THE OBJECTIVE OF THIS STUDY WAS TO EXAMINE THE EFFECTS OF PREGNATAL SUPPLEMENTATION AND DOSE OF RUMEN-PROTECTED CHOLINE (RPC) ON NEO-NATAL CALF GROWTH, METABOLISM, AND VACCINE RESPONSE. PAROUS HOLSTEIN COWS WERE BLOCKED BY CALVING MONTH AND RANDOMLY ASSIGNED WITHIN BLOCK TO RECEIVE 45 G/D OF RPC [20.4 G/D OF CHOLINE IONS (CHOL45), n = 19], 30 G/D OF RPC [13.6 G/D OF CHOLINE IONS (CHOL30), n = 22], OR NO RPC (CON, n = 19) AS A TOP-DRESS, STARTING 24 D BEFORE EXPECTED CALVING. CALF BODY WEIGHS WERE RECORDED FOR THE FIRST 3 WK OF LIFE. ALL CALVES WERE FEED COLOSTRUM REPLACER (300 G OF IGG) AT BIRTH, AND APPARENT EFFICIENCY OF IGG ABSORPTION WAS CALCULATED. ON D 1, 7, 14, AND 21, BLOOD SAMPLES WERE TAKEN TO QUANTIFY PLASMA REACTIVE OXYGEN AND NITROGEN SPECIES, ANTIOXIDANT POTENTIAL, HAPTOGLOBIN, NONESTERIFIED FATTY ACIDS (NEFA), β-HYDROXYBUTYRATE, AND GLUCOSE. CALVES RECEIVED AN INTRANASAL VACCINE AT BIRTH, AND NASAL SECRETIONS WERE COLLECTED ON D 0, 7, 10, 14, AND 21 TO QUANTIFY BOVINE RESPIRATORY SYNCYTIAL VIRUS-SPECIFIC IGA. DATA WERE ANALYZED USING LINEAR MIXED MODELS INCLUDING THE FIXED EFFECTS OF TREATMENT, TIME (WHEN APPLICABLE), CALF SEX, AND PREPARTUM DAM DATA (−24 D) ALONG WITH INTERACTIONS. TREATMENT DID NOT AFFECT CALF BODY WEIGHT, β-HYDROXYBUTYRATE, OR GLUCOSE CONCENTRATIONS. FOR APPARENT EFFICIENCY OF IGG ABSORPTION, TREATMENT INTERACTED WITH THE DAM’S PREPARTUM BODY CONDITION SCORE. WHERE THE DAM’S BODY CONDITION SCORE WAS ≤3.25, IGG ABSORPTION WAS REDUCED IN CALVES BORN FROM CHOL45 DAMS AS COMPARED WITH CALVES FROM EITHER CHOL30 OR CON DAMS. CALVES FROM CHOL30 DAMS HAD A LESSER OXIDATIVE STRESS INDEX (OSI; REACTIVE OXYGEN AND NITROGEN SPECIES/ANTIOXIDANT POTENTIAL) THAN CALVES FROM CON DAMS. HAPTOGLOBIN CONCENTRATIONS WERE LESS IN HEIFER CALVES FROM CHOL45 DAMS AS COMPARED WITH HEIFERS FROM CON DAMS. THE DAM’S PREPARTUM NEFA CONCENTRATION INTERACTED WITH TREATMENT. WHEN DAM NEFA WAS MINIMAL, CALVES FROM CHOL45 AND CHOL30 DAMS HAD GREATER OR TENDED TO HAVE GREATER NEFA, RESPECTIVELY. CONVERSELY, WHEN DAM NEFA WAS GREATER, CALVES FROM CHOL30 AND CHOL45 DAMS HAD LESSER OR TENDED TO HAVE LESSER NEFA THAN CALVES FROM CON DAMS, RESPECTIVELY. FOR VACCINE RESPONSE, TREATMENT INTERACTED WITH THE DAM’S PREPARTUM OSI. AMONG CALVES BORN FROM DAMS WITH A GREATER OSI, CALVES FROM CHOL45 AND CHOL30 DAMS HAD LESSER BOVINE RESPIRATORY SYNCYTIAL VIRUS-SPECIFIC IGA CONCENTRATIONS IN NASAL SECRETIONS AS COMPARED WITH CON. PRENATAL RPC SUPPLEMENTATION DURING LATE GESTATION AFFECTED IGG ABSORPTION, NEONATAL CALF METABOLISM, AND VACCINE RESPONSE WITH SOME EFFECTS DEPENDENT ON THE DAM’S PREPARTUM PARAMETERS.

KEY WORDS: METHYL DONOR, NUTRITIONAL PROGRAMMING, EPIGENETICS

INTRODUCTION

Fetal programming refers to the concept that the factors affecting the health and performance of the pregnant dam can have long-term effects on the offspring. Indeed, Barker’s groundbreaking studies established a causal relationship between prenatal nutrition and the origins of metabolic disease in adult humans (Barker, 2007). Since then, this theory has been applied to numerous other diseases and developmental disorders in humans (Fleming et al., 2015), and has more recently been applied to dairy cattle (Carvalho et al., 2020; Laporta et al., 2020; Swartz et al., 2021).

Although numerous factors affect the neonate’s development, there is a greater appreciation now for the ability of fetal programming to influence the physiology of the dairy calf (Abuelo, 2020). For instance, calves born from dams with elevated nonesterified fatty acid (NEFA) concentrations during late gestation had lesser BW from birth through the first 4 wk of life (Ling et al., 2018). Similarly, calves born from dams with greater oxidative stress markers during late gesta-
tion also had greater serum concentrations of reactive oxygen and nitrogen species themselves during the preweaning period (Ling et al., 2018). Consequently, calves born from dams with elevated oxidative stress markers had greater plasma concentrations of the proinflammatory cytokine, tumor necrosis factor-α, when whole blood was stimulated with LPS, as compared with calves born to dams with low oxidant levels (Ling et al., 2018). In addition to that, adverse events during late gestation, such as heat stress, reduce passive transfer of colostral IgG, as well as impair the development of the neonate’s adaptive immune system (see review article by Dahl et al., 2020). Given that the preweaning period has the highest morbidity (~34%) and mortality rates (~5%) of the dairy cow’s life (Urie et al., 2018), which is largely attributed to their naive immune system (Barrington and Parish, 2001), investigations into strategies to support the health of the neonate via fetal programming are needed.

Although numerous studies have been conducted in nonruminant animals examining nutritional programming of the neonate, research conducted in dairy calves has only just begun. For instance, supplementing methionine during late gestation enhanced neonatal birth weights (Alharthi et al., 2018) and growth during the first 9 wk of the calf’s life (Alharthi et al., 2018, 2019). Similar effects on neonatal ADG have been found when supplementing lysine during late gestation (Wang et al., 2021; Thomas et al., 2022). As such, additional investigation into the effect of prenatal nutrition on the neonate’s performance and health is needed.

Choline is a trimethylated molecule that is recommended as a supplement in the diet of pregnant women because the requirements for choline are speculated to be greater during pregnancy (Caudill, 2010). Health benefits have been identified from prenatal choline supplementation in human and rodent neonates (see review article by Jiang et al., 2014), and increasing prenatal choline supplementation improved the health and survivability of neonatal rats during a Salmonella typhimurium challenge (Newberne et al., 1970). The mechanism behind these effects are not entirely clear; however, it should be noted that choline is a methyl donor and, as a result, can influence DNA methylation, and consequently gene transcription (Caudill, 2010). Many studies have assessed the effects of dietary choline supplementation in the rumen-protected (RPC) form to periparturient dairy cattle, which is known to enhance milk production (meta-analysis, Arshad et al., 2020). Additionally, choline plays a role in regulation of lipid metabolism and hepatic export of very low-density lipoproteins. Indeed, dietary RPC supplementation has been shown to alleviate fatty liver disease (Cooke et al., 2007; Zenobi et al., 2018b), as well as reduce plasma NEFA concentrations (Pinotti et al., 2003; Sun et al., 2016), although these effects are not consistent across studies (Bollatti et al., 2020). Although some of the RPC effects on periparturient dairy cattle are described, little is known about the effect of prenatal dietary RPC supplementation on the calf. Therefore, our objective was to investigate the effects of prenatal supplementation and dose of RPC on neonatal calf growth, metabolism, and vaccine response. We hypothesized that prenatal RPC supplementation would reduce oxidative stress, alter metabolism leading to enhancements in growth, and improve vaccine response in the neonate.

MATERIALS AND METHODS

Experimental procedures were conducted at the Michigan State University Dairy Cattle Teaching and Research Center in East Lansing, Michigan, in accordance with a protocol approved by the Michigan State University Institutional Animal Care and Use Committee (PROTO202000184) from January through August 2021.

Experimental Design and Treatments

Experimental procedures related to study enrollment, treatment application, and the ingredients and composition of the close-up dry cow diet have been reported elsewhere (Swartz et al., 2022). Briefly, close-up dry Holstein parous cows (n = 67) were blocked by expected calving month and randomly assigned within block to receive 1 of 3 treatments. Dietary treatments were top-dressing of 45 g/d of RPC [20.4 g/d of choline ions (CHOL45), n = 23], 30 g/d of RPC [13.6 g/d of choline ions (CHOL30), n = 22], or no RPC (CON, n = 22), starting approximately 24 ± 3 d before expected calving until 21 d postpartum. Using BW as our primary outcome, a power analysis was conducted using α = 0.05, β = 0.80, standard deviation of 3.89 kg, and expected BW differences of 3.75 kg (CHOL45 versus CON) and 2.5 kg (CHOL30 versus CON), 21 calves should be allotted per treatment group. Cows with nonfunctional mammary quarters were excluded from study enrollment. The RPC supplement (Balchem Corporation) used in this study is not currently commercially available. This RPC supplement contained a choline chloride core and a lipid coating with a ruminal protection level of 74.9%, determined using an in situ procedure over a 12-h time frame. Research staff mixed the RPC supplement with ground corn, and the supplement was top-dressed for a total weight of 150 g/d. Control cows received 150 g/d of ground corn.
Calves born from the close-up dry cows were housed in individual calf hutches. At birth, calves were tagged, and 1 mL of injectable vitamin A and D (vitamin AD, MWI Animal Health) and 1 mL of injectable selenium and vitamin E (Bo-Se, Merck Animal Health) was administered intramuscularly in the neck. Calves were fed a total of 300 g of IgG from 2 feedings of a colostrum replacer by the farm staff. The first colostrum feeding was given within 6 h of birth, which contained 200 g of IgG (Bovine IgG Colostrum 200, Saskatoon Colostrum Company Ltd.). The second feeding occurred approximately 6 to 12 h following the first feeding, which contained 100 g of IgG (Bovine IgG Calf’s Total Bronze HiCal Colostrum, Saskatoon Colostrum Company Ltd.). If a calf did not consume the colostrum replacer, an esophageal tube feeder was used. The farm staff recorded the time of the calf’s birth along with the time of the first and second feeding of the colostrum replacer. The first feeding of colostrum replacer occurred, on average, 1.9 ± 1.4, 2.2 ± 1.4, and 1.9 ± 1.5 h (mean ± SD) after birth for CHOL45, CHOL30, and CON calves, respectively. The second feeding of colostrum replacer occurred, on average, 12.1 ± 3.0, 11.9 ± 3.2, and 12.1 ± 4.0 h (mean ± SD) after birth for CHOL45, CHOL30, and CON calves, respectively. Two calves were fed maternal colostrum instead of colostrum replacer and were consequently removed from the study. Only Holstein calves were enrolled, resulting in the exclusion of 3 Holstein-beef crossbred calves. Twins were also excluded (n = 1 calving). Lastly, 1 calf was born dead. As a result, the final sample size included 60 calves (CHOL45, n = 19; CHOL30, n = 22; CON, n = 19). Both male and female calves were enrolled (CHOL45, n = 12 heifers and 7 bulls; CHOL30, n = 14 heifers and 8 bulls; CON, n = 13 heifers and 6 bulls). All data from these calves were recorded for the first 21 d of the calf’s life. Two calves died during this period and the data recorded before death were used in the analyses.

**Feeding, Growth, and Health Measurements**

Calves were fed a 26% CP and 20% crude fat milk replacer containing lasalocid (Cow’s Match ColdFront, BOV MOS) twice daily, which was mixed with water at a rate of 150 g/L. Until 13 d of age, calves were fed 5.7 L/d (or 2.84 L twice daily). From 14 to 21 d of age, calves were fed 7.6 L/d (or 3.79 L twice daily). Milk refusals were recorded by the farm staff. Calves were fed ad libitum grain that contained monensin by the research staff (Kalmbach Commercial Calf Starter Texturized, Kalmbach Feeds). Grain offered and daily refusals were recorded to calculate daily grain intake. A single sample of the grain was analyzed to determine the chemical composition and DM content (22% CP, 90% DM; Cumberland Valley Analytical Services). No other feedstuff was provided.

Calves were weighed by the research staff using a heavy-duty animal scale with a cage at birth, 7, 14, and 21 d of age, and weekly ADG was calculated. The BCS of the dam (1 to 5 scale) was recorded just before treatment application (−24 d) and tested as a covariate in the statistical analyses. Means (± SD) for the dam’s covariates can be found in Supplemental Table S1 (https://doi.org/10.6084/m9.figshare.20815537.v4; Swartz and Bradford, 2022).

Calves were health scored once daily by trained research staff using the Calf Health Scorer application (University of Wisconsin, College of Veterinary Medicine) on an iPad (Apple), which closely follows the criteria of the calf health scoring chart (McGuirk, 2008). Clinical signs of disease are scored on a 4-point scale, ranging from 0 (normal) to 3 (severely abnormal). A calf was determined to have diarrhea when feces had a loose or watery consistency (fecal score ≥2). Using a combination of 5 parameters assessing eye discharge, ear disposition, nasal discharge, coughing, and rectal temperature, respiratory disease was indicated when the sum of these variables was ≥5.

**Plasma Parameters**

Blood samples were collected from the tail vessels of the dam just before treatment application (−24 d), and data recorded from plasma analyses (detailed below) were tested as a covariate in the statistical analyses. For calves, blood samples were collected from the jugular vessel using a 20-mL syringe and an 18-gauge, 3.8-cm needle on d 1 (~24 to 48 h after birth), 7, 14, and 21. Approximately 10 mL of blood was dispensed into K$_2$-EDTA tubes. Blood samples were chilled using ice packs, centrifuged at 2,000 × g for 15 min at 4°C to separate plasma, and then the plasma was aliquoted into 2 mL Eppendorf tubes and stored at −80°C.

**Colostral IgG Absorption**

Total IgG (g/L) was measured in plasma collected on d 1 (~24 to 48 h after birth) using a bovine-specific commercial ELISA kit (Bethyl Laboratories Inc.) according to the manufacturer’s instructions. Apparent efficiency of absorption (AEA) was calculated using the total plasma IgG concentration (g/L), the estimated plasma volume (0.091 × birth weight, kg) in liters, and the IgG intake in grams (300 g for every calf) as
follows: AEA = [plasma (IgG) × plasma volume]/IgG intake (Quigley et al., 1998).

**Parameters Related to Oxidant Status, Metabolism, and Inflammation**

Plasma parameters related to oxidant status, metabolism, and inflammation were measured in both dam (−24 d samples to be tested as a covariate) and all calf samples (d 1, 7, 14, and 21). Assays used to determine oxidant status [reactive oxygen and nitrogen species (RONS) and antioxidant potential (AOP)] were conducted within 2 mo of sample collection. The concentrations of RONS were measured using an in vitro ROS/RNS assay kit (Cell Biolabs Inc.). Free radicals convert a nonfluorescent dichlorodihydrofluorescein probe to the fluorescent 2’,7’-dichlorodihydrofluorescein, and the RONS concentration in the samples is quantified against a hydrogen peroxide standard curve. Background fluorescence was removed by subtracting blank values from sample values. Assay validation was conducted through the assessment of linearity of dilution. Fluorescence quenching was detected in undiluted plasma, whereas diluted plasma displayed linearity. As such, all samples for this assay were diluted 1:10 in PBS. Antioxidant potential was standardized to the reduction capacity of Trolox, a synthetic vitamin E analog, using 2,2’-azinobis-3-ethylbenzothiazoline-6-sulfonic acid as a radical cation, as previously described (Re et al., 1999). The oxidative stress index (OSi) was calculated by dividing RONS by AOP. Finally, glucose (Fujifilm Wako Chemicals), NEFA (NEFA-HR; Fujifilm Wako Chemicals), and BHB (Pointe Scientific) concentrations were determined using enzymatic colorimetric procedures, and haptoglobin was quantified using a bovine-specific commercial ELISA kit (Life Diagnostics).

**Vaccine Response**

On the day of birth, calves were vaccinated with a commercially available intransal vaccine (Inforce 3, Zoetis). This commercial vaccine provides protection against bovine respiratory syncytial virus (BRSV), infectious bovine rhinotracheitis, and bovine parainfluenza 3. The vaccine was prepared according to the manufacturer’s instructions. The freeze-dried vaccine was reconstituted using the sterile diluent provided with a 3-mL syringe. After reconstitution, 1 mL of the vaccine was administered into each nostril using the 3-mL syringe with a cannula. Nasal secretions were collected on d 0 (just before vaccination), 7, 10, 14, and 21, relative to birth. Single-use sponges (L800-D Identi-Plugs, Jaece Industries) were trimmed using scissors to an appropriate size to allow easy insertion into the nostril. A string was tied to the soft sponge, which was used for the removal of the sponge from the nostril following sample collection. The sponge was inserted into a nostril for approximately 10 min or until the sponge was saturated. Afterward, the sponge was removed and placed into a syringe with the plunger removed. The plunger was then reinserted and used to expel the nasal secretions into Eppendorf tubes. Nasal secretions (~1 mL) were stored at −80°C until analysis.

An indirect ELISA was used to quantify BRSV-specific IgA in nasal secretions, as previously described (Díaz et al., 2021). Briefly, high-binding 96-well ELISA plates were coated overnight at 4°C with 100 μL/well of BRSV stock (~10^3 50% tissue culture infectious dose). Negative control wells were coated with 100 μL/well cell culture media prepared fromuninfected bovine turbinate cells. Plates were then washed 4 times (0.05% Tween 20 in PBS) and blocked with 1% nonfat dry milk for 1 h. Nasal secretions were diluted 1:2 with Dulbecco’s PBS and treated with 10 mM dithiothreitol for 1 h at room temperature before performing the ELISA to disrupt the viscosity of the mucus. For assay quality control purposes, we also included a pooled sample of baseline nasal secretions on every plate. All samples were plated in duplicate (100 μL/well), incubated for 2 h at room temperature, and then washed 4 times. Sheep anti-bovine IgA-HRP (0.5 μg/mL, Bethyl Laboratories) was incubated for 30 min (100 μL/well), and then the plate was washed 4 times. Plates were developed using 100 μL/well of TMB Substrate solution (Thermo Fisher Scientific) and the reaction was stopped approximately 15 min later using 100 μL/well of 0.2 M H2SO4. Optical density was determined at 450 nm using a plate reader (Synergy HTX; BioTek Instruments Inc.) and Gen5 software (BioTek Instruments Inc.).

**Statistical Analyses**

Linear mixed models were conducted using PROC GLIMMIX (SAS 9.4, SAS Institute Inc.). The model included the fixed effects of treatment, calf sex (heifer versus bull), time (when applicable), and all 2- and 3-way interactions, with the random effects of block and calf. The dam’s prepartum BCS (−24 d) was tested as a covariate (linear and quadratic terms) along with its interaction with treatment in every outcome, aside from health events. Similarly, the dam’s prepartum plasma parameters (RONS, AOP, OSI, glucose, NEFA, BHB, and haptoglobin) from blood samples collected just before applying treatment (−24 d) were tested as a covariate (linear and quadratic terms) in their respective models (i.e., dam BHB was tested as a predictor for calf BHB, dam NEFA for calf NEFA, and so on), as well as the interaction with treatment. For calf
BW, ADG, milk intake, grain intake, IgG concentration, AEA of IgG, and BRSV-specific IgA, covariates included the linear and quadratic terms of the dam’s prepartum −24 d OSi, NEFA, and BCS, along with their interactions with treatment. Backward elimination was used to remove nonsignificant terms until all variables in the model had a $P \leq 0.05$ or were part of a significant interaction term, except for treatment and time, which were forced into the model regardless of significance. Treatment least squares means were separated using the PDIFF or SLICEDIFF statements with a Tukey adjustment. The autoregressive error structure was used for BW, ADG, and milk intake; moreover, compound symmetry was used for grain intake as the model would not converge when using the autoregressive structure. Because blood parameters and nasal secretion sample time points were not evenly spaced, the spatial power covariance structure was used instead. In all models, residuals were evaluated for normality and outliers (PROC UNIVARIATE). Outliers were removed if the studentized residual was greater than the absolute value of 4. If an outcome variable was not normally distributed, the natural logarithmic transformation was used. Finally, Fisher’s exact test was used to test treatment effects on the incidence of health events. Significance was declared at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$.

RESULTS

Prenatal RPC Effects

Health. Calves were generally in good health, and recorded disease events were typically short-lived and mild. For instance, 37 out of 60 calves were diagnosed with neonatal calf diarrhea (at least 1 d with a fecal score ≥2); however, only 7 of these calves had watery diarrhea (at least 1 d with a fecal score = 3). Similarly, only 1 calf (CHOL45) was diagnosed with bovine respiratory disease. Health event risk did not differ between treatment groups (neonatal calf diarrhea, $P = 0.31$). As mentioned previously, 2 calves died on the trial; 1 calf died due to diarrhea (CON) and the other calf died due to an intestinal rupture (CHOL45).

Colostral Antibody Absorption. Plasma concentrations of IgG were affected by treatment and this effect was dependent on calf sex (interaction, $P = 0.03$; Figure 1A), as well as the dam’s prepartum BCS (interaction, $P < 0.01$; Figure 1B). The average IgG concentration from bull calves born from CHOL45 dams was 22 g/L less than bull calves born from CON dams ($P = 0.01$). Heifer calves from CHOL30 dams tended to have greater IgG concentrations than heifer calves from CON dams ($P = 0.09$). When the dam’s prepartum (−24 d) BCS was either 2.75 or 3, calves born from CHOL45 dams had lesser IgG concentrations than calves born from CHOL30 (both $P < 0.001$) and CON (both $P < 0.01$) dams. When the dam’s BCS was 3.25, calves born from CHOL45 dams had lesser or tended to have lesser IgG concentrations than calves born from CHOL30 ($P = 0.04$) and CON ($P = 0.06$) dams. Plasma IgG concentrations by treatment group (treatment LSM ± SE) were 13.9 ± 4.5, 25.5 ± 4.2, and 25.1 ± 4.6 g/L for calves born from CHOL45, CHOL30, and CON dams, respectively. Because calf birth weights were not different between treatment groups, similar results were found for AEA. Again, treatment interacted with calf sex (interaction, $P = 0.02$; Figure 1C) and the dam’s prepartum BCS (interaction, $P < 0.01$; Figure 1D). Bull calves born from CHOL45 dams had poorer AEA than bull calves born from CON dams ($P = 0.01$). Heifer calves from CHOL30 dams had greater AEA than heifer calves from CON dams ($P = 0.05$). When the dam’s prepartum BCS was ≤3.25, calves born from CHOL45 dams had poorer AEA than calves born from CHOL30 (all $P \leq 0.02$) and CON (all $P \leq 0.04$) dams. Apparent efficiency of IgG absorption by treatment group (treatment LSM ± SE) was 16.8 ± 5.6, 32.4 ± 5.2, and 30.9 ± 5.6% for calves born from CHOL45, CHOL30, and CON dams, respectively.

Growth. Treatment affected ADG ($P = 0.03$; Figure 2A), as calves born from CHOL45 dams had a greater ADG than calves born from CHOL30 dams ($P = 0.03$); however, no difference was seen between either RPC group as compared with CON (both $P \geq 0.12$). Furthermore, treatment did not influence BW through the first 3 wk of the calf’s life ($P = 0.55$; Figure 2B). In agreement, treatment also did not affect feed intake (grain intake, $P = 0.29$; milk intake, $P = 0.68$; Supplemental Figure S1; https://doi.org/10.6084/m9.figshare.20815537.v4; Swartz and Bradford, 2022).

Oxidant Status and Inflammatory Markers. We found no differences between treatment groups for the concentrations of RONS ($P = 0.08$; Figure 3A) and AOP ($P = 0.33$; Figure 3B); however, when taken together (RONS/AOP), treatment did significantly affect OSi ($P < 0.01$; Figure 3C). Calves born from CHOL30 dams had a lesser OSi than calves born from CON dams ($P < 0.01$). Oxidative stress index did not differ between calves from CHOL45 dams as compared with CON dams ($P = 0.67$). For haptoglobin, treatment interacted with calf sex ($P = 0.05$; Figure 3E). Heifer calves born from CHOL45 dams had a lesser haptoglobin concentration than heifer calves born from CON dams ($P < 0.01$). Haptoglobin concentrations by treatment group (treatment LSM ± SE) were 7.56 ±
Metabolism. For NEFA (Figure 4A and 4B), the dam’s prepartum NEFA concentration interacted with treatment ($P = 0.02$). When dam NEFA was minimal (32 μmol/L, 10th percentile), calves from CHOL45 and CHOL30 dams had greater ($P = 0.04$) or tended ($P = 0.10$) to have greater NEFA, respectively. Conversely, when dam NEFA was greater (133 μmol/L, 90th percentile), calves from CHOL45 and CHOL30 dams had lesser ($P = 0.03$) or tended ($P = 0.10$) to have lesser NEFA than calves from CON dams, respectively. Treatment did not affect calf BHB ($P = 0.27$; Figure 4C) or glucose ($P = 0.30$; Figure 4D) concentrations.

Vaccine Response. We found a significant interaction of treatment with the dam’s prepartum (−24 d) OSi (ln) for the concentration of BRSV-specific IgA in nasal secretions (Figure 5). When the dam’s OSi was minimal [−3.63 units (ln), 10th percentile], calves born from CHOL30 and CHOL45 dams tended to have greater concentrations of BRSV-specific IgA compared with calves born from CON dams (both $P = 0.06$). However, when calves were born from dams with a greater OSi [−0.59 to 0.1 units (ln), 70th to 90th percentile], calves born from CHOL45 dams had lesser concentrations of BRSV-specific IgA than CON calves ($P = 0.04$, $P < 0.01$, and $P < 0.001$, at the 70th, 80th, and 90th percentiles, respectively). Similarly, when calves were born from dams with a greater OSi [−0.59 to 0.1 units (ln), 70th to 90th percentile], calves born from CHOL45 dams had lesser concentrations of BRSV-specific IgA than CON calves ($P = 0.04$, $P < 0.01$, and $P < 0.001$, at the 70th, 80th, and 90th percentiles, respectively).
to 0.1 units (ln), 80th and 90th percentile], calves born from CHOL30 dams had lesser concentrations of BRSV-specific IgA than CON calves ($P = 0.05$ and $P = 0.02$ at the 80th and 90th percentiles, respectively). We also found a significant treatment by sex interaction ($P = 0.03$) for the concentration of BRSV-specific IgA in nasal secretions; however, none of the pairwise comparisons were significantly different.

**Maternal Associations with Calf Parameters.** Aside from prenatal choline effects, we detected numerous significant associations between the dam’s (−24 d) prepartum parameters and the calf’s performance. The dam’s prepartum BCS had a quadratic relationship with calf’s IgG concentration ($P = 0.03$; Figure 6A) and AEA ($P = 0.04$; Figure 6B). The dam’s prepartum RONS was positively associated with the calf’s RONS ($P < 0.01$; Figure 6C). Similarly, we found a quadratic relationship between the dam’s prepartum AOP and the calf’s AOP ($P < 0.01$; Figure 6D). Additionally, we found a quadratic relationship between the dam’s prepartum glucose concentration and the calf’s glucose concentration ($P = 0.05$; Figure 6G). Lastly, the dam’s prepartum NEFA concentration was negatively associated with the concentration of BRSV-specific IgA in nasal secretions ($P = 0.02$; Figure 6H).

**DISCUSSION**

Our study assessed prenatal supplementation and dose of RPC on neonatal calf growth, metabolism, and vaccine response. In general, prenatal RPC supplementation reduced markers of oxidative stress, altered metabolism, and affected antibody responses to an intranasal vaccine in the neonate, with some of these effects dependent on choline dose. Moreover, we observed numerous significant associations between maternal parameters (BCS, oxidant status, metabolism, and so on) with neonatal outcomes. In support of that, many of the prenatal RPC effects found were dependent on the dam’s prepartum status before treatment application. As such, our data suggest that neonatal physiology, particularly metabolism, is associated with the dam’s status during late gestation, and, to some extent, the neonate’s physiology can be modified by prenatal choline supplementation.

**Colostral IgG Absorption**

Successful passive transfer of IgG is associated with decreased risk for disease (Raboisson et al., 2016), and is particularly important in dairy calves, as Ig transfer does not occur during gestation. In the present study, all calves were fed 300 g of IgG from a colostrum replacer. Because prenatal choline supplementation can alter choline metabolites (Swartz et al., 2022), as well as IgG concentrations in colostrum (Zenobi et al., 2018a), we decided to feed a colostrum replacer to assess the in utero RPC supplementation effect in the absence of a colostrum effect. Bull calves born from CHOL45 dams had lesser plasma IgG concentrations and poorer AEA than bull calves from CON dams. Similarly, calves born from CHOL45 dams with a BCS ≤3.25 had lesser IgG concentrations and poorer AEA than calves born from CHOL30 or CON dams with a similar BCS. Conversely, heifer calves from CHOL30 dams had greater AEA than heifer calves from CON dams. As such, our data likely demonstrate a quadratic dose response, in addi-
tion to some sex-dependent responses, where moderate levels of prenatal choline supplementation may be beneficial on IgG absorption; however, too much choline may be detrimental. Although not necessarily related to colostral IgG absorption, a quadratic dose response to prenatal choline supplementation has been suggested by others using rodent models for a variety of human diseases (see review article by Jiang et al., 2014). To provide some context, RPC is commonly supplemented at a rate of 12.9 g/d of choline ions, both in a research setting (Arshad et al., 2020) as well as in the dairy industry to periparturient dairy cows. This dose is similar
to the CHOL30 dose (13.6 g/d of choline ions), but less than the CHOL45 dose (20.4 g/d of choline ions) used in the present study. As such, the justification for feeding a greater prenatal choline dose than what is typically used, such as the CHOL45 dose used in the present study, on neonatal calves in the dairy industry is limited at this time.

Growth, Oxidative Stress, Inflammation, and Metabolism

Prenatal choline supplementation did not affect calf BW during the first 3 wk of the calf’s life, and no differences were observed for ADG between the prenatal RPC treatment groups and CON. Nevertheless, we found some indication that prenatal RPC supplementation may alter neonatal oxidant status, inflammation, and metabolism. Specifically, calves born from CHOL30 dams had a lesser OSI than calves born from CON dams. Similarly, haptoglobin, a marker for inflammation and oxidative stress (Ceciliani et al., 2012), was lesser in heifers from CHOL45 dams as compared with heifers from CON dams. Oxidative stress is the imbalance of free radicals with antioxidant defenses and can occur due to rapid growth of tissues or disease challenges. With that said, we saw no differences in either growth or health events for the first 3 wk of life in the present study to support these findings. Dietary RPC supplementation has antioxidant effects in periparturient dairy cattle, which is largely attributed to enhanced antioxidant defenses and greater concentrations of α-tocopherol (Pinotti et al., 2003; Sun et al., 2016); however, we saw only numerical differences in AOP in calves when RPC was supplemented prenatally. Possibly, another mechanism behind the improved oxidant status could be related to mitochondrial function. Indeed, rodent models have demonstrated that choline deficiency results in greater leakage of free radicals from mitochondria (Zeisel, 2012), and methyl donor deficiency reduced ATP production (James et al., 1992). As such, prenatal RPC supplementation may have altered neonatal mitochondrial function, which led to an improvement in oxidant status, although this effect was not substantial enough to enhance calf growth. More...

Figure 4. Effects of prenatal dietary choline supplementation at 45 g/d [20.4 g/d of choline ions (CHOL45), n = 19], 30 g/d [13.6 g/d of choline ions (CHOL30), n = 22], or no supplementation (CON, n = 19) on nonesterified fatty acids (NEFA, μmol/L; A, B), BHB (ln, mmol/L; C), and glucose (mmol/L; D). For NEFA, the effect of treatment interacted with the dam’s prepartum (−24 d) NEFA concentration (B). When dam NEFA was minimal (10th percentile), calves from CHOL45 and CHOL30 dams had greater \( P = 0.04 \) or tended \( P = 0.10 \) to have greater NEFA than calves from CON dams, respectively. Conversely, when dam NEFA was greater (90th percentile), calves from CHOL30 and CHOL45 dams had lesser \( P = 0.03 \) or tended \( P = 0.10 \) to have lesser NEFA than calves from CON dams, respectively. Blood samples were collected on d 1, 7, 14, and 21, relative to birth. Values are LSM ± SE. Trmt = treatment.
over, we cannot rule out the effect of prenatal RPC supplementation on neonatal health, as larger studies are needed for this outcome.

We observed an interaction between the dam’s prepartum NEFA concentration and prenatal RPC supplementation on neonatal NEFA concentration. In general, prenatal RPC supplementation increased or tended to increase neonatal NEFA concentrations when dam NEFA was minimal; however, the opposite was found when dam NEFA was greater. This divergent response largely suggests that prenatal RPC supplementation supports moderation in lipid mobilization in the neonate, such that the extreme NEFA concentrations are less likely to occur. Interestingly, it was particularly evident in the CON calves that the dam’s NEFA concentration was positively associated with the calf’s NEFA concentration. As such, the dam’s metabolism may be associated with the neonate’s metabolism, and this effect may be superseded by prenatal RPC supplementation. We are unaware of any other studies assessing prenatal choline to support these findings; however, it is noteworthy that dietary RPC supplementation is broadly known to regulate lipid metabolism (Cooke et al., 2007; Zenobi et al., 2018b) and in some studies, has reduced NEFA concentrations in periparturient dairy cattle (Pinotti et al., 2003; Sun et al., 2016).

**Vaccine Response**

All calves received a commercially available intranasal vaccine at birth to protect against a variety of respiratory pathogens, one of which is BRSV. Because vaccine response is poor in young calves (Chase et al., 2008) and oxidative stress during gestation can affect neonatal immune function (Ling et al., 2018), we sought to determine whether prenatal RPC supplementation could enhance vaccine response. We found a significant interaction of treatment with the dam’s prepartum OSi for the concentration of BRSV-specific IgA in nasal secretions. Calves born from either CHOL45 or CHOL30 dams had lesser concentrations of BRSV-specific IgA than CON calves ($P = 0.04$, $P < 0.01$, and $P < 0.001$, at the 70th, 80th, and 90th percentiles, respectively). Similarly, when calves were born from dams with a greater OSi (80th and 90th percentile), calves born from CHOL30 dams had lesser concentrations of BRSV-specific IgA than CON calves ($P = 0.05$ and $P = 0.02$ at the 80th and 90th percentiles, respectively). Calves were vaccinated with an intranasal vaccine (Inforce 3, Zoetis) at birth and nasal secretions were collected on d 0 (just before vaccination), 7, 10, 14, and 21, relative to birth. Values are LSM ± SE. Trmt = treatment.

**Figure 5.** Effects of prenatal dietary choline supplementation at 45 g/d [20.4 g/d of choline ions (CHOL45), n = 19], 30 g/d [13.6 g/d of choline ions (CHOL30), n = 22], or no supplementation (CON, n = 19) on bovine respiratory syncytial virus (BRSV)-specific IgA [optical density (OD); A, B]. The effect of treatment interacted with the dam’s prepartum (−24 d) oxidative stress index (OSi; B). For calves born from dams with a greater OSi (70th to 90th percentile), calves from CHOL45 dams had lesser concentrations of BRSV-specific IgA than CON calves ($P = 0.04$, $P < 0.01$, and $P < 0.001$, at the 70th, 80th, and 90th percentiles, respectively). Similarly, when calves were born from dams with a greater OSi (80th and 90th percentile), calves born from CHOL30 dams had lesser concentrations of BRSV-specific IgA than CON calves ($P = 0.05$ and $P = 0.02$ at the 80th and 90th percentiles, respectively). Calves were vaccinated with an intranasal vaccine (Inforce 3, Zoetis) at birth and nasal secretions were collected on d 0 (just before vaccination), 7, 10, 14, and 21, relative to birth. Values are LSM ± SE. Trmt = treatment.
Taken together, it can be speculated that these data suggest that choline promotes a T-helper 1 response at the expense of an antibody response. With that said, we acknowledge that there are limitations in measuring IgA produced in response to a vaccine rather than a novel exposure such as ovalbumin. Specifically, the selection of a vaccine does not allow us to discriminate between IgA produced in response to the vaccine versus IgA produced in response to a natural exposure of BRSV. As such, interpretation of these data should be done with caution and future studies are needed to assess the effects of prenatal RPC on vaccine efficacy during a BRSV challenge.

**Maternal Associations with Calf Parameters**

Aside from prenatal RPC effects, we found numerous significant associations between the dam’s prepartum parameters and the calf’s performance, and this was particularly evident for metabolic outcomes. Specifically, we detected a relationship between the dam’s prepartum AOP, RONS, NEFA, and glucose concentrations with the neonate’s respective metabolic measures. Similarly, greater prepartum BCS was associated with reduced IgG absorption, lesser plasma haptoglobin concentrations, and lesser BHB concentrations, a marker for rumen development, in the neonate. Lastly, greater prepartum NEFA concentrations were associated with lesser BRSV-specific IgA concentrations in nasal secretions following vaccination. Although there are limited data in this area of bovine research, our findings related to oxidative stress agree with a past study in dairy calves (Ling et al., 2018). Moreover, metabolic programming is a well-accepted concept in human research (Barker, 2007; Thompson and Al-Hasan, 2012; Castro-Rodríguez et al., 2020). Taken together, the dam’s prepartum status was associated with numerous neonatal outcomes, and this may have consequences on IgG absorption, rumen development, and vaccine response in the neonate. Nevertheless, much of the dairy cattle research in this area is still in the infancy stage and further investigation is merited.

**CONCLUSIONS**

Prenatal RPC supplementation reduced markers of oxidative stress and altered lipid mobilization in calves, although no effect was found on BW during...
the first 3 wk of the calf’s life. Furthermore, caution is warranted when feeding a greater dose of prenatal RPC (20.4 g/d of choline ions) as it may reduce colostral IgG absorption in neonatal calves. Similarly, prenatal RPC supplementation reduced the antibody response (BRSV-specific IgA) toward an intranasal vaccine if calves were born from dams with a greater OSi, although the implications of this are unknown.

Moreover, we found several significant associations between maternal prepartum parameters, such as BCS and metabolic parameters, with neonatal metabolism. Accordingly, many of the prenatal RPC effects found were dependent on the dam’s prepartum status before treatment application. As such, our data suggest that neonatal physiology, especially metabolism, is associated with the dam’s status during late gestation, and, to some extent, the neonate’s physiology can be modified by prenatal choline supplementation.

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