	1.85	0.04	0.14	0.11	0.28	<0.01	<0.01	<0.01	<0.01	
FCM/DMI	1.91	2.03	1.89	1.92	0.05	0.18	0.13	0.36	<0.01	<0.01	0.08	<0.01	
Milk composition, %													
Fat	3.95	3.80	3.73	3.83	0.07	0.18	0.70	0.09	<0.01	0.01	0.20	0.21	
True protein	2.99	2.91	2.95	3.00	0.03	0.46	0.64	0.06	<0.01	0.60	0.99	<0.01	
SCC, ×1,000 cells/mL ²	58.1	65.2	61.8	61.0	16.1	0.99	0.85	0.81	<0.01	0.61	0.76	0.58	
Lactose	4.82	4.81	4.80	4.80	0.03	0.55	0.81	0.99	<0.01	0.02	0.70	0.72	
Energy balance, Mcal/d	–1.1	–2.1	0.0	–0.3	0.70	0.03	0.34	0.58	<0.01	0.07	0.08	0.19	
BW, kg	660	660	643	668	11.9	0.68	0.29	0.29	<0.01	0.05	0.44	0.72	
BW loss, kg	38.9	50.9	36.6	41.1	5.50	0.25	0.12	0.48	—	—	—	—	
BCS	3.30	3.35	3.19	3.29	0.05	0.07	0.10	0.56	<0.01	0.69	0.67	0.26	
BCS loss	0.19	0.19	0.29	0.21	0.05	0.15	0.30	0.37	—	—	—	—	

¹E = EXE vs. MNE; RPC = –RPC vs. +RPC; E × RPC = interaction of energy and RPC diets.

²SCC data were log-transformed.

interaction, $P = 0.07$; Figure 6A). Both treatments reached positive EB by 8 wk postpartum. In agreement, mean negative EB over the first 41 DIM postpartum was greater for cows fed an excess rather than a maintenance amount of calories prepartum (Graugnard et al., 2013). In addition, multiparous cows overconsuming calories prepartum took longer to reach positive EB after calving compared with those fed to maintenance in their prepartum period [due to a slower increase in DMI after calving (Agenäs et al., 2003) or due to a tendency for greater yield of 3.5% FCM (Janovick and Drackley, 2010)].

Mean BCS tended ($P = 0.07$) to be slightly greater for EXE than MNE (3.32 vs. 3.24; Table 5); however, prepartum dietary energy did not affect loss of BW from calving to EB nadir at 4 wk postpartum (Figure 7A). Nevertheless, cows fed MNE steadily gained 31 kg between 5 and 15 wk postpartum, whereas cows fed EXE only gained 8 kg (energy by time interaction, P

Figure 2. (A) Effect of prepartum dietary energy density [maintenance or excess (1.40 or 1.63 Mcal of NE_L /kg of feed DM)] on postpartum DMI. Effects of dietary energy ($P < 0.01$) and dietary energy $\times$ time interaction ($P < 0.01$) were detected. (B) Effect of periparturient supplementation with or without ruminally protected choline (+RPC or -RPC, respectively) on postpartum DMI. Effects of RPC ($P = 0.91$) or RPC $\times$ time interaction ($P = 0.50$) were not detected. At each week of lactation, differences between dietary treatments were found: * $P \leq 0.05$, † $0.05 > P \leq 0.10$. Error bars represent SEM.

Figure 3. (A) Effect of prepartum dietary energy density [maintenance or excess (1.40 or 1.63 Mcal of NE_L /kg of feed DM)] on production of ECM for first 15 wk postpartum. Effects of dietary energy ($P = 0.38$) and dietary energy $\times$ time interaction were detected ($P < 0.01$). (B) Effect of periparturient supplementation with or without ruminally protected choline (+RPC or -RPC, respectively) on production of ECM for first 15 wk postpartum. Effect of RPC was detected ($P = 0.08$), whereas effect of RPC $\times$ time interaction was not detected ($P = 0.21$). At each week of lactation, differences between dietary treatments were found: * $P \leq 0.05$. Error bars represent SEM.

= 0.05; Figure 7A). The increased BW gain after 5 wk postpartum was caused primarily by increasingly greater consumption of calories beyond that needed for maintenance and milk production during a time when yield of ECM was decreasing in cows fed MNE compared with those fed EXE. Although the negative effect of overconsumption of calories prepartum on postpartum BW change has not been reported consistently, it has occurred when cows were overfed calories for the last 3 wk of pregnancy (Zhang et al., 2015) or for most of the dry period (Kunz et al., 1985; Rukkwamsuk et al., 1998; Agenäs et al., 2003).

As stated previously, cows fed EXE were in more negative EB during the first 5 wk postpartum, which resulted in marked differences in concentrations of metabolites in plasma. Feeding EXE tended ($P = 0.10$) to increase the mean concentration of plasma fatty acids (502 vs. 453 μM) postpartum (Table 6). Mean concentrations of fatty acids were 742 and 697 μM on the day

of calving for cows fed EXE and MNE, respectively, but quickly decreased to around $550 \mu\text{M}$ by 7 DIM in both treatments. Increased plasma concentrations of fatty acids postpartum have resulted from dramatically overfeeding calories prepartum during most of the dry period (Kunz et al., 1985; Rukkwamsuk et al., 1998; Holtenius et al., 2003; Janovick et al., 2011; Graugnard et al., 2013; Mann et al., 2015) or during the last 3 wk of gestation (Ji et al., 2012; Zhang et al., 2015). Mean concentration of plasma BHB was greater across weeks (7.4 vs. 5.5 mM) for cows fed EXE compared with those fed MNE, but only differences at wk 1 and 3 postpartum were identified as significant (energy by time interaction, $P = 0.01$; Table 6; Figure 8A). Mean concentrations of BHB peaked at 7 DIM at 1.0 mM for cows fed EXE (Figure 8A), but few cows were above the 1.2 mM value proposed by McArt et al. (2012) as

evidence of subclinical ketosis. Janovick et al. (2011), Ji et al. (2012), and Mann et al. (2015) reported this same positive relationship of prepartum caloric intake and postpartum circulating BHB response. Plasma concentrations of fatty acids and BHB typically increase as cows experience a greater negative EB (as did the cows fed EXE) due to the mobilization of fat from adipose and the production of ketones by the liver via incomplete oxidation of fatty acids. Circulating concentrations of glucose were unaffected (64.2 vs. 63.7 mg/dL), although those of insulin tended ($P = 0.10$) to be increased postpartum (0.17 vs. 0.13 ng/mL) in cows fed EXE compared with those fed MNE (Table 6). Likewise, greater insulin concentrations were reported for postpartal cows if greatly overfed calories prepartum (Kunz et al., 1985; Grum et al., 1996; Holcomb et al., 2001; Graugnard et al., 2013; Zhang et al.,

Figure 4. (A) Effect of prepartum dietary energy density [maintenance or excess (1.40 or 1.63 Mcal of NE_L /kg of feed DM)] on milk production for first 40 wk postpartum. Effects of dietary energy ($P = 0.53$) or dietary energy $\times$ time interaction ($P = 0.59$) were not detected. (B) Effect of periparturient supplementation with or without ruminally protected choline (+RPC or -RPC, respectively) on milk production for first 40 wk postpartum. Effect of RPC was detected ($P = 0.09$) whereas effect of RPC $\times$ time interaction was not detected ($P = 0.74$). Error bars represent SEM.

2015). Offering diets that excessively exceeded rather than slightly exceeded energy requirements prepartum stimulated insulin release starting in the prepartum period and carried over to the postpartum period in the current study. The elevated insulin concentrations of postpartal cows fed EXE was surprising, because cows fed EXE were in a greater negative EB than cows fed MNE for the first 5 wk postpartum. This increased insulin could mediate an increase in circulating concentrations of leptin from adipose tissue (Asakuma et al., 2003), a hormone known to depress feed intake via the hypothalamus (Houseknecht et al., 1998). In addition, prepartum concentrations of plasma leptin tended to be greater in cows fed excess rather than restricted amounts of calories prepartum (Holtenius et al., 2003). Thus, postpartum DMI may have been lower in cows fed EXE prepartum because of the greater presence of

leptin starting prepartum and the stimulating influence of insulin on leptin release from adipose postpartum.

Dietary energy density prepartum did not affect concentrations of hepatic TAG at 2 wk prepartum, which averaged 2.3 and 2.7% of liver DM in cows fed EXE or MNE, respectively (Figure 9A). However, mean concentration of TAG from 3 weekly biopsies starting at 7 DIM was greater in liver of cows fed EXE compared with those fed MNE (10.6 vs. 8.5% $P = 0.04$; 7.3 vs. 5.2 $\mu\text{g}/\mu\text{g}$ of DNA, $P < 0.01$; Table 6), with the values at 7 (11.1 vs. 8.7% of DM) and 21 DIM (10.1 vs. 7.6% of DM) detected as different (energy by time interaction, $P = 0.10$; Figure 9A), matching the effect of prepartum energy intake on concentration of plasma BHB. In their review article, Bobe et al. (2004) reported that plasma concentrations of BHB and fatty acids were positively and strongly associated with fatty liver, metabolites that are elevated as cows experience greater negative EB. Increases in plasma BHB, fatty acids, and nega-

Figure 5. (A) Effect of prepartum dietary energy density [maintenance or excess (1.40 or 1.63 Mcal of NE_L/kg of feed DM)] on efficiency of converting feed DM into ECM. Effect of dietary energy ($P = 0.14$) was not detected but effect of dietary energy $\times$ time interaction was detected ($P < 0.01$). (B) Effect of periparturient supplementation with or without ruminally protected choline (+RPC or -RPC, respectively) on efficiency of converting feed DM into ECM. Effect of RPC was not detected ($P = 0.11$), but effect of RPC $\times$ time interaction was detected ($P < 0.01$). At each week of lactation, differences between dietary treatments were found: * $P \leq 0.05$, † $0.05 > P \leq 0.10$. Error bars represent SEM.

Figure 6. (A) Effect of prepartum dietary energy density [maintenance or excess (1.40 or 1.63 Mcal of NE_L/kg of feed DM)] on postpartum energy balance. Effects of dietary energy ($P = 0.03$) and dietary energy $\times$ time interaction were detected ($P = 0.07$). (B) Effect of periparturient supplementation with or without ruminally protected choline (+RPC or -RPC, respectively) on postpartum energy balance. Effect of RPC was not detected ($P = 0.34$), but effect of RPC $\times$ time interaction was detected ($P = 0.08$). At each week of lactation, differences between dietary treatments were found: * $P \leq 0.05$, † $0.05 > P \leq 0.10$. Error bars represent SEM.

Figure 7. (A) Effect of prepartum dietary energy density [maintenance or excess (1.40 or 1.63 Mcal of NE_L/kg of feed DM)] on postpartum BW. Effect of dietary energy was not detected ($P = 0.68$) but effect of dietary energy $\times$ time interaction was detected ($P = 0.05$). (B) Effect of periparturient supplementation with or without ruminally protected choline (+RPC or -RPC, respectively) on postpartum BW. Effects of RPC ($P = 0.29$) and RPC $\times$ time interaction ($P = 0.44$) were not detected. At each week of lactation, differences between dietary treatments were found: $\dagger 0.05 > P \leq 0.10$. Error bars represent SEM.

tive EB are in agreement with the greater hepatic TAG detected for cows fed EXE prepartum. Incidence of hepatic lipidosis based on TAG $>5\%$ of wet tissue (Gaal et al., 1983) tended to be greater ($P = 0.07$) in cows overfed energy prepartum (22.6 vs. 8.1% for EXE and MNE, respectively). Excessive feeding of calories for most of the dry period resulted in greater concentrations of hepatic TAG at 1, 14, and 21 DIM (Janovick et al., 2011). Andersen et al. (2002) reported that insulin suppressed β -oxidation of long-chain fatty acids by the liver of lactating dairy cows. A similar action by elevated plasma insulin in the current experiment, along with the elevated plasma concentration of fatty acids in cows fed EXE, may have led to increased hepatic esterification of fatty acids and would help explain the increased concentrations of hepatic TAG. Activity of carnitine palmitoyltransferase was reduced at 1 DIM in hepatic mitochondria of multiparous cows fed excess compared with those fed moderate amounts of calories

in the prepartum period (Douglas et al., 2006), which supports a reduced β -oxidation by hepatic tissue from overfed prepartum cows.

Mean concentration of tCa (8.5 vs. 8.7 mg/100 mL) and prevalence of SCH (19.3 vs. 16.7%) did not differ between EXE and MNE treatments, respectively (Table 6). Based upon ultrasonography, energy intake prepartum did not influence the proportion of cows cycling by 40 ± 3 DIM (78.7 vs. 82.5%) for EXE and MNE treatments, respectively. Neither average days to pregnancy (138 vs. 134 DIM) nor pregnancy at first AI (32 vs. 32% for MNE vs. EXE groups, respectively) were affected by prepartum energy intake.

Yield of colostrum (8.9 vs. 9.2 kg) and concentration of IgG (76.1 vs. 78.9 g/L) were not affected by amount of energy consumed prepartum (EXE and MNE, respectively; Table 7). Recently, Mann et al. (2016) reported greater concentration of IgG in colostrum from multiparous cows fed at 112 versus 157% of NE_L requirement during the prepartum period.

To examine the influence of in utero exposure to maternal intake of 2 amounts of energy, the growth of 35 heifers (18 from EXE dams and 17 from MNE dams) were followed for 1 yr. All heifers were managed the same with the exception that the source of colostrum fed was not controlled except that it exceeded 22% Brix. Body weights at birth, weaning, and at 50 wk of age were not different due to dietary treatment, such that all measures of ADG were unaffected (Table 7).

Choline Supplementation During the Periparturient Period

The RPC was supplemented for 17 ± 4.6 d prepartum through 21 DIM for a total period of approximately 38 d. All cows received RPC for a minimum of 7 d prepartum, with 42 cows (91.3%) supplemented for a minimum of 11 d prepartum.

Incidence of the individual diseases and disorders listed in Table 3, as well as morbidity as a whole, were not affected by feeding RPC in our study of 93 cows. In a field experiment involving 357 primiparous and multiparous cows, incidence of clinical ketosis, in particular, and general morbidity (retained fetal membranes, metritis, clinical ketosis, displaced abomasum, and mastitis) were reduced (57.0 vs. 38.4%) by feeding RPC from -25 to 80 DIM (Lima et al., 2012). In their second field experiment of 573 primiparous cows, feeding RPC only during the last 22 d of pregnancy resulted in reduced incidence of retained fetal membranes (6.7 vs. 2.1%) and mastitis cases per cow (0.09 vs. 0.04), but general morbidity tended to increase (33.7 vs. 41.2%; Lima et al., 2012). Supplemental choline may need to be fed in the prepartum and postpartum periods when

Table 6. Postpartum responses of blood plasma metabolites and liver triacylglycerol (TAG) of multiparous Holstein cows fed prepartum diets formulated for caloric intake that greatly exceeded (EXE) or met requirement (MNE) of net energy and supplemented with (+) or without (–) rumen-protected choline (RPC) during the perinatal period

Variable	EXE			MNE			Contrasts and <i>P</i> -value ¹						
	–RPC	+RPC		–RPC	+RPC	SEM	E	RPC	E × RPC	Time (T)	E × T	RPC × T	E × RPC × T
Fatty acids, μM	471	533		446	461	32	0.10	0.19	0.42	<0.01	0.83	0.86	0.14
BHB, mM	0.49 (0.61)	0.55 (0.81)		0.42 (0.50)	0.43 (0.55)	0.03	<0.01	0.36	0.43	<0.01	0.01	0.17	0.67
Glucose, mg/dL	64.7	63.8		64.4	63.0	1.1	0.55	0.23	0.81	<0.01	0.32	0.75	0.04
Insulin, ng/mL	0.17	0.17		0.14	0.12	0.02	0.10	0.57	0.77	0.09	0.72	0.95	0.32
Urea N, mg/dL	9.8	9.4		10.4	9.9	0.4	0.14	0.30	0.84	0.02	0.88	0.56	0.60
Liver ³													
TAG, % of DM	10.2	11.1		8.0	9.0	1.0	0.04	0.36	0.99	<0.01	0.10	0.29	0.12
TAG, $\mu g/\mu g$ of DNA	6.8	7.8		4.8	5.6	0.8	0.03	0.43	0.83	<0.01	0.68	0.47	0.13
Hepatic lipidosis, %	22.1	23.1		7.5	8.8	7.5	0.07	0.86	0.93	—	—	—	—
Total Ca, mg/dL	8.4	8.6		8.5	8.9	0.13	0.11	0.02	0.43	<0.01	0.39	0.27	0.54
Incidence of SCH, %	77.3	72.0		80.0	47.6	9.2	0.52	0.31	0.42	—	—	—	—
Prevalence of SCH, %	24.2	14.5		26.9	6.5	6.0	0.35	<0.01	0.20	<0.01	0.38	0.05	0.81

¹E = EXE vs. MNE; RPC = –RPC vs. +RPC; E × RPC = interaction of energy and RPC diets.²Log-transformed; LSM in parenthesis are nontransformed.³Biopsies at 7, 14, and 21 DIM.⁴>5% TAG wet basis (Gaal et al., 1983) at 7, 14, or 21 DIM.⁵Subclinical hypocalcemia defined as Ca <8.0 mg/dL of plasma (Reinhardt et al., 2011) at 0, 1, 3, or 7 DIM. Cow was diagnosed as positive or negative for SCH once during the first 7 DIM.⁶Subclinical hypocalcemia defined as Ca <8.0 mg/dL of plasma (Reinhardt et al., 2011) at 0, 1, 3, or 7 DIM. Cow was diagnosed as positive or negative for SCH more than once during the first 7 DIM.

Figure 8. (A) Effect of prepartum dietary energy density [maintenance or excess (1.40 or 1.63 Mcal of NE_L/kg of feed DM)] on postpartum concentrations of BHB. Effects of dietary energy ($P < 0.01$) and dietary energy $\times$ time interaction were detected ($P = 0.01$). (B) Effect of periparturient supplementation with or without ruminally protected choline (+RPC or -RPC, respectively) on postpartum concentrations of BHB. Effects of RPC ($P = 0.36$) and RPC $\times$ time interaction ($P = 0.17$) were not detected. At each week of lactation, differences between dietary treatments were found: $0.05 > P \leq 0.10$. Error bars represent SEM.

Figure 9. (A) Effect of prepartum dietary energy density [maintenance or excess (1.40 or 1.63 Mcal of NE_L/kg of feed DM)] on concentration of liver triacylglycerol (TAG). Effects of dietary energy ($P = 0.04$) and dietary energy $\times$ time interaction were detected ($P = 0.10$). (B) Effect of periparturient supplementation with or without ruminally protected choline (+RPC or -RPC, respectively) on concentration of liver TAG. Effects of RPC ($P = 0.36$) and RPC $\times$ time interaction ($P = 0.39$) were not detected. At each day relative to parturition, differences between dietary treatments were found: $*P \leq 0.05$. Error bars represent SEM.

Table 7. Yield and quality of colostrum and growth of heifers born to multiparous Holstein cows fed prepartum diets formulated for caloric intake that greatly exceeded (EXE) or met requirement (MNE) of net energy and supplemented with (+) or without (-) rumen-protected choline (RPC) during the perinatal period

Variable	EXE		MNE		SEM	Contrasts and P -value ¹		
	-RPC	+RPC	-RPC	+RPC		Energy	RPC	E $\times$ RPC
Colostrum, kg	8.6	9.3	8.8	9.6	1.4	0.84	0.61	0.97
IgG, g/L	68.2	83.9	68.1	89.8	8.4	0.73	0.03	0.73
IgG, g/cow	503	695	477	709	99	0.95	0.04	0.84
Number of heifers for ADG	9	9	8	9				
BW, kg								
Calving	40.8	37.4	40.1	39.3	1.2	0.63	0.07	0.25
Weaning ²	78.4	73.8	75.0	81.0	2.6	0.47	0.80	0.05
50 wk of age ³	325	332	320	339	6.4	0.91	0.05	0.37
ADG, kg/d								
From calving to weaning	0.63	0.60	0.59	0.65	0.04	0.93	0.67	0.27
From weaning to 50 wk	0.85	0.89	0.85	0.89	0.02	0.88	0.05	0.87
From calving to 50 wk	0.81	0.84	0.80	0.85	0.02	0.94	0.06	0.61

¹E = EXE vs. MNE; RPC = -RPC vs. +RPC; E $\times$ RPC = interaction of energy and RPC diets.

²61.1 $\pm$ 3.3 d of age.

³350.0 $\pm$ 2.2 d of age.

EB is negative and the immune system is under the greatest stress to maximize potential health benefits.

Mean BW was 725 and 747 kg and mean BCS was 3.53 and 3.58 when dried off and assigned to the -RPC and +RPC treatments, respectively, and did not differ (Table 4). Mean DMI during the last 15 d of gestation did not differ (10.5 vs. 11.0 kg/d and 1.50 vs. 1.54% of BW for the -RPC and +RPC groups, respectively; Table 4), nor did mean EB. Plasma concentrations of fatty acids, BHB, glucose, and insulin were unaffected by RPC supplementation during the last 3 wk of gestation (Table 4).

The mean DMI during the postpartum period (15 wk) averaged 23.2 kg/d for -RPC and 23.8 kg/d for +RPC groups and did not differ (Table 5; Figure 2A). Mean yields of milk (43.5 vs. 41.3 kg/d), ECM (44.2 vs. 42.0 kg/d), and 3.5% FCM (45.4 vs. 43.1 kg/d) tended to be greater ($P < 0.10$) for the +RPC compared with the -RPC group (Table 5; Figure 3B). When milk yield was analyzed up to 40 wk, again the +RPC group tended to produce more milk (36.9 vs. 34.8 kg/d, $P = 0.08$; Figure 4B), demonstrating a carry-over effect after the supplementation ended at 21 DIM due to greater peak yield of milk. Choline supplementation also tended to increase yields of milk fat ($P = 0.09$) and protein ($P = 0.07$) due to increased milk yield. Mean concentration of fat and protein tended to decrease in the milk of cows fed EXE, but increased in the milk of cows fed MNE due to RPC supplementation ($P < 0.10$, Table 5). Cows categorized as the bottom 50% in milk yield in the current experiment had a 1.1 kg/d greater milk response to RPC than the top 50%. Because RPC was fed at a constant amount of 60 g/d, the ratio of RPC intake per unit of milk production was greater for lower producers compared with that of the higher-producing cows. Hence, the amount of RPC fed in the current experiment may not have been sufficient to capture all of the benefit of RPC for higher-producing cows. If only cows with BCS of ≤ 3.50 at calving were considered ($n = 76$), milk response to RPC supplementation was 2.7 kg/d ($P = 0.09$), indicating that moderately conditioned cows responded well to RPC.

Others have reported that yield of milk or FCM increased or tended to increase when RPC was fed with methionine-adequate diets (Piepenbrink and Overton, 2003; Lima et al., 2012; Sun et al., 2016), although these groups fed RPC starting prepartum through 60 to 80 d postpartum; only Sun et al. (2016) fed RPC for 21 d postpartum, as was done in the current experiment. In addition, others fed diets considered inadequate in methionine and still reported greater milk yield due to supplementation with RPC (Pinotti et al., 2003; Zahra et al., 2006; Amrutkar et al., 2015). Some authors reported no change in milk yield with RPC supplementa-

tion (Zom et al., 2011; Zhou et al., 2016); the milk yield response detected in the current trial (2.2 kg/d) is comparable to the average of all those trials.

Although mean efficiency of production of ECM and FCM was unaffected ($P = 0.11$ to 0.13 , Table 5) by RPC, efficiency of ECM production was greater at wk 1, 2, 3, and 6 when RPC was fed (RPC by time interaction, $P < 0.01$; Table 5; Figure 5B). Likewise, mean EB did not differ due to RPC supplementation but EB was more negative at wk 2 (-8.9 vs. -6.6 Mcal/d) and 3 (-11.4 vs. -8.7 Mcal/d) and tended to be more negative at wk 6 postpartum for cows fed RPC (RPC by time interaction, $P = 0.08$; Figure 6B). Both groups reached positive EB by wk 8 postpartum. The mean BW and BCS and the pattern of loss and gain of BW and body condition (Figure 7B) were not different between the 2 treatment groups. Therefore, the improved efficiency of milk production may have been due partially to improved utilization of feed nutrients. Total-tract digestibility of OM (including ether extract) was increased for lactating cows fed 15 or 30 g/d of RPC compared with those not fed RPC chloride (Mohsen et al., 2011). Lactating rats fed diets deficient in choline had shorter villi in the jejunum and a reduced capacity to absorb fat compared with rats supplemented with choline or PC, possibly due to choline's role in chylomicron assembly (da Silva et al., 2015).

Despite the fact that cows fed RPC were in a more negative energy state during wk 2 and 3 postpartum (Figure 6B), supplementation of RPC had no effect on the mean concentrations of plasma fatty acids, BHB, glucose, insulin, and BUN (Table 6; Figure 8B). Literature reporting the effect of supplementing RPC on the concentration of these metabolites is equivocal. Postpartum plasma concentrations of glucose, fatty acids, and BHB did not differ between control and RPC-supplemented lactating cows (Janovick Guretzky et al., 2006; Zom et al., 2011), although others have reported decreased plasma concentrations of BHB (Elek et al., 2013; Sun et al., 2016) and fatty acids (Sun et al., 2016) but greater plasma concentrations of glucose (Sun et al., 2016) when RPC was supplemented.

Prepartum hepatic TAG values were 2.3 and 2.7% of DM and 1.50 and 1.58 $\mu\text{g}/\mu\text{g}$ of DNA for cows not fed or fed RPC, respectively (Figure 9B). Just as RPC intake did not affect postpartum circulating concentrations of fatty acids and BHB, RPC did not influence mean concentrations of hepatic TAG postpartum (9.1 vs. 10.0% of DM and 5.8 vs. 6.7 $\mu\text{g}/\mu\text{g}$ of DNA; Table 6). The pattern of changing hepatic TAG over the first 3 wk postpartum was not affected by RPC (Figure 9B). Likewise, prevalence of lipidosis was not different between -RPC and +RPC groups (13.2 vs 14.5%, respectively). Lack of lipidosis in cows fed RPC

was somewhat surprising because of the cow's greater negative EB during the first 2 to 3 wk postpartum. The greater extent of negative EB during wk 2 to 3 postpartum of cows fed RPC, extending down to -11.5 Mcal/d, would suggest a likely increase in hepatic fat in the current experiment. It is possible that feeding RPC may have prevented greater accumulation of fat by the liver of cows in greater negative EB. When cows were split into 2 groups of most negative EB ($n = 46$) and least negative EB ($n = 47$) averaged across wk 2 and 3, RPC supplementation did not affect liver TAG concentration averaged across wk 2 and 3 in either group. Piepenbrink and Overton (2003) and Zahra et al. (2006) reported no effect of RPC on hepatic TAG, although the former reported increased concentrations of glycogen with increasing consumption of RPC. However, esterified products tended to decrease when liver slices from cows fed increasing amounts of RPC were incubated in vitro with carbon 14-labeled palmitic acid (Piepenbrink and Overton, 2003), indicating increasing production of lipoproteins such as very low density lipoproteins, which is necessary to export TAG from the liver. Alternatively, several studies have reported a reduction of TAG in liver of early-lactation multiparous cows fed RPC (Santos and Lima, 2009; Zom et al., 2011; Elek et al., 2013).

Our hypothesis was that supplemental RPC would have a more positive effect on cows prone to develop hepatic lipidosis; that is, cows fed the EXE diet prepartum. Indeed, cows fed EXE had greater hepatic TAG compared with those fed MNE (Table 6), but RPC supplementation did not preferentially reduce hepatic TAG in those cows fed EXE. A lack of consistency by supplemental RPC to reduce hepatic TAG across experiments may result from (1) differences in choline status of cows across studies, (2) insufficiency of a 1.5-g sample of liver tissue collected via biopsy to consistently represent a 7 to 9 kg liver, and (3) a lack of sufficient hepatic lipidosis in control cows to allow a benefit from supplemental RPC. The mean concentration of hepatic TAG of control cows in the current study was 9.1% (DM basis; Table 6) averaged across the first 21 DIM. This concentration converts to 2.3% (wet basis), assuming that liver is 25% DM. The hepatic concentration of TAG in control cows was 7.5 and 10% (wet tissue basis) in 2 experiments (Zom et al., 2011; Elek et al., 2013) that reported a reduction in hepatic TAG due to RPC supplementation, whereas concentrations were approximately 16 and 11.5% (wet basis, averaged between 1 and 21 DIM) in control cows that did not respond to supplemental RPC (Piepenbrink and Overton, 2003; Zahra et al., 2006). In these 4 studies, TAG concentration of control cows were relatively high (Bobe et al., 2004) and similar in value, and therefore

would not account for lack of RPC effect. Control cows in the current study may not have accumulated enough TAG to allow RPC to provide a detectable benefit.

Consumption of RPC tended ($P = 0.09$) to increase milk by 2.2 kg/d per cow despite no apparent improvement in liver function. This improvement may have been through a combination of improved DMI and energy mobilization. Cows fed RPC had numerically greater intake of feed DM (0.6 kg/d), greater loss of BW (46.0 vs. 37.8 kg), and a greater oxidation of fatty acids for energy in early lactation, thus sparing some glucose for increased synthesis of lactose for milk production. An increased efficiency or extent of fatty acid oxidation may result from choline's documented effects upon carnitine. Carnitine is required to transport long-chain fatty acids into the mitochondria for β -oxidation of fatty acids. Choline is a methyl donor for the hepatic synthesis of carnitine (Bremer, 1983). Second, expression of mRNA of 2 key proteins (fatty acid transporter 5 and organic cation-carnitine transporter SLC22A5) for transporting carnitine into hepatocytes were increased in cows supplemented with RPC (Goselink et al., 2013). Increased dietary intake of carnitine by periparturient Holstein cows reduced concentration of TAG in liver by $>50\%$ and increased in vitro production of glucose from alanine by liver tissue collected via biopsy (Carlson et al., 2007). Circulating concentrations of glucose have been increased in dairy cows fed RPC (Baldi and Pinotti, 2006; Soltan et al., 2012; Sun et al., 2016), but not in others (Zahra et al., 2006; Zom et al., 2011; Elek et al., 2013). If choline is increasing the synthesis of carnitine, which may allow more fatty acids to undergo oxidation, the plasma concentration of ketones may be reduced if their oxidation is completed. Only Elek et al. (2013) and Sun et al. (2016) reported lower concentration of plasma BHB in fresh cows fed RPC, whereas others reported no effect of RPC supplementation on circulating BHB (Piepenbrink and Overton, 2003; Zahra et al., 2006; Zom et al., 2011).

Cows supplemented with RPC had greater mean concentration of tCa in plasma in the first 7 DIM compared with cows that did not received RPC (8.7 vs. 8.4 mg/dL, respectively, $P = 0.02$; Table 6; Figure 10). Across treatments, 70% of all cows had tCa in plasma below 8 mg/dL during at least one time point in the first 7 DIM, but RPC did not influence the incidence rate of SCH. This incidence rate is greater than that of 48% reported by Reinhardt et al. (2011), but cows in their study were sampled only 1 time within the first 48 h postcalving. Prevalence of SCH, defined as tCa <8.0 mg/dL in the first 7 DIM, was reduced by 15 percentage points during the first week postpartum (25.5 vs. 10.5% for $-$ RPC vs. $+$ RPC, respectively; $P < 0.01$; Table 6), when cows were supplemented with RPC. The treat-

ment difference in plasma tCa was significant only on d 0 and 3 postpartum (Figure 10), indicating that more cows had returned to Ca homeostasis in blood by 3 DIM if fed RPC. Supplemental choline may have improved Ca status through (1) improved activity of vitamin D (fat-soluble vitamin); (2) improved absorption of Ca or vitamin D if synthesis of intestinal chylomicrons was increased with additional PC; (3) increased mobilization of Ca from bone; or (4) increased retention of Ca by the kidney. For example, activity of CaATPase, which is involved in vitamin D-dependent active transport of Ca, was reduced in absorptive cells of small intestine of rats fed a choline-deficient diet (Takahashi et al., 1982). The effects of RPC on Ca status are quite profound, and further research on potential effects of choline on Ca homeostasis may prove fruitful.

Supplementation with RPC did not influence the proportion of cows cycling by 40 ± 3 DIM (80.5 vs. 80.9% for -RPC and +RPC treatments, respectively). Feeding RPC tended to increase the proportion of cows pregnant at first AI ($P = 0.09$; 41.3 vs. 23.6%) but not at 40 wk postpartum (69.8 vs. 62.5%). The hazard of pregnancy for +RPC and -RPC cows was 1.29 (95% CI = 0.44 to 1.35). The number of days open were 143 versus 123 d for -RPC and +RPC groups, respectively. Cox's proportional analysis of average days to pregnancy from the end of the voluntary waiting period until 210 DIM indicated that treatments did not affect ($P = 0.14$) the rate at which cows became pregnant (Figure 11). Others have reported reproductive benefits from supplementing with RPC, such as improved pregnancy rate (29.0 vs. 58.1%; $n = 62$; Scheer Oelrichs, 2003), 33 fewer days open ($n = 27$; Amrutkar et al., 2015), and a greater proportion of multiparous but not primiparous cows cycling by 61 DIM ($n = 333$; Lima et al., 2012); pregnancy was numerically but not statistically greater in these latter 2 studies. Supplemental choline may enhance the local production of apolipoproteins by ovarian granulosa cells, which may provide a protective effect through binding by the lipoproteins with LPS, thus preventing an inflammatory response within the ovary. Because ovarian granulosa cells produce apolipoproteins (Gautier et al. 2010), and because lipoproteins can bind to LPS (Vreugdenhil et al., 2001), supplemental choline may help minimize an inflammatory response with the ovary, resulting in a more robust oocyte for fertilization. Reduced inflammation through supplemental RPC may have contributed toward improved pregnancy. Although not significant, incidence of RFM, metritis, puerperal metritis, and endometritis were lower in cows fed RPC (Table 3). Cows with more uterine disease are less likely to become pregnant (Riberio et al., 2012). Relative transcription abundance of several cytokines were lower in blood cells stimulated

Figure 10. Effect of periparturient supplementation with or without ruminally protected choline (+RPC or -RPC, respectively) on prevalence of subclinical hypocalcemia (SCH). Effects of RPC ($P < 0.01$) and RPC $\times$ time interaction were detected ($P = 0.05$). At each day relative to parturition, differences between dietary treatments were found: * $P \leq 0.05$. Error bars represent SEM.

in vitro with LPS from cows fed RPC (Zenobi et al., 2017). Lima et al. (2012) reported that cows fed RPC had lower morbidity in a field study in which cows were fed RPC pre- and postpartum.

Yield of colostrum did not differ between -RPC and +RPC treatment groups (8.7 vs. 9.4 kg, respectively;

Figure 11. Survival curves for days to pregnancy up to 210 DIM for multiparous Holstein cows supplemented with or without ruminally protected choline (+RPC or -RPC, respectively). Only cows synchronized and AI were included in the analysis ($n = 91$). Effect of RPC ($P = 0.14$) was not detected. Average (143 vs. 123) and median (160 vs. 112) days to pregnancy for -RPC and +RPC treatments, respectively. Hazard ratio was 1.29 (95% CI = 0.74–2.26). Pregnancy by 210 DIM was 55.4 vs. 59.7% for -RPC vs +RPC, respectively.

Table 7); however, yield of IgG was greater (490 vs. 702 g/cow; $P = 0.04$) due to numerically greater yield of colostrum and greater concentration of IgG in colostrum of cows fed RPC (68.2 vs. 86.9 g/L, $P = 0.03$, respectively). The implications of increased intake of IgG on a calf's immune system and subsequent lactation performance needs further study.

Retrospective data were analyzed for the uterine effect of RPC (uterine effect) on performance of the offspring. Only 35 females were born in a single delivery ($n = 17$ and 18 for -RPC vs. +RPC, respectively) and were weighed at birth, weaning, and 300 d of age. Calves exposed in utero to a richer choline environment during the last 17 d of gestation tended ($P = 0.07$) to be lighter at birth (40.4 vs. 38.3 kg) but weighed more by 300 d of age (335 vs. 321 kg; $P = 0.05$; Table 7), which tended ($P = 0.06$) to result in a greater ADG from calving to 300 d of age (0.81 vs. 0.85 kg/d) compared with heifers born from cows not fed RPC (Table 7).

Work with rats demonstrated the benefits of feeding adequate amounts of lipotropes (choline, methionine, and vitamin B₁₂) to the pregnant dam on the immunity of their pups. Offspring from dams fed diets deficient in lipotropes weighed less at birth and weaning than pups born from rats fed the diet adequate in lipotropes (Newberne et al., 1970). In addition, pup survival from *Salmonella* Typhimurium infection was not improved if pups were fed the lipotropes after birth compared with pups born to dams fed adequate amounts of lipotropes before and after parturition. In addition, choline supplementation during late gestation may improve gluconeogenesis, the most important metabolic pathway for endogenous glucose generation from substrates such as propionate, lactic acid, and gluconeogenic AA, after weaning. Cai et al. (2014) reported that supplementing sows throughout gestation with betaine (a metabolite of choline; 3 g/kg of diet) improved hepatic gluconeogenesis in newborn piglets. Specifically, newborn piglets from betaine-supplemented sows had greater serum concentrations of lactic acid and gluconeogenic AA, including serine, glutamate, methionine, and histidine. In addition, liver tissue from these piglets contained greater glycogen concentration (0.16 vs. 0.13 g/g) and cytoplasmic phosphoenolpyruvate carboxykinase enzyme activity, as well as greater protein expression of several gluconeogenic enzymes, including pyruvate carboxylase, cytoplasmic phosphoenolpyruvate carboxykinase, mitochondrial phosphoenolpyruvate carboxykinase, and fructose-1,6-bisphosphatase, compared with control piglets. Feeding RPC during late gestation to pregnant ruminants may provoke changes in expression of gluconeogenic genes in the liver of preruminants, causing long-term positive effects in glucose homeostasis later in ruminant life.

CONCLUSIONS

Consuming calories at approximately 140% of requirement during the entire dry period did not affect BW or BCS at calving but did result in lower DMI and greater negative EB postpartum, resulting in greater circulating concentrations of fatty acids and BHB, greater concentration of liver TAG, and greater time to regain prepartum BW compared with cows fed energy to maintenance. Supplementation of 17.3 g/d of choline chloride (12.9 g/d of choline ions) as RPC for approximately 3 wk before and 3 wk after parturition tended to improve yield of milk (15 and 40 wk postpartum) and milk components (15 wk postpartum) without affecting DMI. As a result, EB was more negative at wk 2 and 3 postpartum but without changes in plasma concentrations of fatty acids and BHB or liver TAG concentrations. Additional benefits of RPC supplementation included reduction in prevalence of SCH, improved conception at first AI, improved output of IgG in colostrum, and improved ADG of heifers through 300 d of age. These positive effects of RPC were independent of prepartum caloric intake. If these results are confirmed in future studies, the case for choline as an essential nutrient for high-producing ruminants will be strengthened.

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