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Timing of initiation and duration of feeding rumen-protected choline affects performance of lactating Holstein cows

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ABSTRACT

Objectives were to evaluate the effects of altering timing of initiating and duration of supplementing rumen-protected choline (RPC) on lactation performance in dairy cows. The hypothesis was that RPC increases yields of milk and milk components, regardless of when supplementation is initiated, and that the effects of supplementing RPC starting prepartum and continuing post-transition would be additive. Cows at 241 ± 2.2 d of gestation were blocked by parity group (49 entering lactation 2, 50 entering lactation >2) and 305-d milk yield and, within block, assigned randomly to 1 of 4 treatments arranged as a 2 × 2 factorial with 2 levels of choline in transition, from 21 d pre- to 21 d postpartum, and 2 levels of choline in post-transition, from 22 to 105 d postpartum. The 2 levels of RPC supplemented were either 0 g/d or 12.9 g/d of choline ion fed as 60 g/d of an RPC product that was top-dressed onto the total mixed ration. Thus, treatments were as follows: NN (n = 25): no choline in transition or post-transition; NC (n = 25): no choline in transition and choline in post-transition; CN (n = 25): choline in transition and no choline in post-transition; CC (n = 24): choline in transition and in post-transition. Prepartum, treatments were supplemented (mean ± SD) for the last 18.8 ± 5.7 and 19.2 ± 5.0 d of gestation in treatments with 0 or 12.9 g/d of choline ion, respectively. Supplementing RPC prepartum did not affect dry matter intake (DMI), body weight (BW), or body condition score (BCS) in the last 3 weeks of gestation. Likewise, RPC did not affect the yield or the composition of colostrum. Supplementation with RPC during transition increased fat percent by 0.02 percentage units, fat yield by 0.16 kg/d, and energy-corrected milk (ECM) by 3.1 kg/d in the first 21 d postpartum, and increased fat yield by 0.10 kg/d and ECM by 2.4 kg/d from 22 to 105 d postpartum. Supplementing RPC during transition did not affect DMI postpartum, but it improved feed efficiency, and cows produced 0.11 kg/d more ECM per kg of DMI. Changes in BW and BCS during the first 21 d postpartum did not differ between treatments. Cows fed RPC during transition had more negative net energy balance and 0.1 unit smaller BCS in the first 105 d postpartum than non-supplemented cows. Supplementing RPC in post-transition did not influence productive performance in dairy cows, and choline supplementation during transition or post-transition did not affect measures of reproduction. Collectively, supplementing RPC to supply 12.9 g/d of choline ion benefited productive performance in dairy cows when supplementation occurred during the transition period, but no additional benefit was observed from supplementing RPC past 22 d postpartum.

Key words: choline, dairy cow, transition, lactation

INTRODUCTION

Choline (trimethyl-β-hydroxyethylammonium), an essential nutrient with multiple functions in mammalian cells, has been proposed to be a limiting nutrient for milk production in high-yielding dairy cows, especially at the onset of lactation (Erdman and Sharma, 1991; Pinotti et al., 2002). Based on these considerations, the effects of rumen-protected choline (**RPC**) supplementation to transition dairy cows have been investigated in several experiments, and results have been compiled in some reviews (Pinotti et al., 2010; Sales et al., 2010; Arshad et al., 2020). Zenobi et al. (2018a) evaluated RPC supplementation in transition parous Holstein cows, from 21 d before to 21 d after calving. Supplementing RPC to supply 12.9 g/d of choline ion increased yields of milk and ECM without changes in DMI during the first 15 wk of lactation. Furthermore, the increase in milk yield extended up to the first 40 wk postpartum. This carryover effect from supplementation with RPC during the transition period might

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be related to potential benefits to health, intermediary metabolism, or perhaps effects on the mammary gland. Additionally, RPC increased IgG concentration in colostrum and tended to increase the proportion of cows pregnant at first AI (Zenobi et al., 2018a).

Recently, Arshad et al. (2020) conducted a systematic literature review and meta-analysis from randomized experiments in which RPC was supplemented during the transition period to parous cows. The experiments used in the meta-analysis supplemented diets of cows for at least 7 d prepartum (range of 7 to 40 d), and postpartum duration ranged from 15 to 120 d. The authors observed a 2.18 kg/d increase in ECM yield. Their study confirmed the benefits of RPC supplementation to transition dairy cows, but it remains unexplored whether extending supplementation beyond the transition period will affect dairy cow performance at doses of choline ion typically fed to transition cows. Some experiments (Piepenbrink and Overton, 2003; Elek et al., 2008; Ardalan et al., 2010) have extended RPC past the first 3 wk postpartum; however, their results cannot separate the effects of RPC supplemented after 3 wk of lactation from those of the peripartum supplementation because, within the same experiment, no treatment started supplementing RPC after the transition period.

Few experiments have evaluated the effects of RPC supplementation beginning after the transition period, with cows in either early or mid-lactation (Erdman and Sharma, 1991; Davidson et al., 2008; Mohsen et al., 2011). In these experiments, RPC supplementation resulted in greater yields of milk or FCM, or both. These results indicate that the benefits of supplementing RPC might not be restricted to cows during the transition period. Therefore, it is possible that extending or even initiating supplementation past the first 3 wk postpartum might benefit lactation performance in dairy cows. In contrast, if choline requirements are met very early in lactation, at least based on re-establishment of concentrations of plasma choline metabolites (Artegoitia et al., 2014; Imhasly et al., 2015), then supplementing past 3 wk postpartum may not further improve productive performance. Thus, we suggest that the optimal feeding strategy for RPC remains unclear and deserves to be investigated within the same experiment.

We hypothesized that RPC increases yields of milk and milk components regardless of when supplementation is initiated. We further hypothesized that the effects of supplementing RPC prepartum and continuing post-transition would be additive, resulting in the greatest benefit to yields of milk and milk components. Thus, we designed this experiment with the objective of evaluating the effects of timing of initiation and duration of supplementation of RPC on lactation performance in dairy cows. The treatments designed differed in the timing of initiation (prepartum vs. post-transition) and duration of feeding (only transition or transition and post-transition). This is the first of 2 companion papers on the effects of RPC on lactation performance, metabolism, and aspects of inflammatory responses in dairy cows (Bollatti et al., 2020).

MATERIALS AND METHODS

The experiment was conducted at the University of Florida Dairy Unit (Gainesville) from November 2016 to September 2017. All procedures involving cows in the experiment were carried out according to the University of Florida's Institutional Animal Care and Use Committee.

Cows and Housing

At 241 ± 2.2 d of gestation (mean ± SD; range 241 to 251), 113 pregnant nonlactating Holstein cows that had completed at least 1 lactation were enrolled in the experiment. Selection criteria included apparently healthy pregnant cows with no recent history of disease in the 90 d preceding enrollment.

All prepartum cows were housed together in a freestall barn with sand-bedded stalls, and each cow was randomly assigned to an individual feeding gate (American Calan Inc., Northwood, NH). Cows that remained in the experiment fully learned to use their assigned feeding gate within 1 d of training. Intake was recorded daily after enrollment, and data for the last 21 d of gestation were analyzed (mean ± SD, 19.0 ± 5.4 d). Intake measured before treatment administration was used as covariate for statistical analysis of DMI. Immediately after calving, cows were moved to and housed together in another adjacent pen. Cows were re-assigned to an individual feeding gate (American Calan Inc.) for the first 105 d postpartum for treatment administration and measurement of DMI.

The experimental pens were equipped with 2 rows of fans (1 fan/6 linear meters) placed above the beds, and a water soaker line with nozzles was placed above the feedbunk for evaporative cooling of cows. Fans and water nozzles were controlled by thermostats and activated when ambient temperature reached 18°C.

Feeding Management

Cows were fed for ad libitum intake once daily prepartum at 0930 h and twice daily postpartum at 0600 h and 1100 h. Amounts of feed offered to individual cows were adjusted daily to result in at least 5% refusals, which were weighed once daily, before the morning feeding, and daily DM intakes were calculated. Two diets were

fed, one prepartum and another postpartum (Tables 1 and 2). All cows were fed the same diet within period, and treatments were administered daily as top-dress, concurrent with the morning feeding. Diets pre- and postpartum were formulated using a source of blood meal containing ruminally-protected methionine and lysine (Perdue AgriBusiness, Salisbury, MD). Additionally, the lactating mineral-vitamin mixture contained 21.2% of an undegradable protein supplement rich in methionine and lysine (Spectrum Agriblue 50, Perdue AgriBusiness). The objective was to provide diets with adequate concentrations of metabolizable methionine pre- and postpartum. The contents of metabolizable methionine and lysine in the diets were, respectively, 2.10 and 6.14% of the MP prepartum, and 2.04 and 6.31% of the MP postpartum (Table 2). The ratios of metabolizable lysine to methionine pre- and postpartum were 2.9:1 and 3.1:1, respectively (NRC, 2001).

Forages and wet brewers grains were sampled every 3 d, and concentrate mixtures were sampled weekly. Samples were dried at 55°C for 48 h in a forced-air oven, and dry weights were recorded. Dried feed samples were ground to pass a 1-mm screen of a Wiley mill (Thomas Scientific, Swedesboro, NJ), composited for every 6-wk period, and analyzed using wet chemistry procedures (AOAC methods; Dairyland Laboratories Inc., Arcadia, WI). The same ingredients were dried at 105°C for 24 h to determine DM content, to calculate daily DMI of individual cows. The forage-to-concentrate ratios of the TMR fed were adjusted every 3 d using the rolling average of the DM values of the wet feeds and concentrate mixtures.

Treatments

The experiment followed a randomized block design with cow as the experimental unit. Weekly cohorts of parous cows were blocked by parity group prepartum (lactation 1 vs. lactation >1) and, within parity group, ranked by 305-d milk yield from lowest to highest, such that every 4 cows within parity group and level of milk yield were assigned to a block. Within block, cows were assigned randomly to 1 of the 4 treatments in a 2 × 2 factorial arrangement (Figure 1). Treatments were labeled by 2 letters, the first indicating the supplementation during the transition period, from 21 d pre- to 21 d postpartum, and the second letter indicating the treatment received during the post-transition period, from 22 to 105 d postpartum. The 4 treatments were **NN** (n = 27): 0 g/d of choline ion in transition and post-transition; **NC** (n = 28): 0 g/d of choline ion in transition and 12.9 g/d of choline ion post-transition; **CN** (n = 29): 12.9 g/d of choline ion in transition and 0 g/d of choline ion post-transition; and **CC** (n = 29): 12.9 g/d of choline ion in transition and in post-transition. Of the 113 cows initially enrolled, 14 were removed from the experiment, and any data from those animals were excluded before statistical analyses. Reasons for removal are provided in Supplemental Table S1 (https://doi.org/10.3168/jds.2019-17293). Removed cows were those that developed severe illnesses in the

Table 1. Ingredient composition of diets fed during the prepartum and lactating periods

Ingredient, % of DM	Prepartum[1]	Lactation[2]
Corn silage	45.7	49.5
Bermuda hay	25.1	—
Alfalfa hay	—	5.5
Soybean meal	3.5	16.0
Corn grain, finely ground	—	10.5
Brewers grains, wet	14.0	—
Citrus pulp, dried	4.3	5.8
Soybean hulls	—	7.1
LysAAMet[3]	1.3	0.2
Saturated free fatty acids[4]	—	1.6
Mycotoxin binder[5]	—	0.5
Acidogenic supplement[6]	1.9	—
Prepartum mineral-vitamin mixture[7]	4.2	—
Postpartum mineral-vitamin mixture[8]	—	3.3

[1]Diet fed for an average (±SD) of 19.0 ± 5.4 d prepartum.

[2]Diet fed from calving to 105 d postpartum.

[3]Spray-dried blood meal product enriched with rumen-protected lysine and methionine (LysAAMet, Perdue AgriBusiness, Salisbury, MD).

[4]Energy-Booster Mag (fat supplement containing 95.8% fatty acids and 2.3% Mg, Milk Specialties Global, Eden Prairie, MN).

[5]Novasil Plus (BASF Corp., Florham Park, NJ).

[6]Soy-Chlor: acidogenic feed supplement (West Central Cooperative, Ralston, IA).

[7]A mixture containing 69.01% corn gluten feed, 17.50% magnesium sulfate × 7 H_2O, 4.70% magnesium oxide, 4.90% sodium chloride, 1.90% ClariFly Livestock Premix 0.67% (Central Garden and Pet Co., Walnut Creek, CA), 0.70% vitamin E 227,000 kIU/kg, 0.47% Sel-Plex 2000 (Alltech Biotechnology, Nicholasville, KY), 0.39% Rumensin 90 (Elanco Animal Health, Greenfield, IN), 0.30% IntelliBond Vital 4 (Micronutrients USA LLC, Indianapolis, IN), 0.035% vitamin A concentrate 650,000 kIU/kg, 0.013% vitamin D_3 concentrate 500,000 kIU/kg, and 0.002% EDDI 80%. Each kilogram contained (DM basis): 0.21% Ca, 0.79% P, 4.48% Mg, 0.97% K, 2.48% S, 2.02% Na, 3.04% Cl, 814 mg Zn; 183 mg Cu; 580 mg Mn; 10.1 mg Se; 4.6 mg Co; and 16.8 mg I, 242 kIU of vitamin A, 68 kIU of vitamin D, 3,526 IU of vitamin E, 771 mg of monensin, and 128 mg of diflubenzuron.

[8]A mixture of 23.70% sodium bicarbonate, 21.18% Spectrum Agriblue (Perdue AgriBusiness, Salisbury, MD), 12.40% DCAD Plus (Arm and Hammer Animal Nutrition, Princeton, NJ), 10.40% calcium carbonate, 6.10% magnesium oxide, 10.50% mono-dicalcium phosphate, 5.11% sodium chloride, 4.40% potassium chloride red, 3.67% wheat middlings, 0.64% USA Lysine (Purina Animal Nutrition, St. Louis, MO), 0.82% ClariFly Livestock Premix (Central Garden and Pet Co.), 0.315% Sel-Plex 2000 (Alltech Biotechnology), 0.26% vitamin E 227,000 kIU/kg, 0.19% Rumensin 90 (Elanco Animal Health), 0.19% IntelliBond Vital 4 (Micronutrients USA LLC), 0.1% biotin (2%), 0.017% vitamin A concentrate 650,000 kIU/kg, 0.005% vitamin D_3 concentrate 500,000 kIU/kg, and 0.0017% calcium iodate. Each kilogram contained (DM basis): 18.9% CP, 5.94% Ca, 2.32% P, 3.4% Mg, 8.27% K, 9.42% Na, 5.3% Cl, 0.54% S, 2,256 mg of Fe, 504 mg of Zn, 118 mg of Cu, 423 mg of Mn, 6.3 mg of Se, 11.82 mg of Co, 10.8 mg of I, 110 kIU of vitamin A, 26.7 kIU of vitamin D_3, 1,322 IU of vitamin E, 345 mg of monensin, and 49.8 mg of diflubenzuron.

first 2 wk postpartum; such cows did not recover after treatment and, consequently, had to be moved to a hospital pen and left the treatment diets, to comply with the guidelines of the University of Florida's Institutional Animal Care and Use Committee. Therefore, 99 cows contributed data to the experiment, 25 NN, 25 NC, 25 CN, and 24 CC (Figure 1). Because of removal of cows, the number of cows in lactation 2 or lactation >2 was not the same among treatments (Supplemental Table S2, https://doi.org/10.3168/jds.2019-17293).

The RPC product supplemented (60 g/d ReaShure, Balchem Corp., New Hampton, NY) contained 28.8% choline chloride as per manufacture information, which supplied supplemented cows a daily dose of 12.9 g of choline ion. The RPC product was mixed with ground corn and dried molasses in a 30:56:14 ratio (as-is basis) and top-dressed at 200 g/d onto the TMR concurrent with the morning feeding to supplemented cows. Cows assigned to the 0 g/d of choline ion treatment received 200 g/d of a top-dress containing ground corn and dried molasses in an 80:20 ratio.

Because of the experimental design, data up to 21 d postpartum were analyzed with 2 treatments: no choline in transition (**NT** = 50; including treatments NN and NC), or cows supplemented with choline in transition (**CT** = 49; including treatments CN and CC; Figure 1).

Starting on d 106 postpartum, cows were moved to another freestall barn and housed together, remaining there until at least 180 d postpartum. From wk 15 to 25 postpartum, all cows as a group were fed the same diet, a TMR not supplemented with RPC, under the exactly same management conditions.

BW and BCS

Cows were weighed on the day of experiment enrollment and then once weekly prepartum, in the morning before feeding, until calving. Body condition was scored on the day of enrollment and then once weekly by the same 2 trained evaluators using a 1-to-5 scale with increments of 0.25 units, as depicted in the Elanco BCS chart (Elanco Animal Health, 2009). During the postpartum period, immediately after each milking, cows were weighed on a walk-though scale (AfiWeigh, SAE Afikim, Israel) located on the exit lane of the milking parlor. Body condition was scored once weekly as described previously.

Measurement and Analysis of Colostrum

Cows were milked within the first 2 h after calving, and colostrum yield in the first milking was measured

Table 2. Chemical composition of diets (mean ± SD) fed during the prepartum and lactating periods

Item, DM basis	Prepartum[1]	Lactation[2]
NE_L,[3] Mcal/kg	1.54	1.66
CP, %	15.8 ± 0.5	16.6 ± 0.3
MP,[3] g/d	1,237	2,648
Metabolizable Met,[3] g/d	26	54
Metabolizable Lys,[3] g/d	76	167
Ratio of metabolizable Lys to Met[3]	2.9:1	3.1:1
NDF, %	45.4 ± 0.7	29.9 ± 0.7
ADF, %	25.9 ± 0.5	19.9 ± 0.4
Lignin, %	3.9 ± 0.1	2.21 ± 0.3
Starch, %	18.4 ± 0.9	26.7 ± 1.0
Ether extract, %	3.4 ± 0.2	2.9 ± 0.4
Ash, %	5.9 ± 0.2	6.8 ± 0.2
Ca, %	0.60 ± 0.02	0.79 ± 0.03
P, %	0.34 ± 0.01	0.36 ± 0.01
Mg, %	0.57 ± 0.04	0.29 ± 0.05
K, %	1.05 ± 0.06	1.55 ± 0.08
S, %	0.34 ± 0.02	0.17 ± 0.01
Na, %	0.16 ± 0.02	0.42 ± 0.04
Cl, %	0.81 ± 0.06	0.46 ± 0.07
Mn, mg/kg	64.4 ± 10.0	37.4 ± 3.8
Zn, mg/kg	78.5 ± 10.1	51.6 ± 11.1
Cu, mg/kg	21.6 ± 3.6	13.5 ± 1.8
Fe, mg/kg	188.5 ± 12.2	376.5 ± 29.7
DCAD,[4] mEq/kg	−104 ± 17	341 ± 48

[1]Diet fed for an average (±SD) of 19.0 ± 5.4 d prepartum.

[2]Diet fed from calving to 105 DIM.

[3]Calculated using NRC (2001) software according to DM intake pre- and postpartum, 11.5 and 22.4 kg/d, respectively.

[4]DCAD calculated as follows: [(mEq of K) + (mEq of Na)] − [(mEq of Cl) + (mEq of S)].

and sampled (AfiFlo milk meters, SAE Afikim). Two representative aliquots of colostrum were collected. One of the aliquots was kept at −20°C for further analysis, whereas the other was diluted 1 to 1 with skim milk, and bronopol-B-14 (Advanced Instruments, Norwood, MA) was added as a preservative. Skim milk and diluted colostrum samples were analyzed for concentrations of fat, true protein, lactose, and SCC at the Southeast Dairy Herd Improvement milk laboratory in Belleview, Florida. Component concentrations in the original colostrum samples were calculated based on the concentrations of each component in skim milk and in the diluted samples and the 1-to-1 dilution factor.

The aliquot of frozen-thawed colostrum was analyzed for concentration of IgG via radial immunodiffusion assay (Triple J Farms, Bellingham, WA) per manufacturer's protocol. Briefly, colostrum was diluted 1 to 5 in 0.9% saline, such that the concentration of IgG would fall within the linear range of the standard curve of the assay. The diluted samples were pipetted into the bovine anti-bovine IgG antibody plate and incubated for 27 h on a flat surface protected from light. The diameter of the precipitin ring was measured using a 7× scale loupe (no. 1975, Peak Optics, GWJ Co., La Quinta, CA) and used to calculate the IgG concentrations. The intra- and interassay coefficients of variation were, respectively, 1.2 and 5.4%.

Measurements of Yields of Milk and Milk Components

Cows were milked twice daily at 1100 h and 2300 h, and yields of milk were recorded automatically (AfiFlo, SAE Afikim). Samples of milk were collected weekly until 105 DIM, on Mondays, from 2 sequential milkings, morning and night, for measurements of concentrations of fat, true protein, lactose, and SCC at the Southeast Dairy Herd Improvement milk laboratory. The SCC was transformed to SCS for statistical analysis according to the following formula: $SCS = Log_{10}(SCC/12.5)/Log_{10}(2)$.

Milk yield and composition from each sampling were used to calculate the final concentrations of milk components for each week. Yields of milk corrected for 3.5% fat and for energy were calculated according to NRC (2001) as follows: 3.5% FCM, kg/d = (0.4324 × milk yield) + (16.218 × milk fat yield); ECM, kg/d = [(0.3246 × milk yield) + (12.86 × fat yield) + (7.04 × protein yield)].

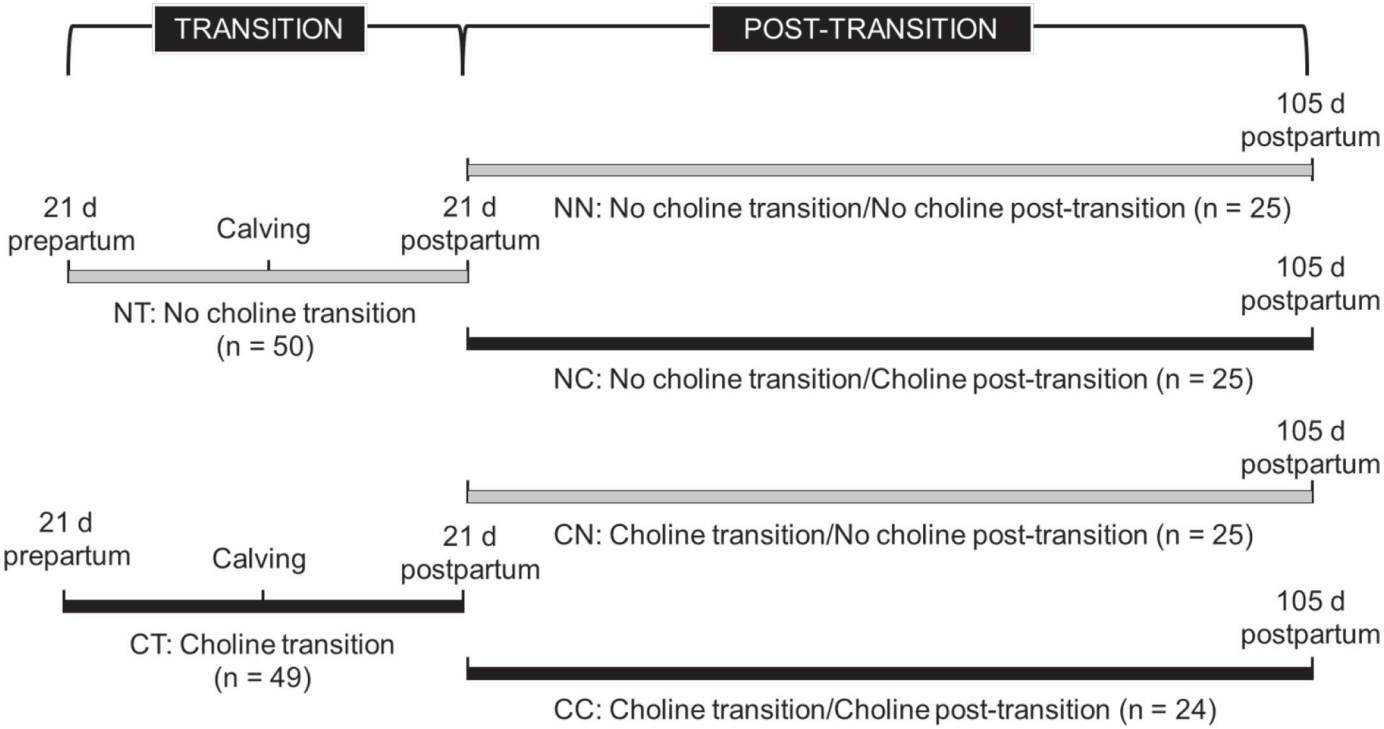

Figure 1. Diagram of the experiment. Cows were blocked by parity group prepartum (lactation 1 vs. lactation >1) and 305-d milk yield; within block, they were assigned randomly to the following treatments: NN = 0 g/d of choline ion in transition (21 d pre- to 21 d postpartum) or post-transition (22 to 105 d postpartum); NC = 0 g/d of choline ion in transition and 12.9 g/d of choline ion post-transition; CN = 12.9 g/d of choline ion in transition and 0 g/d of choline ion post-transition; CC = 12.9 g/d of choline ion in transition and in post-transition.

Milk yield was also measured for the first 25 wk of lactation, although no milk samples were collected after 105 d postpartum. The rationale for measuring milk yield after 105 d postpartum was to detect whether carryover effects occurred past the period of treatments. Milk yield data were collected up to 25 wk because 95 cows contributed with data on all weeks up to 25 wk postpartum. After that, removal from the herd resulted in an unequal number of cows contributing with data for each of the 4 treatments.

Measurement of Net Energy Balance

Energy balance was calculated using daily caloric intake from DMI and the energy content of the diets according to NRC (2001) and using the NE_L system. Needs for maintenance were calculated based on metabolic BW [net energy (**NE**) maintenance, Mcal/d = $BW^{0.75} \times 0.08$]. Calories required for gestation of prepartum cows were calculated based on the birth BW of calves and adjusted for day of gestation (NRC, 2001). The NE_L required for milk synthesis was calculated according to yields of fat, protein, and lactose, based on NRC (2001), as follows: {milk yield × [(0.0929 × fat %) + (0.0563 × protein %) + (0.0395 × lactose %)]}.

Reproductive Management and Reproductive Responses

Detection of corpus luteum via transrectal ultrasonography was performed on d 28 ± 3 and 40 ± 3 postpartum to assess resumption of estrous cyclicity. Cows were considered to be cyclic when a visible corpus luteum was detected via transrectal ultrasonography on either of the 2 scanning days. Cows without a corpus luteum both days were considered to be anovular.

All cows were subjected to the double Ovsynch protocol for first AI (Souza et al., 2008). Briefly, cows received an i.m. injection of 100 μg of GnRH (Cystorelin, 50 μg/mL gonadorelin diacetate tetrahydrate; Merial, Duluth, GA) at 53 ± 3 d postpartum, followed by an injection of 25 mg of $PGF_{2\alpha}$ (Lutalyse Sterile Solution, 5 mg/mL dinoprost as tromethamine salt; Zoetis, Florham Park, NJ) at 60 ± 3 d postpartum, and another injection of 100 μg of GnRH at 63 ± 3 d postpartum. Seven days later, at 70 ± 3 d postpartum, the same sequence of injections was repeated, with the final GnRH administered in the afternoon of 79 ± 3 d in lactation, and timed AI performed the morning of d 80 ± 3 postpartum, approximately 14 to 16 h after the final GnRH treatment. Pregnancy was diagnosed on d 32 after each AI, based on the presence of an amniotic vesicle with an embryo with heartbeat, detected by transrectal ultrasonography. Nonpregnant cows had the estrous cycle resynchronized for timed AI with the Ovsynch protocol, to be reinseminated 10 d after the nonpregnancy diagnosis. Pregnant cows on d 32 after AI were re-evaluated for pregnancy on d 74 after AI. For statistical analyses, the diagnosis performed on d 74 after AI was used to determine whether a cow became pregnant at the first or subsequent AI. Days open to 280 d postpartum were recorded. Days open refer to the number of days a cow was eligible to be inseminated and included the interval from calving to pregnancy, "do not inseminate" status, sale, or death, whichever happened first up to 280 d postpartum. Cows that became "do not inseminate," were sold or died, or remained nonpregnant by 280 d postpartum were censored during survival analysis.

Statistical Analyses

Statistical analyses of continuous data were performed using the MIXED procedure of SAS version 9.4 (SAS/STAT, SAS Institute Inc., Cary, NC). Continuous data were tested for distribution of residuals using Shapiro-Wilk and for homogeneity of variance by plotting residuals against predicted values after fitting the statistical models. Data that deviated from the assumptions of normality were subjected to Box-Cox transformation according to the TRANSREG procedure of SAS before analyses.

Because of the experimental design, data analyzed during the transition period, from 21 d prepartum to 21 d postpartum, considered only 2 levels of treatment, NT or CT. Also, during the transition period, data were analyzed separately for the pre- and postpartum periods. After 21 d postpartum, the factorial arrangement with 4 treatments, including 2 levels of choline in transition (0 vs. 12.9 g/d) and 2 levels of choline post-transition (0 vs. 12.9 g/d), were included in the statistical models.

For the transition period, the statistical models included the fixed effects of RPC (NT vs. CT), time (day or week) of measurement, and the interaction between treatment and time, and the random effect of block. Data with repeated measurements included the random effect of cow nested within transition treatment. Data collected from cows before treatment were used as covariates in the statistical models of data analyzed prepartum. Body weight at enrollment and calf category (singleton male, singleton female, or twin) were covariates in all statistical models for data analyzed during the transition period.

For the post-transition period, data were analyzed as a 2 × 2 factorial arrangement of treatments. The models included the fixed effects of RPC in transi-

tion, RPC post-transition, interaction between RPC in transition and post-transition, week of measurement, and interactions of treatments with week postpartum, and the random effects of block and cow nested within treatment. Three contrasts were evaluated: (1) effect of RPC fed in transition (NN + NC vs. CN + CC); (2) effect of RPC fed post-transition (NN + CN vs. NC + CC); and (3) effect of the interaction of feeding RPC in transition and post-transition (NC + CN vs. NN + CC). Body weight at enrollment and calf category (singleton male, singleton female, or twin) were covariates in all statistical models analyzed during the post-transition period. For the carryover period in wk 16 to 25, milk yield was analyzed as a 2 × 2 factorial arrangement of treatments, using the same model described for the post-transition period and evaluating the same contrasts.

In all models, the Kenward-Roger method was used to calculate the approximate denominator degrees of freedom for the F tests. In all statistical models with repeated measures, the REPEATED statement was used for dependent variables measured over time. Cow nested within treatment was the error term for testing the effects of treatment. The covariance structure with the smallest Akaike information criterion was selected for each variable. When an interaction between treatment and time (day or week) resulted in $P < 0.10$, then treatment means at different time points were partitioned using the SLICE command of SAS.

Binary responses were analyzed via logistic regression using the GLIMMIX procedure of SAS. The statistical model included the fixed effects of RPC in transition, RPC post-transition, interaction between RPC in transition and post-transition, and the random effects of block. Time to event, such as days open by 280 d postpartum, was analyzed with Cox's proportional hazard regression model using the PHREG procedure of SAS, with a model that included the effects of RPC in transition, RPC post-transition, and interaction between RPC in transition and RPC post-transition. Nonpregnant cows at 280 d postpartum and those that left the herd nonpregnant were censored at 280 d or when they left the herd, respectively. The adjusted hazard ratio and respective 95% confidence interval were calculated. Statistical significance was considered at $P \leq 0.05$ and tendency at $0.05 < P \leq 0.10$.

RESULTS

Descriptive statistics of measures collected at the time of enrollment and at parturition for the 99 cows completing according to treatment are presented in Supplemental Table S2 (https://doi.org/10.3168/jds.2019-17293). In the prepartum period, treatments were supplemented (mean ± SD) for the last 18.8 ± 5.7 and 19.2 ± 5.0 d of gestation in NT and CT cows, respectively. The range of days on treatments prepartum were 7 to 30 in NT and 9 to 30 in CT. The variability among cows for days fed the prepartum treatments was caused primarily by differences in gestation length. Nevertheless, gestation length did not differ between transition treatments and averaged (±SD) 273.2 ± 6.1 in NT and 273.5 ± 4.6 in CT (Supplemental Table S2, https://doi.org/10.3168/jds.2019-17293).

Because of the experimental design, data collected during the transition period were analyzed considering 2 levels of treatment, NT versus CT, and the results herein are presented in this sequence. For the post-transition period, data were analyzed with the factorial arrangement, and results are presented with the following sequence: NN, NC, CN, CC.

Transition Period

Prepartum Intake and Measures of Energy Status. Supplementation with RPC during the last 3 wk of gestation did not affect DMI (Figure 2A), BW, or BCS (Table 3). Consequently, NE balance was unaffected by treatments (Figure 2C).

Colostrum Yield and Composition. Supplementing RPC prepartum did not affect the yield of colostrum or the concentration of IgG in colostrum (Table 4). Furthermore, the content and yield of nutrients in colostrum did not differ between NT and CT. The SCS was greater ($P = 0.02$) in NT than in CT (Table 4).

Dry Matter Intake, Production Performance, and Energy Status. Intake of DM and milk yield did not differ between transition treatments in first 21 d postpartum (Table 5; Figures 2A and 2B); however, supplementing RPC during transition increased ($P \leq 0.05$) yields of ECM and 3.5% FCM in the first 21 d postpartum (Table 5; Figure 2D). The increments in yields of ECM and FCM were caused mostly by a combination of numerically greater milk yield that tended ($P = 0.08$) to have greater fat concentration, resulting in greater ($P = 0.02$) fat yield in CT than in NT (Table 5). The concentrations and yields of true protein and lactose did not differ with transition treatment, and no effect of RPC was observed for yield of ECM per kilogram of DMI. The increased yield of ECM in CT compared with NT, with no effect on DMI, resulted in cows fed CT having slightly ($P = 0.09$) more negative NE balance in early lactation than did those in NT (Figure 2C; Table 5). The mean BW and BCS and the changes in BW and BCS did not differ with transition treatment.

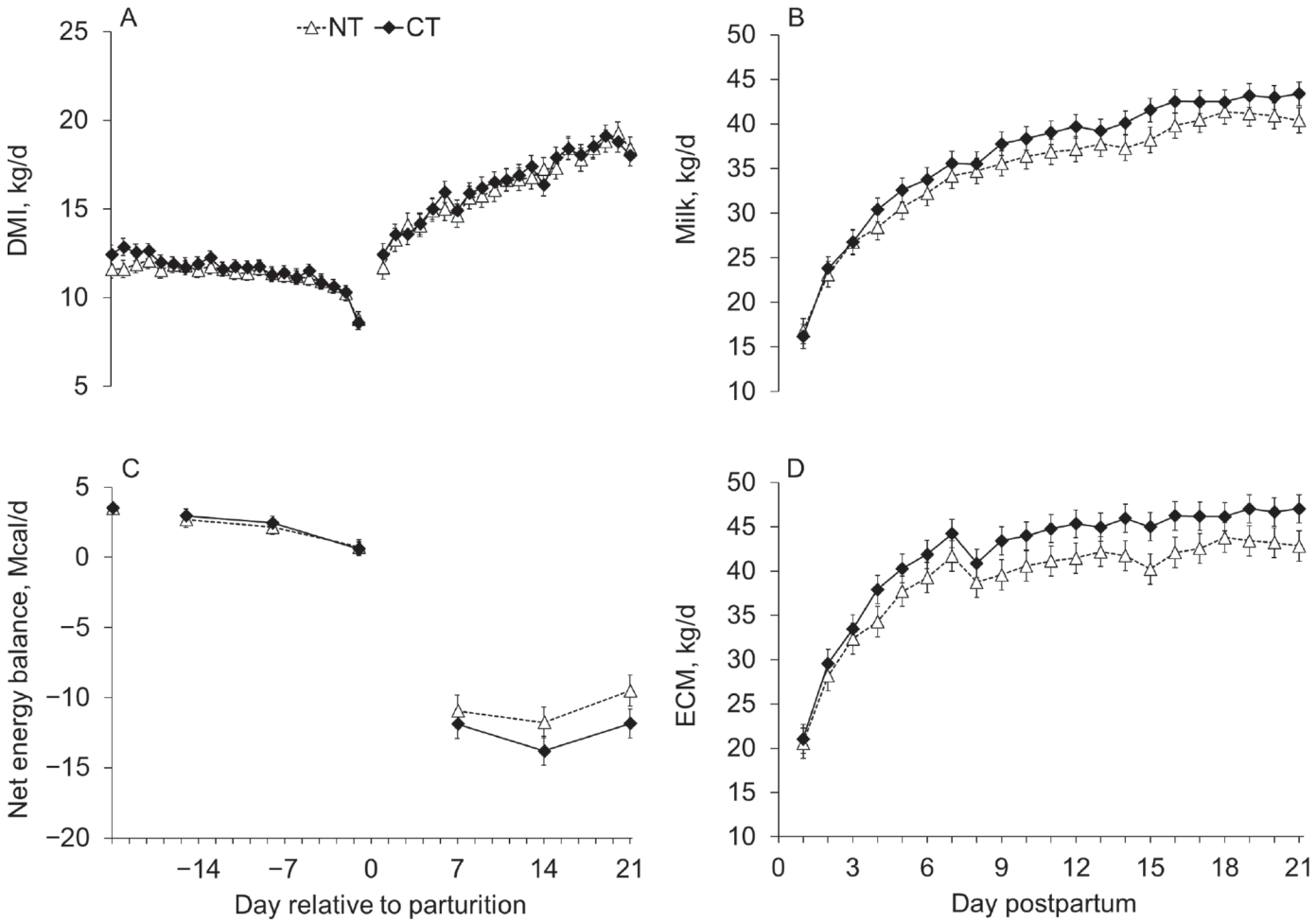

Figure 2. Effect of supplementing diets of transition cows with either 0 (Δ, NT) or 12.9 g/d of choline ion (◆, CT) as rumen-protected choline (RPC) on DMI (A), milk yield (B), net energy balance (C), and ECM yield (D) of parous dairy cows. Panel A, prepartum: effects of RPC ($P = 0.42$) and interaction between RPC and day ($P = 0.93$). Panel A, postpartum: effects of RPC ($P = 0.77$) and interaction between RPC and day ($P = 0.29$). Panel B: effects of RPC ($P = 0.17$) and interaction between RPC and day ($P = 0.54$). Panel C, prepartum: effects of RPC ($P = 0.75$) and interaction between RPC and day ($P = 0.66$). Panel C, postpartum: effects of RPC ($P = 0.09$) and interaction between RPC and day ($P = 0.41$). Panel D: effects of RPC ($P = 0.05$) and interaction between RPC and day ($P = 0.59$). Error bars represent SEM.

Table 3. Effect of supplementing diets of transition cows with rumen-protected choline (RPC) on prepartum performance

	Treatment[1]			*P*-value[2]	
Item	NT	CT	SEM	Tr	Tr × time
DMI, kg/d	11.3	11.6	0.3	0.42	0.93
BW, kg	770.3	775.7	5.4	0.28	0.92
BCS, 1 to 5	3.48	3.45	0.04	0.56	0.84
Net energy balance, Mcal/d	1.86	2.00	0.48	0.75	0.66

[1]Cows were blocked by parity group prepartum (lactation 1 vs. lactation >1) and 305-d milk yield; within block, they were assigned randomly to the following treatments: NN = 0 g/d of choline ion in transition (21 d pre- to 21 d postpartum) or post-transition (22 to 105 d postpartum); NC = 0 g/d of choline ion in transition and 12.9 g/d of choline ion post-transition; CN = 12.9 g/d of choline ion in transition and 0 g/d of choline ion post-transition; CC = 12.9 g/d of choline ion in transition and in post-transition. Treatment NT represents NN and NC, and treatment CT represents CN and CC.

[2]Tr = effect of supplementing RPC in transition (NT vs. CT); Tr × time = interaction between Tr and time prepartum (day or week).

Post-Transition Period

Dry Matter Intake, Production Performance, and Energy Status. We detected no interactions between supplementing RPC in transition and post-transition (Table 6; Figure 3). Therefore, results will be presented focusing on the main effects of RPC supplementation during transition or post-transition.

Supplementing RPC in transition or post-transition did not affect DMI, which averaged 23.4 kg/d between 22 and 105 d postpartum (Table 6; Figure 4A). Cows supplemented with RPC during transition produced 2.4 kg/d more ECM (NT = 43.1 ± 1.2 vs. CT = 45.5 ± 1.2 kg/d; $P = 0.05$) and 2.6 kg/d more 3.5% FCM (NT = 44.2 ± 1.3 vs. CT = 46.8 ± 1.2 kg/d; $P = 0.04$) than did NT cows (Table 6; Figure 4B). Milk fat content did not differ among treatments, but supplementing RPC in transition tended ($P = 0.07$) to increase fat yield in cows after 21 d postpartum (NT = 1.53 ± 0.06 vs. CT = 1.63 ± 0.05 kg/d). Concentration and yield of true protein did not differ among treatments, but cows fed RPC in transition tended ($P = 0.09$) to increase lactose yield (NT = 2.14 ± 0.06 vs. CT = 2.24 ± 0.05 kg/d). Concentration of lactose decreased ($P = 0.05$) with RPC in post-transition (no choline = 4.80 ± 0.03 vs. choline = 4.75 ± 0.03%), and the SCS of milk tended ($P = 0.06$) to increase with supplementation with RPC post-transition (no choline = 1.67 ± 0.49 vs. choline = 2.57 ± 0.44). Feeding RPC in transition increased ($P = 0.01$) efficiency of feed utilization by 0.11 kg of ECM per kg of DMI (NT = 1.86 ± 0.05 vs. CT = 1.97 ± 0.04; Table 6; Figures 3C and 4C); however, feed efficiency tended ($P = 0.10$) to decrease with RPC post-transition by 0.07 kg/kg (no choline = 1.95 ± 0.05 vs. choline = 1.88 ± 0.04; Table 6; Figure 3C). Consequently, the NE balance of cows was less ($P = 0.02$) with supplementing RPC during the transition period (NT = −1.39 ± 0.73 vs. CT = −3.05 ± 0.66 Mcal/d) and tended to be greater ($P = 0.06$) with supplementing RPC post-transition (no choline = −2.91 ± 0.72 vs. choline = −1.53 ± 0.66 Mcal/d; Table 6; Figures 3D and 4D). The differences in NE balance resulted in smaller ($P = 0.04$) BCS in cows fed RPC during transition (NT = 3.08 ± 0.05 vs. CT = 2.98 ± 0.04; Figures 3E and 4E) and less ($P = 0.02$) BW gain after d 21 postpartum (NT = 0.24 ± 0.07 vs. CT = 0.07 ± 0.07 kg/d; Table 6). In spite of the differences in energy balance and BW change, we found no differences in mean BW throughout the experiment because of supplementation with RPC in transition (Figure 4F) or in post-transition (Figure 3F).

Carryover Effect on Production Past Wk 15 Postpartum. Supplementing RPC during transition did not affect milk yield from 16 to 25 weeks of lactation (Figure 5). Also, similar to the first 15 wk postpartum, supplementing RPC post-transition did not affect milk yield after supplementation stopped, from 16 to 25 wk (no choline = 39.5 ± 1.5 vs. choline = 38.4 ± 1.4 kg/d). During the first 25 wk postpartum, the mean milk yields were 40.9 and 42.9 kg/d for NT and CT, respectively (Figure 5).

Reproduction and Survival. Supplementing RPC did not influence the proportion of cows with corpus luteum by 40 ± 3 d postpartum, an indicator of resumption of estrous cyclicity (Table 7). Furthermore, feeding RPC did not affect the proportion of cows pregnant at first or second AI, or the proportion of cows pregnant by 280 d postpartum. The lack of differences in pregnancy per AI resulted in no effect of supplementing RPC on the rate of pregnancy and median days open (Table 8; Supplemental Figure S1, https://doi.org/10.3168/jds.2019-17293).

The rate of removal from the herd up to 280 d postpartum tended ($P = 0.09$) to be less for NT than for CT (adjusted hazard ratio = 0.25; 95% CI = 0.05 to 1.22) resulting in 2 NT and 7 CT removed from the herd by 280 d postpartum (NN = 1, NC = 1, CN = 2, CC = 5). Reasons for removal included 1 NN and 1 NC sold on d 275 and 265 postpartum, respectively, because of low milk production; 2 cows in CN were sold on d 143 and 210 postpartum, 1 because of trauma

Table 4. Effect of supplementing diets of transition cows with rumen-protected choline on colostrum yield and composition[1]

Item	Treatment[2]		SEM	*P*-value
	NT	CT		
Yield, kg	4.94	4.27	0.74	0.38
IgG				
g/L	120.2	116.0	8.1	0.62
Total, g	551.5	465.7	75.0	0.26
Fat				
%	4.52	4.59	0.35	0.85
kg	0.22	0.21	0.04	0.68
True protein				
%	14.33	13.84	0.49	0.33
kg	0.69	0.57	0.09	0.23
Lactose				
%	3.18	3.28	0.08	0.22
kg	0.16	0.14	0.26	0.55
Somatic cells				
× 1,000/mL	2,353	1,436	—	—
Score	6.93	6.14	0.31	0.02

[1]Colostrum from first milking harvested in the first 2 h after calving.

[2]Cows were blocked by parity group prepartum (lactation 1 vs. lactation >1) and 305-d milk yield; within block, they were assigned randomly to the following treatments: NN = 0 g/d of choline ion in transition (21 d pre- to 21 d postpartum) or post-transition (22 to 105 d postpartum); NC = 0 g/d of choline ion in transition and 12.9 g/d of choline ion post-transition; CN = 12.9 g/d of choline ion in transition and 0 g/d of choline ion post-transition; CC = 12.9 g/d of choline ion in transition and in post-transition. Treatment NT represents NN and NC, and treatment CT represents CN and CC.

Table 5. Effect of supplementing diets of transition cows with rumen-protected choline (RPC) on performance in the first 21 d postpartum

	Treatment[1]			*P*-value[2]	
Item	NT	CT	SEM	Tr	Tr × time
DMI, kg/d	16.2	16.4	0.6	0.77	0.29
Yield, kg/d					
Milk	34.8	36.5	1.2	0.17	0.54
ECM	38.9	42.0	1.5	0.05	0.59
3.5% FCM	39.6	43.1	1.5	0.03	0.66
ECM:DMI, kg/kg	2.43	2.58	0.09	0.12	0.60
Fat					
%	4.42	4.62	0.11	0.08	0.60
kg/d	1.52	1.68	0.06	0.02	0.76
True protein					
%	3.42	3.38	0.08	0.55	0.89
kg/d	1.17	1.22	0.04	0.30	0.87
Lactose					
%	4.67	4.66	0.03	0.92	0.38
kg/d	1.64	1.71	0.06	0.24	0.81
Somatic cells					
× 1,000/mL	323.9	236.1	—	—	—
Score	2.56	2.32	0.36	0.53	0.18
Net energy balance, Mcal/d	−10.7	−12.5	1.0	0.09	0.41
BW					
kg	667	674	7.0	0.35	0.20
Change, kg/d	−2.28	−2.78	0.40	0.26	0.59
Body condition					
Score, 1 to 5	3.23	3.20	0.04	0.47	0.28
Change[3]	−0.25	−0.30	0.04	0.23	—

[1]Cows were blocked by parity group prepartum (lactation 1 vs. lactation >1) and 305-d milk yield; within block, they were assigned randomly to the following treatments: NN = 0 g/d of choline ion in transition (21 d pre- to 21 d postpartum) or post-transition (22 to 105 d postpartum); NC = 0 g/d of choline ion in transition and 12.9 g/d of choline ion post-transition; CN = 12.9 g/d of choline ion in transition and 0 g/d of choline ion post-transition; CC = 12.9 g/d of choline ion in transition and in post-transition. Treatment NT represents NN and NC, and treatment CT represents CN and CC.

[2]Tr = effect of supplementing RPC in transition (NT vs. CT); Tr × time = interaction between Tr and time postpartum (day or week).

[3]Difference between BCS on d 21 postpartum and BCS before parturition.

and 1 because of toxic mastitis; and 1 cow in CC died on d 247 postpartum, and 4 cows were sold, 1 because of toxic mastitis on d 106, 1 because of lameness on d 115, and 2 because of low production on d 243 and 247 postpartum. Supplementing RPC post-transition did not affect ($P = 0.27$) the rate of removal from the herd (adjusted hazard ratio = 0.46; 95% CI = 0.11 to 1.85).

DISCUSSION

Several experiments have been conducted to evaluate whether supplementing RPC to dairy cows influences lactation performance. In most cases, diets of cows were supplemented with RPC starting prepartum and continued postpartum. In the present experiment, the timing of starting and duration of RPC supplementation were evaluated, and parous cows fed diets supplemented with 12.9 g/d of choline ion from 21 d pre- to 21 d postpartum produced 3.1 kg/d more ECM and 0.16 kg/d more fat in the first 21 d postpartum. Furthermore, the benefits of supplementing RPC in transition carried over in the post-transition period. Cows in CT produced 2.2 kg/d more milk, 2.4 kg/d more ECM, and 0.10 kg/d more fat from 4 to 15 wk postpartum than did NT cows. On the other hand, supplementing RPC post-transition did not affect production performance of dairy cows in the first 105 d postpartum.

Choline is known to play a role in intermediary metabolism, in particular as a component of phospholipids and lipoproteins, which are critical for lipid absorption and transport, and might be limiting in early lactation, thereby making transition cows responsive to supplementation. In fact, the pattern of concentrations of plasma choline biomolecules in dairy cows seems to be lowest prepartum, reaching a nadir in the week of calving and then increasing with week postpartum (Imhasly et al., 2015). Measurements of total choline biomolecules in plasma among dairy cows almost doubled from 1 to 3 wk postpartum and increased 6- to 12-fold from 1 wk postpartum to mid- and late lacta-

tion (Artegoitia et al., 2014). Although supplementing 12.9 g/d choline ion as RPC had small effects, increasing concentrations of choline biomolecules in dry cows subjected to negative nutrient balance (Zenobi et al., 2018b) and mid-lactation cows (de Veth et al., 2016), it is possible that the effect of RPC on improving concentrations of choline biomolecules in circulation is greater in cows during the transition period. Transition cows have smaller concentrations of circulating choline biomolecules than do late-lactation or dry cows, and therefore dietary supplementation might have a greater effect in increasing plasma concentrations of cows in transition cows than at any stage of lactation. It is possible that these differences might partially explain the benefits to productive performance of supplementing choline to transition cows.

It is well known that milk yield increases during early lactation as a result of an increase in mammary cell number followed by an increase in secretory activity per cell (Capuco et al., 2003). The differences in milk yield in the present experiment seemed to start very early postpartum and continued for at least 12 wk after RPC withdrawal. Indeed, other manipulations during the transition period, such as increasing milking frequency (Bar-Peled et al., 1995) or reducing prepartum heat stress (Tao et al., 2011), also reportedly resulted in carryover effects on milk yield that were linked to increased mammary cell proliferation (Capuco et al., 2003; Tao et al., 2011). Choline kinase, an enzyme involved in the conversion of choline to phosphocholine, regulates mammary cell proliferation (Oka and Perry, 1979; Ramírez de Molina et al., 2004). It is possible that an increased supply of choline in early lactation might have stimulated the enzyme to enhance mitosis in mammary cells in CT cows. In addition, choline may exert some endocrine control on the mammary gland and influence nutrient partition toward milk synthesis mediated by increases in growth hormone (Kawamura et al., 2012). In laboratory animals, supplementation with glycerophosphocholine, an intermediate of choline

Table 6. Effect of altering the timing of initiation and duration of feeding rumen-protected choline (RPC) on performance from 22 to 105 d postpartum

	Treatment[1]					*P*-value[2]					
Item	NN	NC	CN	CC	SEM	Tr	PT	Tr × PT	Tr × Wk	PT × Wk	Tr × PT × Wk
DMI, kg/d	22.7	24.2	23.3	23.3	0.6	0.79	0.12	0.13	0.73	0.56	0.18
Yield, kg/d											
Milk	44.9	44.3	47.1	46.5	1.4	0.06	0.62	0.96	0.99	0.43	<0.01
ECM	43.0	43.1	45.8	45.1	1.5	0.05	0.84	0.76	0.84	0.20	0.23
3.5% FCM	44.1	44.2	47.1	46.4	1.6	0.04	0.82	0.76	0.90	0.34	0.31
ECM:DMI, kg/kg	1.91	1.80	1.98	1.95	0.05	0.01	0.10	0.35	0.91	0.11	0.48
Fat											
%	3.41	3.50	3.51	3.48	0.12	0.66	0.73	0.53	0.53	0.23	0.72
kg/d	1.52	1.54	1.64	1.62	0.07	0.07	0.97	0.66	0.71	0.32	0.73
True protein											
%	2.84	2.90	2.85	2.83	0.05	0.46	0.60	0.38	0.38	0.98	0.75
kg/d	1.27	1.28	1.33	1.32	0.04	0.15	0.85	0.73	0.98	0.54	0.06
Lactose											
%	4.81	4.75	4.80	4.75	0.03	0.82	0.05	0.98	0.60	0.41	0.12
kg/d	2.17	2.11	2.26	2.21	0.07	0.09	0.35	0.94	0.98	0.58	<0.01
Somatic cells											
× 1,000/mL	300.7	478.6	272.4	450.5	133.1	—	—	—	—	—	—
Score	1.68	2.78	1.67	2.35	0.61	0.65	0.06	0.68	0.88	0.89	0.54
Net energy balance, Mcal/d	−2.55	−0.23	−3.27	−2.84	0.90	0.02	0.06	0.21	0.99	0.06	0.65
BW											
kg	660.6	668.6	651.6	659.1	9.9	0.21	0.28	0.98	0.95	0.10	0.37
Change, kg/d	0.176	0.308	0.039	0.110	0.089	0.02	0.15	0.68	0.69	0.18	0.14
Body condition											
Score, 1 to 5	3.03	3.13	3.00	2.95	0.06	0.04	0.55	0.13	0.08	0.11	0.22
Change[3]	−0.18	−0.02	−0.21	−0.11	0.06	0.21	0.01	0.56	—	—	—

[1]Cows were blocked by parity group prepartum (lactation 1 vs. lactation >1) and 305-d milk yield; within block, they were assigned randomly to the following treatments: NN = 0 g/d of choline ion in transition (21 d pre- to 21 d postpartum) or post-transition (22 to 105 d postpartum); NC = 0 g/d of choline ion in transition and 12.9 g/d of choline ion post-transition; CN = 12.9 g/d of choline ion in transition and 0 g/d of choline ion post-transition; CC = 12.9 g/d of choline ion in transition and in post-transition.

[2]Tr = effect of supplementing RPC in transition (NN plus NC vs. CN plus CC); PT = effect of supplementing RPC in post-transition (NN plus CN vs. NC plus CC); Tr × PT = interaction between Tr and PT (NN plus CC vs. NC plus CN); Tr × Wk = interaction between Tr and week after calving; PT × Wk = interaction between PT and Wk; Tr × PT × Wk = interaction between Tr, PT and Wk.

[3]Difference between BCS on d 105 postpartum and BCS on d 21 postpartum.

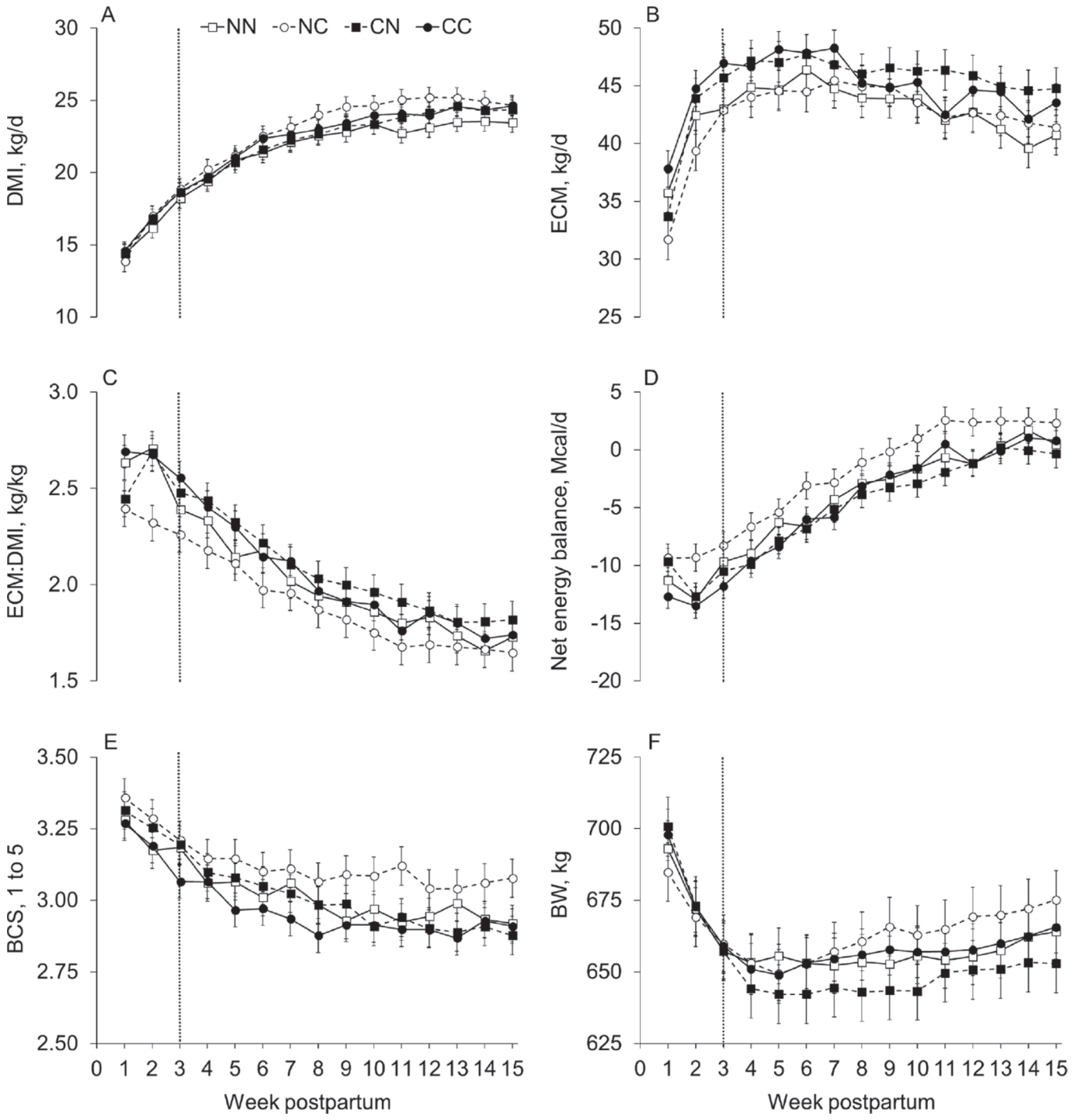

Figure 3. Effect of altering the timing of initiation and duration of feeding rumen-protected choline (RPC) on DMI (A), ECM yield (B), feed efficiency (C), net energy balance (D), BCS (E), and BW (F) of parous dairy cows. Cows were blocked by parity group prepartum (lactation 1 vs. lactation >1) and 305-d milk yield; within block, they were assigned randomly to the following treatments: NN (□) = 0 g/d of choline ion in transition (21 d pre- to 21 d postpartum) or post-transition (22 to 105 d postpartum); NC (○) = 0 g/d of choline ion in transition and 12.9 g/d of choline ion post-transition; CN (■) = 12.9 g/d of choline ion in transition and 0 g/d of choline ion post-transition; CC (●) = 12.9 g/d of choline ion in transition and in post-transition. Vertical dashed lines indicate when the 2 × 2 factorial arrangement of treatments starts. Statistical results refer to analysis of data from wk 4 to 15 postpartum. Tr = effect of supplementing RPC in transition; PT = effect of supplementing RPC post-transition; Tr × PT = interaction between Tr and PT. Panel A = effects of Tr ($P = 0.79$), PT ($P = 0.12$), and Tr × PT ($P = 0.13$). Panel B = effects of Tr ($P = 0.05$), PT ($P = 0.84$), and Tr × PT ($P = 0.76$). Panel C = effects of Tr ($P = 0.01$), PT ($P = 0.10$), and Tr × PT ($P = 0.35$). Panel D = effects of Tr ($P = 0.02$), effects of PT ($P = 0.06$), and Tr × PT ($P = 0.21$). Panel E = effects of Tr ($P = 0.04$), PT ($P = 0.55$), and Tr × PT ($P = 0.13$). Panel F = effects of Tr ($P = 0.21$), PT ($P = 0.28$), and Tr × PT ($P = 0.98$). Error bars represent SEM.

metabolism in tissues, increased plasma concentrations of free choline and growth hormone (Kawamura et al., 2012). These changes increased lipolysis and ketogenesis, which could supply nutrients for milk synthesis. In the current experiment, cows supplemented with RPC in transition had increased yields of ECM and milk

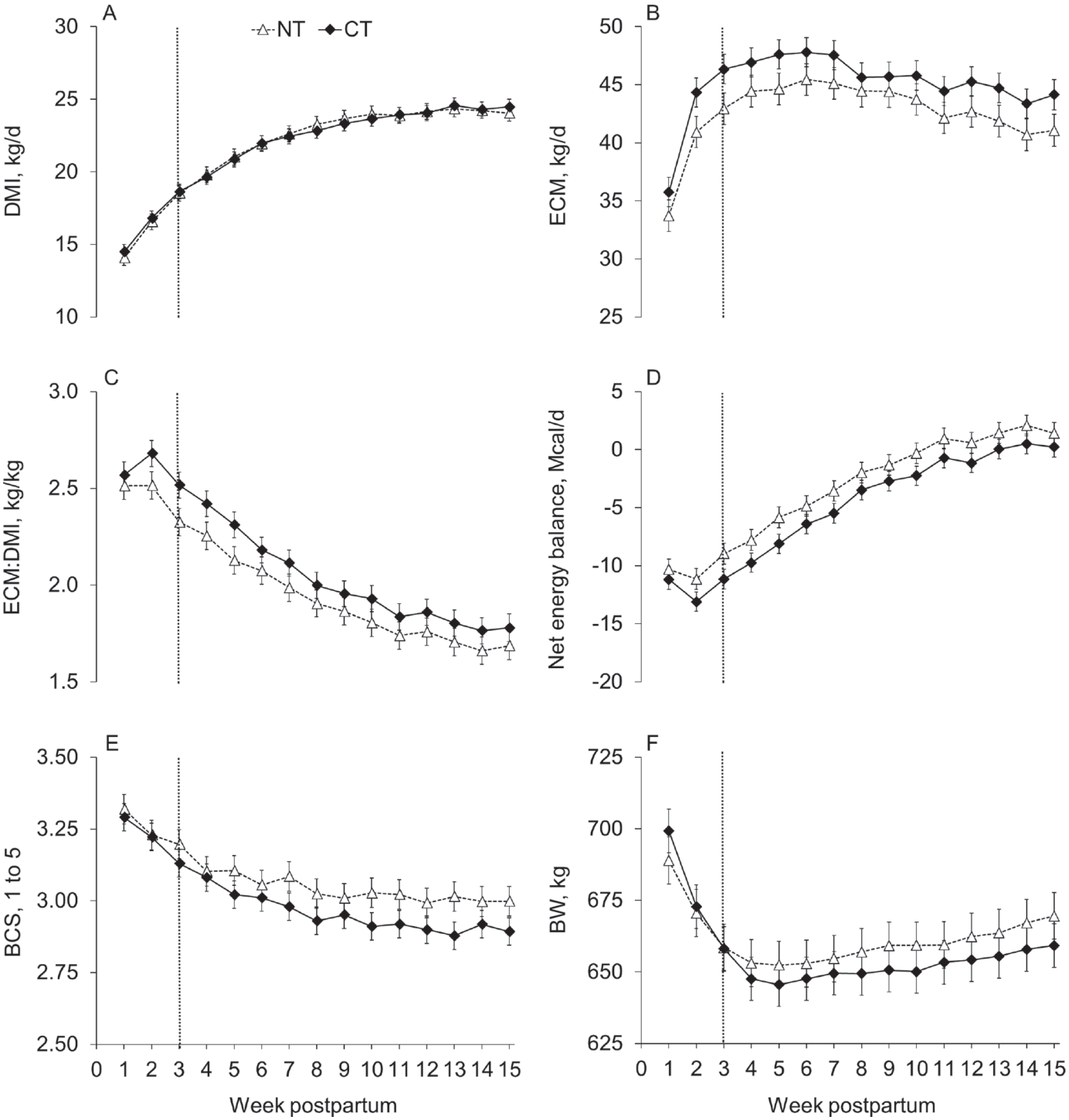

Figure 4. Effect of supplementing diets of transition cows with either 0 (Δ, NT) or 12.9 g/d of choline ion (◆, CT) as rumen-protected choline (RPC) on DMI (A), ECM (B), feed efficiency (C), net energy balance (D), BCS (E), and BW (F) of parous dairy cows. Vertical dashed line indicates when supplementation with RPC in transition stopped. Statistical results refer to analysis of data from wk 4 to 15 postpartum and the effects of RPC in transition. Panel A = effect of RPC ($P = 0.79$). Panel B = effect of RPC ($P = 0.05$). Panel C = effect of RPC ($P = 0.01$). Panel D = effect of RPC ($P = 0.02$). Panel = effect of RPC ($P = 0.04$). Panel F = effect of RPC ($P = 0.21$). Error bars represent SEM.

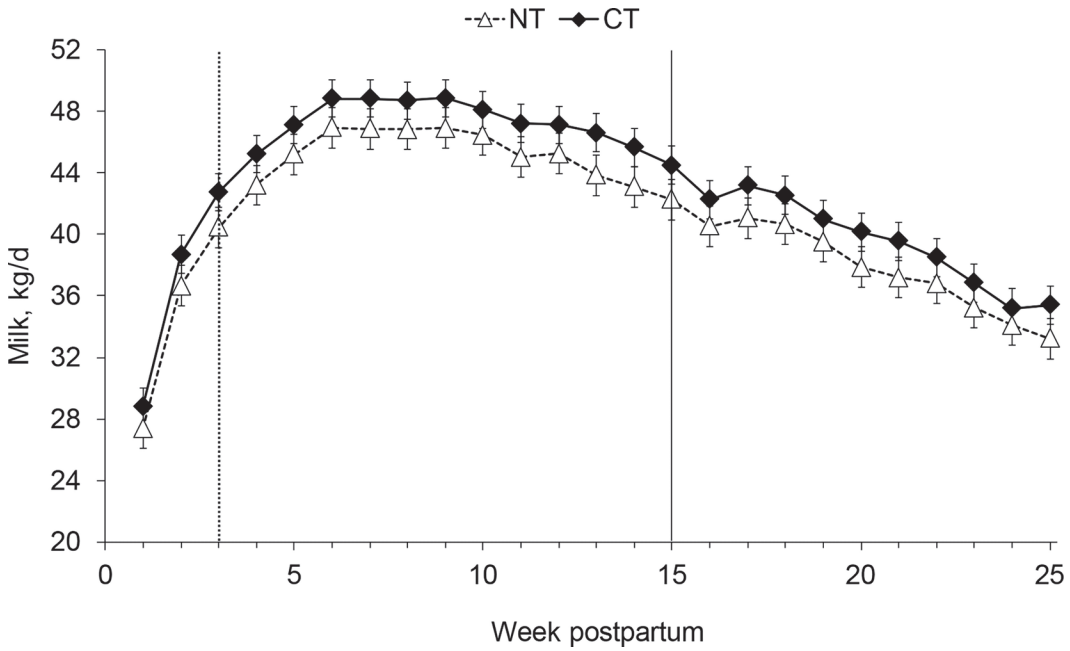

Figure 5. Effect of supplementing diets of transition cows with either 0 (Δ, NT) or 12.9 g/d of choline ion (◆, CT) as rumen-protected choline (RPC) from 21 d pre- to 21 d postpartum on milk yield up to 25 wk postpartum. Vertical dashed line indicates when supplementation with RPC in transition stopped (21 d postpartum), and the 2 × 2 factorial arrangement of treatments started until 105 d postpartum (solid line), after which all cows were housed in the same pen and received the same diet without RPC. Statistical results refer to separate analysis of data, wk 1 to 3, 4 to 15, and 16 to 25 postpartum. The LSM ± SEM for NT and CT (kg/d) were, respectively, 34.8 ± 1.2 and 36 ± 1.2 for wk 1 to 3 ($P = 0.17$); 44.6 ± 1.0 and 46.8 ± 1.1 for wk 4 to 15 ($P = 0.06$); and 38.2 ± 1.5 and 39.7 ± 1.4 for wk 16 to 25 ($P = 0.26$). The mean milk yields from wk 1 to 25 were, respectively, 40.9 and 42.9 kg/d for NT and CT.

fat concurrent with reduced body condition after 21 d postpartum.

It has been suggested that the decline in DMI prepartum might alter the physical structure of the gastrointestinal mucosal lining that could potentially facilitate the entry of bacteria or toxins into the bloodstream and stimulate an inflammatory response (Kvidera et al., 2017b). Phospholipids are indispensable to maintain the function of the gastrointestinal barrier (Braun et al., 2009). Phosphatidylcholine, in particular, is not only a critical component of chylomicrons (Takahashi et al., 1982) needed for lipid absorption and transport,

Table 7. Effect of altering the timing of initiation and duration of feeding rumen-protected choline (RPC) on reproductive performance

	Treatment[1]				*P*-value[2]		
Item	NN	NC	CN	CC	Tr	PT	Tr × PT
Estrous cyclic,[3] % (no.)	68.0 (17/25)	76.0 (19/25)	84.0 (21/25)	75.0 (18/24)	0.40	0.84	0.34
Pregnant,[4] % (no.)							
First AI	32.0 (8/25)	44.0 (11/25)	20.0 (5/25)	18.2 (4/22)	0.30	0.75	0.63
Second AI	23.5 (4/17)	28.6 (4/14)	35.0 (7/20)	23.5 (4/17)	0.83	0.83	0.59
280 d postpartum	76.0 (19/25)	96.0 (24/25)	80.0 (20/25)	75.0 (18/24)	0.39	0.41	0.33
Days open							
LSM ± SEM	151 ± 14	135 ± 14	164 ± 15	173 ± 16	—	—	—
Median (95% CI)	128 (80–202)	102 (80–185)	135 (113–199)	161 (113–218)	—	—	—

[1]Cows were blocked by parity group prepartum (lactation 1 vs. lactation >1) and 305-d milk yield; within block, they were assigned randomly to the following treatments: NN = 0 g/d of choline ion in transition (21 d pre- to 21 d postpartum) or post-transition (22 to 105 d postpartum); NC = 0 g/d of choline ion in transition and 12.9 g/d of choline ion post-transition; CN = 12.9 g/d of choline ion in transition and 0 g/d of choline ion post-transition; CC = 12.9 g/d of choline ion in transition and in post-transition.

[2]Tr = effect of supplementing RPC in transition (NN plus NC vs. CN plus CC); PT = effect of supplementing RPC in post-transition (NN plus CN vs. NC plus CC); Tr × PT = interaction between Tr and PT (NN plus CC vs. NC plus CN).

[3]Based on detection of corpus luteum by transrectal ultrasonography on d 28 ± 3 or 40 ± 3 postpartum.

[4]Pregnancy based on diagnosis performed 74 d after insemination.

but is also involved in maintenance of intestinal villus integrity (da Silva et al., 2015). Supplementing choline to dry dairy cows subjected to feed restriction and subsequent fat challenge increased the plasma concentration of triacylglycerol compared with non-supplemented controls (Zenobi et al., 2018b). Increased plasma triacylglycerol after a fatty acid challenge suggests that choline enhances the absorptive capacity of the gastrointestinal tract, and therefore it is possible that nutrient absorption is improved, particularly absorption of fatty acids. Cows in the present experiment had improved efficiency of conversion of DMI into ECM, which might be related to improved gastrointestinal function, in particular nutrient absorption with RPC supplementation. Also, it is possible that by having a less disrupted gastrointestinal barrier, the nutritional costs necessary to support an activated immune system might be reduced (Kvidera et al., 2017a), which would provide more nutrients for milk synthesis. Furthermore, choline plays a role in modulating immune response (Vailati-Riboni et al., 2017; Garcia et al., 2018), and it might attenuate inflammation (Sun et al., 2016), which would benefit transition cows, which are known to suffer from numerous inflammatory diseases. Collectively, these potential mechanisms might explain why supplementing RPC during the transition period improved lactation performance in dairy cows.

Supplementing RPC during the transition period improved milk fat yield, as has been shown by others (Arshad et al., 2020). In addition to a potential effect of RPC improving supply of nutrients through absorption and transport of lipids, it is known that choline compounds are actively secreted into milk (Pinotti et al., 2003), and these choline-related phospholipids are part of the fat globule membrane (McPherson and Kitchen, 1983). Choline might improve formation of the milk fat globule membrane and, thus, facilitate packaging and transport of milk triacylglycerols through the mammary cell. Improving the formation of milk fat globules might protect milk fat and facilitate secretion into milk (Jensen and Nielsen, 1996). Also, the effect of RPC on milk fat might occur by increasing the precursors for mammary lipid secretion such as absorbed fatty acids transported by chylomicrons, or by increasing hepatic secretion of very low density lipoprotein (Chandler and White, 2017) and export of hepatic fatty acids (Piepenbrink and Overton, 2003). Both chylomicrons and very low density lipoprotein can deliver long-chain fatty acids to the mammary gland for milk fat synthesis. Recently, Coleman et al. (2019) demonstrated that abomasal infusion of choline in dairy cows increased hepatic concentrations of carnitine and betaine compared with no infusion of choline. Choline may increase carnitine by sparing other methyl donors for the hepatic synthesis of carnitine (Bremer, 1983) or regulating urinary excretion (Daily et al., 2002). In other species, choline increases skeletal muscle concentrations of carnitine and reduces total body fat (Daily et al., 1998). Thus, choline may stimulate the release and transport of long-chain fatty acids that eventually will be taken up by the mammary gland for milk fat synthesis.

Supplementing RPC after the transition period did not improve performance of dairy cows. One possible explanation may be that dietary choline requirements are greatest during the last weeks of gestation and first weeks of lactation, based on the patterns of plasma choline biomolecules shown by Artegoitia et al. (2014) and Imhasly et al. (2015). Cows in the first weeks of

Table 8. Cox's hazard regression model for days open according to treatments with rumen-protected choline (RPC) during transition or post-transition[1]

	Transition		Post-transition		*P*-value[2]	
Item	No choline	Choline	No choline	Choline	Tr	PT
Cows, no.	50	49	50	49	—	—
Pregnant,[3] %	86.0	77.6	78.0	85.7	—	—
Cows censored, no.	7	11	11	7	—	—
Days open						
Median (95% CI)	114 (80–157)	147 (115–198)	132 (113–193)	133 (102–185)	—	—
Mean ± SEM	145.7 ± 10.4	169.1 ± 11.0	160.2 ± 10.8	153.4 ± 10.6	—	—
AHR[4] (95% CI)	Referent	0.79 (0.51–1.23)	Referent	1.32 (0.85–2.05)	0.29	0.21

[1]Cows were blocked by parity group prepartum (lactation 1 vs. lactation >1) and 305-d milk yield; within block, they were assigned randomly to the following treatments: NN = 0 g/d of choline ion in transition (21 d pre- to 21 d postpartum) or post-transition (22 to 105 d postpartum); NC = 0 g/d of choline ion in transition and 12.9 g/d of choline ion post-transition; CN = 12.9 g/d of choline ion in transition and 0 g/d of choline ion post-transition; CC = 12.9 g/d of choline ion in transition and in post-transition.

[2]Tr = effect of supplementing RPC in transition; PT = effect of supplementing RPC post-transition. The interaction between Tr and PT was not significant ($P = 0.25$) and was dropped from the final model.

[3]Pregnancy based on diagnosis performed 74 d after each AI.

[4]AHR = adjusted hazard ratio.

lactation undergo a period of negative nutrient balance, which likely limits the availability of precursors for endogenous synthesis of choline. A small portion of choline in lactating goats is derived from methionine (Emmanuel and Kennelly, 1984), and early-lactation cows have relatively low DMI, which limits the supply of methionine, either from microbial origins or from escape protein, that can be used for synthesis of choline. During the post-transition period, DMI averaged 23.4 kg/d, which resulted in 3,885 g/d of CP, 2,772 g/d of MP, and 56 g/d of metabolizable methionine, based on NRC (2001). Post-transition experiments in which RPC increased lactation performance usually fed diets with low CP content (13.5%; Mohsen et al., 2011) or with limited supply of metabolizable methionine (42 g/d; Davidson et al., 2008), or supplemented choline ion in quantities larger than 22 g/d (Erdman and Sharma, 1991; Davidson et al., 2008; Mohsen et al., 2011). When dietary protein was moderate to high, 17.2% (Rahmani et al., 2014) or 21% of the diet DM (Deuchler et al., 1998), or dose of choline ion was less than 15 g/d (Erdman and Sharma, 1991), cows in post-transition did not increase yields of milk or FCM with choline supplementation. Thus, the lack of response to RPC post-transition observed in the present experiment might be related to either the adequate supply of metabolizable methionine or the relatively low dose of choline ion supplemented.

Supplementing RPC during the transition period did not influence yield or composition of colostrum. It is noteworthy that the mean concentration of IgG in colostrum was high, 118 g/L, 50% greater than previously reported (Zenobi et al., 2018a). Limited literature is available on the effects of supplementing RPC to prepartum cows on colostrum yield and composition. Zenobi et al. (2018a) reported greater concentration and yield of IgG (68.2 vs. 86.9 g/L; 490 vs. 702 g) in colostrum when cows received RPC prepartum. In pigs, supplementing graded levels of soybean lecithin in the prepartum diet increased the concentration of phosphatidylcholine and total phospholipids in milk and tended to increase total Ig content of colostrum (Shi et al., 2019). The mammary gland produces Ig but primarily transfers large amounts of plasma IgG across the mammary barrier to epithelial cells for secretion in colostrum (Hine et al., 2019). The mechanisms by which choline or derived phospholipids influence colostrum IgG content are unknown, and the lack of effect in the present experiment, which had half of the colostrum yield, but 50% greater IgG content than the results of Zenobi et al. (2018a) cannot be explained at this point. Possible areas to be explored are proliferation of mammary cells (Oka and Perry, 1979; Ramírez de Molina et al., 2004) or enhanced transport of Ig from plasma to the mammary epithelial cell (Hine et al., 2019).

Supplementing RPC did not affect pregnancy per AI or days open. Zenobi et al. (2018a) reported that supplementing RPC during the transition period tended to increase pregnancy at first AI postpartum. In general, heterogeneity exists for the effect of choline on reproduction in dairy cows, with some experiments describing benefits (Ardalan et al., 2010; Amrutkar et al., 2015; Zenobi et al., 2018a) but others showing no effect (Erdman and Sharma, 1991; Lima et al., 2012). Properly powered experiments are needed to elucidate the effects of supplemental choline on reproduction in dairy cows.

CONCLUSIONS

Supplementing 12.9 g/d choline ion top-dressed as RPC to parous cows from 21 d before to 21 d postpartum increased milk fat content by 0.20 percentage units, milk fat yield by 0.16 kg/d, and ECM yield by 3.1 kg/d in the first 21 d postpartum. Additionally, supplementing choline during transition increased yields of fat, milk and ECM, respectively, 0.10, 2.2, and 2.4 kg/d from 22 to 105 d postpartum, although supplementation stopped at 21 d postpartum. The increases in productive performance were not followed by increases in DMI, which resulted in improved efficiency of feed conversion into ECM in the first 105 d postpartum. As a result, cows supplemented with RPC in transition had slightly less BCS after 21 d postpartum. At the dose fed, we found no effects of RPC supplementation post-transition on productive performance of dairy cows. Previous research showed that supplementing larger doses of choline to mid-lactation cows, particularly when diets had low CP content, increased milk or FCM yield; therefore, it is possible that the lack of response to choline supplementation post-transition in the present experiment might be related to the dose fed, 12.9 g/d, or to the intake of MP and metabolizable methionine. At the current supplemental dose of RPC and levels of dietary protein and metabolizable methionine fed, extending supplementation past 21 d or starting at 21 d postpartum are not warranted.

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