ABSTRACT

The objectives were to investigate whether supplementation with rumen-protected choline (RPC) during late pregnancy in Holstein cows affects offspring immunity and growth, and whether effects are uteroplacental, colostrum dependent, or both. A total of 105 multiparous Holstein cows were assigned randomly to a prepartum diet (1.54 Mcal of NEL/kg of DM, and 15.8% CP) without (control) or with added RPC (12.9 g/d of choline ion). Calves (n = 111) were blocked by sex and assigned randomly to colostrum from control cows or colostrum from RPC cows, resulting in 4 treatments in a 2 × 2 factorial arrangement: (1) calves born and fed colostrum from non-supplemented dams (NN; n = 33); (2) calves from non-supplemented dams and fed colostrum from RPC-fed cows (NC; n = 25); (3) calves from RPC-supplemented dams and colostrum from non-supplemented dams (CN; n = 28); and (4) calves from RPC-supplemented dams and colostrum from RPC-fed cows (CC; n = 25). Growth, intakes, and immunity of females were evaluated up to 56 d of age. Growth and intake of male calves was evaluated up to 35 d of age, and physiological and immune responses to intravenous LPS challenge were evaluated from 21 to 35 d of age. Effects of prenatal and colostrum treatments and interactions between treatments were analyzed using mixed models. Calves fed colostrum from RPC-supplemented dams had a 17.4% increase in apparent efficiency of absorption of IgG compared with calves fed colostrum from control dams (27.4 vs. 23.3%). Incidence of fever in the first 21 d of age tended to be less in females born from RPC-supplemented dams compared with females born from control dams (31 vs. 58%). Prenatal RPC females had increased hematocrit and concentrations of red blood cells, leukocytes, neutrophils, and lymphocytes in blood compared with prenatal females born from control dams. Compared with prenatal control females, prenatal RPC females had greater intake of milk replacer (704 vs. 748 ± 9.9 g/d) and starter (45.4 vs. 60.2 ± 5.9 g/d) during the first 21 d of age. In male calves, mean intake of DM was greater (1,074 vs. 976 ± 45 g/d) after the LPS challenge (0 to 8 d) by calves born from dams fed RPC compared with males born from control dams. Calves born from RPC-fed dams also had lower mean rectal temperature (39.0 vs. 39.2°C) and mean respiration rate (35.6 vs. 39.3 breaths/min) compared with males born from control dams. Moreover, serum concentrations of metabolites (i.e., β-hydroxybutyric acid, fatty acids, and glucose), cytokines (i.e., tumor necrosis factor-α) and acute phase proteins (i.e., serum amyloid A) were consistent with less-severe inflammatory response to LPS in males born from dams fed RPC compared with control. Source of colostrum and interaction between prenatal and colostrum treatments had minimal effects on calf responses to LPS. Overall, maternal RPC supplementation during late gestation suggests a positive effect on immunity, in that colostrum from RPC-fed dams increased efficiency of IgG absorption and maternal supplementation with RPC during late gestation, regardless of colostrum source, attenuated responses to LPS.

Key words: choline, calf, colostrum, immunity

INTRODUCTION

Preweaning dairy calves are at a high risk of morbidity and mortality, with recorded rates as high as 50% and 11%, respectively (USDA, 2007). Not surprisingly, diarrhea and respiratory diseases are the most significant causes of calfhood morbidity and mortality (Johnson et al., 2011; Uetake, 2013). Hence, it is crucial to find solutions to reduce the incidence of these problems and improve profitability of dairy farms and wellbeing of animals. Disease during the preweaning...
period has long-term negative impacts resulting in poor calf performance. For instance, respiratory disease in Norwegian dairy calves increased the risk of death up to 1 yr of age (Gulliksen et al., 2009). Heifers that had episodes of calfhood respiratory disease were less likely to complete their first lactation compared with those that were never diagnosed with a respiratory disease (Bach, 2011). Therefore, a reduction in diseases in calves likely results in long-term benefits.

Appreciation for the effects of maternal factors on calf health and growth has increased in recent years. Maternal nutrition and environmental stressors during gestation have been reported to affect growth of beef and dairy heifers (Funston et al., 2010; Monteiro et al., 2016; Burdick Sanchez et al., 2017). Maternal choline supplementation also offers potential benefits for calf growth and health. In addition to the positive effects of supplementing rumen-protected choline (RPC) to transition dairy cows on subsequent lactation performance (Zenobi et al., 2018a; Arshad et al., 2020; Bollatti et al., 2020), choline may be transferred to offspring via placenta or colostrum. Choline acquired by dietary absorption or endogenous synthesis is transferred from the maternal circulation to the fetus mediated by choline transporter-like protein 1, which is encoded by the \textit{SLC44A1} gene (Lee et al., 2009). Maternal choline supplementation has been shown to influence placental nutrient transporter abundance and nutrient metabolism in late-gestation mice, thereby influencing nutrient supply to the fetus (Kwan et al., 2017). The key role of choline on placental function is supported by an elegant study using a mouse model of placental insufficiency, where choline supplementation ameliorated the detrimental effects of placental insufficiency on fetal growth (King et al., 2017) and positively influenced the development of the offspring’s immune system (Lewis et al., 2017; Delschaft et al., 2018). Furthermore, rat pups born to dams fed choline-deficient diets had greater mortality rates after a challenge with \textit{Salmonella enterica} serovar Typhimurium than controls, even if the pups were fed choline-adequate diets after weaning (Newberne et al., 1970).

Alternatively, supplementing dairy cows with choline in late gestation may improve success of offspring via colostrum. Choline concentration is relatively high in colostrum (Waugh et al., 1947), suggesting a significant need for choline by the offspring, and greater choline intake by women during lactation increased concentration of choline in breast milk (Fischer et al., 2010; Davenport et al., 2015). Moreover, feeding RPC for the last 21 d of gestation increased concentration and total production of IgG in colostrum (Zenobi et al., 2018a). Calves with adequate passive transfer of immunity through colostrum have decreased morbidity and mortality (Smith and Little, 1922; McEwan et al., 1970). We hypothesized that calves born from dams fed RPC during late gestation and fed colostrum produced by dams fed RPC during late gestation will have improved immunity, health, and overall growth. Our objective was to evaluate effects of prenatal and colostrum effects of choline on transfer of passive immunity, immune status and response, and growth performance of the offspring.

**MATERIALS AND METHODS**

The experiment was conducted at the University of Florida Dairy Research Unit (Gainesville, FL) from November 2016 to August 2017. Mean (± standard deviation, SD) and range of daily temperatures were, respectively, 20.1 ± 5.5°C and 1.1 to 28.5°C; those of daily relative humidity were 78.8 ± 10.4% and 46 to 97%, respectively. All procedures involving cows and calves in the experiment were approved by the University of Florida’s Institutional Animal Care and Use Committee (protocol no. 201609647).

**Cows, Housing, Prepartum Diets, Feeding Management, and Chemical Analyses**

At 241 ± 2.2 d of gestation, pregnant, nonlactating Holstein cows with at least 1 previous lactation were enrolled in the experiment. Cows were moved to an open-sided freestall barn with sand-bedded stalls, equipped with fans mounted above the beds and a water sprinkler line in the feed bunk area for cooling. Each cow was trained to an individual feeding gate (Calan Broadbent feeding system, American Calan Inc.). Cows fully learned to open their assigned Calan gate within 1 d of training. Selection criteria included apparently healthy cows with no history of chronic disease before enrollment. Parous Holstein cows (n = 105) and their offspring (n = 111; 59 heifers and 52 bulls) were enrolled.

The prepartum diet was corn-silage based and contained 1.54 Mcal of NE\textsubscript{d}/kg of diet, 15.8% CP, and a DCAD of −10.4 mEq/100 g [DM basis; calculated using NRC (2001) software; Supplemental Table S1, https://original-ufdc.uflib.ufl.edu/1/IR00011924/00001]. The diet was formulated using a source of blood meal containing ruminally protected methionine and lysine (LysAAMet, Perdue AgriBusiness) so that the ratio of lysine to methionine was 2.9 according to NRC (2001). Diets were fed as a TMR once daily at 0930 h and were consumed ad libitum. The amount of TMR offered to individual cows was adjusted daily to result in at least 5% refusals. Refusals were weighed once daily before the morning feeding.
Sample Size Calculation and Experimental Design

A 2-sided sample size was calculated using the POWER procedure of SAS (SAS/STAT version 9.4, SAS Institute Inc.) based on productive responses of dams receiving or not receiving RPC during the transition period, reported in Bollatti et al. (2020). The samples size was calculated for the cow portion of the experiment reported in Bollatti et al. (2020) to provide sufficient power (\(\alpha = 0.05; \beta = 0.20\)) to detect differences in ECM yield in the first 21 DIM when the difference between the 2 treatments, 0 or 12.9 g/d of choline ion fed as RPC, was 2 kg/d and considering an SD of 3.5 kg. For that, a total of 98 experimental units were needed. We anticipated that 50% of calves born to cows in the present experiment would be female and 50% male. A sample size calculation for heifer calf performance, considering a typical daily BW gain of 0.8 kg/d with SD of 0.2 kg/d for female preweaning calves at the University of Florida Dairy, and an experiment with 4 treatments in a factorial arrangement, anticipated that 48 female calves would allow us to detect a difference in average daily gain of 0.16 kg/d (2-sided test, \(\alpha = 0.05; \beta = 0.20\)) for the main effects of in utero or colostrum choline exposure, or the interaction term in the model. For evaluation of immune competence using an LPS challenge model on male calves, considering an increase in rectal temperature of 1.6°C with SD of 0.2°C in response to an LPS challenge (Carroll et al., 2009), the sample size anticipated that 48 male calves would allow us to detect a difference in rectal temperature of 0.17°C after LPS challenge (2-sided test, \(\alpha = 0.05; \beta = 0.20\)) for the main effects of in utero or colostrum choline exposure, or the interaction term in the model.

The experiment was a randomized block design with \(2 \times 2\) factorial arrangement of treatments. Cows were blocked by previous yield of 305-d mature milk equivalent and assigned to receive 1 of 2 dam treatments: RPC fed at 0 or 12.9 g/d of choline ion per cow from 21 d before expected calving date until calving. The choline ion was fed as part of 60 g/d of ReaShure (Balchem Corp.). The RPC was mixed with ground corn and dried molasses in a 30:56:14 ratio (as-is basis) and top-dressed (200 g/d) once daily onto the TMR of individual cows. Cows assigned to 0 g/d of choline ion received 200 g/d of top-dressed ground corn and dried molasses (80:20 ratio, as-is basis). A total of 111 calves were born in the experiment, 12 of which were twins. No female-male sets of twins were used in the experiment. At birth, calves were blocked by sex and assigned randomly to colostrum collected and stored from experimental dams supplemented with or without RPC. Therefore, the treatments imposed on calves were calves born to and fed colostrum from non-supplemented dams (NN, \(n = 33\)); calves born from non-supplemented dams and fed colostrum from RPC-fed cows (NC, \(n = 25\)); calves born from RPC-supplemented dams and fed colostrum from non-supplemented cows (CN, \(n = 28\)); and calves born from RPC-supplemented dams and fed colostrum from RPC-fed cows (CC, \(n = 25\)). The numbers of male and female calves in each treatment are reported in Table 1.

Colostrum Collection and Analyses

Colostrum was harvested from individual cows at the first milking, within 6 h of calving, and poured into 1.9-L bottles. Immediately after collection, bottles were identified and transported to the laboratory where 2 representative samples were collected. The remaining colostrum was stored at \(-20^\circ\text{C}\) until needed for feeding calves. As an estimate of IgG concentration, degrees Brix were measured using a refractometer (MA871, Milwaukee Instruments Inc.). Colostrum that was \(<22\%\) Brix was not fed to calves (equivalent to 50 g/L of IgG; Bielmann et al., 2010). One sample of colostrum without preservative was kept at \(-20^\circ\text{C}\) for determination of concentration of IgG. The second sample was diluted in 1-to-1 ratio (as-is) using commercial skim milk, and both skim milk and the skim milk-diluted colostrum sample mixture were sent to Southeast Milk Dairy Laboratory (Belleview, FL) for analysis of concentrations of fat, true protein, and lactose using a Bentley 2000 infrared component analyzer (Bentley Instruments Inc.). Concentration of somatic cells was determined using a Bentley 500 Somacount flow cytometer (Bentley Instruments Inc.). Bronopol-B-14 (Advanced Instruments) was used as a milk preservative during shipping. Concentrations of nutrients in the original colostrum samples were calculated after accounting for their dilution with skim milk and the composition of skim milk.

Efficiency of Immunoglobulin Absorption

Blood was sampled from the jugular vein between 24 and 36 h after colostrum feeding. Blood was sampled into evacuated tubes without anticoagulant (Vacutainer, Becton Dickinson) and centrifuged at 1,125 \(\times g\) for 20 min at room temperature (Allegra X-15R centrifuge, Beckman Coulter Inc.). Serum was harvested and stored at \(-20^\circ\text{C}\) until analysis. Colostrum and serum samples were analyzed for total IgG concentration by single radial immunodiffusion (Triple J Farms, Bellingham, WA). Briefly, serum and colostrum samples were diluted 1:1 and 1:5, respectively, in 0.9% saline to fall within the linear range of the 3-point standard curve. The diluted samples were pipetted into the bovine anti-
bovine IgG antibody plate and incubated for 24 h on a flat surface at 24°C without exposure to light. The diameter of the precipitin ring was measured 3 times, rotating the ring to 3 different positions using a 7× scale loop (no. 1975, Peak Optics), with the average reading used to calculate IgG concentration. Intra- and interassay coefficients of variation (CV) were 1.2 and 5.4%, respectively. Colostrum IgG concentrations in grams were used to calculate intake of IgG. When colostrum from more than 1 dam was fed to a calf, the proportion of colostrum from each dam was used in a weighted sum to calculate intake of IgG.

The apparent efficiency of IgG absorption (AEA, %) was calculated according to Quigley et al. (1998) using the following equation: 

\[
\text{AEA}\% = 100\% \times \frac{[(\text{IgG concentration in serum at 24 h of life, in g/L}) \times (0.091 \times \text{BW at birth, in kg})]}{\text{IgG intake, in g}} 
\]

× 100.

Feeding and Management of Calves, and Measurements of BW and Height

Calves were born in the open-sided freestall barn. All dams were monitored constantly for initial signs of calving between 0530 and 1900 h, and then every 2 h between 1900 and 0530 h. Cows showing signs of calving were closely monitored. Within 2 h of birth, calves were weighed and ear-tagged, and the umbilical cord was disinfected with a solution of 7% iodine (Triodine-7, First Priority Inc.). Immediately after weighing, 3.8 L of good-quality (>22 Brix) colostrum was warmed to 36° to 38°C in hot water and fed to each calf using an esophageal feeding tube. Calves did not receive colostrum from their dam, and colostrum fed to each calf originated from 1 to 3 dams whenever a cow did not produce more than the 3.8 L of colostrum to be fed as designed in the experiment. Colostrum was

### Table 1. Number of calves, gestation length, colostrum composition, IgG intake, and passive immunity of calves

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>Sex</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NN</td>
<td>NC</td>
<td>CN</td>
</tr>
<tr>
<td>Females born, n</td>
<td>16</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>Females died, n</td>
<td>3</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Males born, n</td>
<td>17</td>
<td>9</td>
<td>13</td>
</tr>
<tr>
<td>Males died, n</td>
<td>7</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Total death, n</td>
<td>10</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Total death, %</td>
<td>30.3</td>
<td>16.0</td>
<td>17.9</td>
</tr>
<tr>
<td>Gestation length, d</td>
<td>272</td>
<td>274</td>
<td>272</td>
</tr>
<tr>
<td>Calf BW, kg</td>
<td>40.9</td>
<td>41.4</td>
<td>41.1</td>
</tr>
<tr>
<td>First colostrum, % min</td>
<td>94.6</td>
<td>92.9</td>
<td>94.1</td>
</tr>
<tr>
<td>Colostrum, %</td>
<td>Fat</td>
<td>4.63</td>
<td>5.21</td>
</tr>
<tr>
<td></td>
<td>True protein</td>
<td>13.3</td>
<td>13.3</td>
</tr>
<tr>
<td></td>
<td>SCS⁴</td>
<td>6.37</td>
<td>6.17</td>
</tr>
<tr>
<td></td>
<td>Lactose</td>
<td>3.25</td>
<td>3.28</td>
</tr>
<tr>
<td></td>
<td>IgG intake, g</td>
<td>394</td>
<td>406</td>
</tr>
<tr>
<td></td>
<td>24 h after birth</td>
<td>20.3</td>
<td>26.3</td>
</tr>
<tr>
<td>Serum IgG, g/L</td>
<td>21.8</td>
<td>28.9</td>
<td>24.2</td>
</tr>
<tr>
<td></td>
<td>AEA⁵, %</td>
<td>6.06</td>
<td>6.44</td>
</tr>
<tr>
<td></td>
<td>STP⁶, g/dL</td>
<td>29</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>Serum IgG, g/L</td>
<td>20.3</td>
<td>26.3</td>
</tr>
<tr>
<td></td>
<td>AEA, %</td>
<td>6.06</td>
<td>6.44</td>
</tr>
<tr>
<td></td>
<td>STP, g/dL</td>
<td>29</td>
<td>39</td>
</tr>
</tbody>
</table>

¹Calves were born to cows fed prepartum diet without (N) or with (C) rumen-protected choline. At birth calves were assigned colostrum from N or C cows in a 2 × 2 factorial arrangement, resulting in 4 treatments: calves born to and fed colostrum from N cows (NN); calves born from N cows and fed colostrum from C cows (NC); calves born from C cows and fed colostrum from N cows (CN); and calves born to and fed colostrum from C cows (CC).

²PT = prenatal treatment (NN + NC vs. CN + CC); CT = colostrum treatment (NN + CN vs. NC + CC); PT × CT = interaction between PT and CT (NN + CC vs. NC + CN).

³Minutes from birth to colostrum feeding.

⁴Somatic cell score = Log10(SCC/12.5)/Log10(2).

⁵Apparent efficiency of IgG absorption, % = {IgG concentration in serum at 24 h of life (g/L) × [0.091 × BW at birth (kg)]}/IgG intake (g) × 100.

⁶Serum total protein.

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selected randomly to be fed to calves according to the treatment assigned, and source of colostrum fed to each calf was recorded.

Calves were housed temporarily in individual pens (1 × 1 m) equipped with a heat lamp before moving to individual wire pens (1 × 1.5 m) bedded with sand within 12 h of birth. Female calves were housed in the calf unit of the University of Florida Dairy Research Unit and subjected to the care management of the farm staff and research personnel; male calves were housed in a separate barn at the University of Florida Dairy, and feeding and care were managed by research personnel. Starting 12 h after colostrum feeding, all calves were fed 0.87 kg/d of milk replacer DM (MR; SMI 28/15 BOVA/DFB Calf Milk Replacer Medicated, Strauss Feeds; Supplemental Table S1) prepared in a solution at 15% DM to result in 5.8 L, offered by bucket, split into 2 meals of 2.9 L each at 0600 and 1430 h. Milk replacer was added to 46°C water and mixed using an electric drill with a wire whisk attachment. The MR solution was cooled to 38°C ± 1°C for feeding. Each calf was monitored to ensure that the MR was consumed within 10 min of feeding. The few calves not willing to drink MR within 10 min during the first 3 d of life were fed using a nipple bottle. A pelleted grain mix (AmpliCalf, Purina Animal Nutrition LLC; Supplemental Table S1) and water were offered by bucket ad libitum starting on d 0. Orts for MR were recorded 30 min after each feeding, whereas orts of pelleted grain mix, targeted at 10% of the amount offered, were recorded once daily. The amount of MR offered to heifers was 5.8 L/d from birth to 42 d of age. Starting on d 43, females were offered a single daily feeding of MR with 2.9 L/d until 50 d of age, and heifers were weaned from MR on d 51 of age, when they only received the pelleted grain mix and data collection for DMI continued until d 56 of age. The amount of MR offered to male calves was the same as females from birth until end of the experiment. Each bag of MR was sampled upon opening. The pelleted grain mix was sampled weekly. Both sets of samples were composited monthly (n = 6) and analyzed for chemical composition (Dairyland Laboratories Inc., Arcadia, WI). Results are reported in Supplemental Table S1. Females were weighed, heart girth measured, and wither heights recorded as measures of growth on d 0, 14, 28, 42, and 56 d of age, whereas males were weighed at d 0 and at the beginning and end of the LPS challenge (approximately 21 and 35 d of age).

Calf Scoring for Health Assessment and Incidence of Health Disorders

Nasal, eye, and ear discharge, cough, respiratory score, and fecal consistency were evaluated daily after the morning feeding of MR by a single observer according to the University of Wisconsin’s Calf Health Score Chart (McGuirk, 2008).

Incidence of health disorders and death were recorded daily for individual females. Rectal temperature was measured daily after the morning feeding from 0 to 56 d of age. Females with a rectal temperature ≥39.5°C at any given measurement were categorized as febrile. A calf was considered pneumonic if it had fever with at least one clinical sign of respiratory disease (mucopurulent nasal discharge, cough, increased respiratory rate, increased respiratory effort, or abnormal lung sounds auscultated by the herd veterinarian). A navel was considered infected if it was enlarged, firm, and painful on palpation regardless of discharge. Calves were vaccinated and treated by farm personnel according to protocols established by the herd veterinarian. Briefly, at birth, calves received an intranasal modified-live vaccine against bovine rhinotracheitis and parainfluenza virus-3 (TSV-2, Zoetis). Then, at 3 and 5 wk of age, calves were vaccinated with an injectable modified-live vaccine against bovine rhinotracheitis, parainfluenza virus-3, bovine viral diarrhea virus, and bovine respiratory syncytial virus (Bovi-Shield Gold, Zoetis). Scours were treated with electrolytes (Gener-Lyte, Bio-Vet Inc.) and bismuth subsalicylate (Bismusol, First Priority Inc.). Of the 59 heifers, 7 died between 5 and 11 d of age and 1 died at 24 d of age. The mean and median days at death were 9.6 and 7.5, respectively. Analyses of intake, health events, and blood hematology included only the 51 heifers that completed the first 56 d of age. Reasons for death included congenital heart defect (1 female calf), dislocation of stifle joint that resulted in euthanasia (1 female calf), and septicemia (6 female calves).

Sample Collection and Laboratory Analyses for Female Calves

Blood was sampled from the jugular vein at 0, 7, 14, 21, 35, and 56 d of age into evacuated tubes containing lithium heparin as anticoagulant, inverted several times, and immediately placed on ice. Within 60 min of collection, chilled samples were centrifuged at 1,125 × g for 20 min at room temperature, plasma was harvested, and multiple aliquots of 1.5 mL were frozen at −20°C until analysis. Plasma samples were analyzed in duplicate, and samples from the 4 treatments were included in each assay with a plasma sample of known concentration for the analyte. A Technicon Autoanalyzer (Technicon Instruments Corp.) was used to measure plasma concentrations of glucose (Bran and Luebbe Industrial Method 339-19; Gochman and Schmitz, 1972) and urea N (Bran and Luebbe Industrial Method 339-01; Marsh
et al., 1965). Intra- and interassay CV were 2.5 and 2.7% for glucose and 1.7 and 1.8% for plasma urea N, respectively. Concentration of triacylglycerol (TAG) was analyzed using a commercial enzymatic assay (Stanbio Triglycerides LiquiColor Procedure No. 2100, Stanbio Laboratory), and the intra- and interassay CV were 3.7 and 8.9%, respectively. Plasma concentrations of total IGF-1 were determined by a commercial ELISA kit (Quantikine ELISA Human IGF-1 Immunoassay, R&D Systems Inc.) as previously reported (Ribeiro et al., 2016). Intra- and interassay CV were 2.8 and 4.5%, respectively. Plasma concentrations of haptoglobin were measured using a colorimetric procedure that measures haptoglobin-hemoglobin complexing by estimating differences in peroxidase activity (Makimura and Suzuki, 1982). The absolute absorbance values at 450 nm were multiplied by 100 and used for statistical analysis. Intra- and interassay CV were 1.9 and 9.3%, respectively. Plasma concentrations of fibrinogen were determined on d 0, 7, 14, and 21 of age using a fibrinogen determination kit (TriniCLOT Fibrinogen Kit No. T1301, Tcoag Ireland Ltd.) by estimating clotting time using a BBL Fibrometer coagulation analyzer (Rankin Biomedical Corp.). The intra- and interassay CV of the duplicate samples were 2.2 and 8.2%, respectively. Plasma concentrations of serum amyloid A (SAA) were measured on d 0, 7, 14, and 21 of age using a commercial solid-phase sandwich ELISA phase assay (TP-802, Tri-Delta Diagnostics Inc.) with samples diluted 1:500 in 1× diluent buffer to fit within the standard curve of the assay. The intra- and interassay CV were 5.9 and 3.9%, respectively. For assessment of blood hematology, whole blood was collected via venipuncture of the jugular vein from heifers into vacutainer tubes containing K2EDTA at 0, 7, 14, and 21 d of age. Immediately after collection, samples were inverted several times, placed on ice, and transported to the laboratory within 4 h of collection to be analyzed using the Idexx ProCyte Dx Analyzer (Idexx Laboratories Inc.). The blood cells of interest included red blood cells, reticulocytes, platelets, white blood cells, monocytes, lymphocytes, neutrophils, eosinophils, and basophils, as well as hematocrit and hemoglobin.

Cell-Mediated Hypersensitivity Test for Female Calves

To evaluate immune competence of female calves, cell-mediated hypersensitivity to immunization with a novel antigen was evaluated. Epidermal injections of 0 and 200 µg of phytohaemagglutinin (PHA; L1668, Sigma Aldrich Co.) prepared in sterile saline were administered intradermally to heifers at 28.8 ± 1.9 and 56.3 ± 1.6 d of age. The treated shoulder was shaved and cleaned with 70% alcohol before subcutaneous injections using insulin syringes. Skin-fold thickness was measured from both injections by the same person in duplicate at 0, 6, 24, and 48 h relative to time of injection using a digital caliper (Pittsburgh 152-mm Composite Digital Caliper, Virtuabotix LLC). These time points were selected based on a pilot trial and previous experience of our laboratory to capture the maximum responses. The precision of the caliper is reported to be of ±0.03 mm. The diameter (mm) of the skin-fold thickness was recorded to determine the delayed-type hypersensitivity response to PHA. Delayed-type hypersensitivity was calculated as the difference between the increase in diameter of the skin-fold thickness from the 0- and 200-µg injection sites measured at the same time after injection.

Lipopolysaccharide Challenge in Bull Calves

The day before the LPS challenge (mean ± SD, 21.5 ± 2.3 d of age), male calves were weighed (52.1 ± 7.7 kg), and a clinical examination was conducted to assess the health condition. If males were in healthy condition (full intake of MR, no sign of diarrhea, fever, or dehydration), a 16-gauge 7.5-cm extended-use indwelling catheter (Mila International Inc.) and 53.3-cm standard bore extension set with removable side clamp (Smiths Medical ASD Inc.) with pre-pierced resealing short male adapter plug with Luer lock (Hospira Inc.) was placed aseptically in the jugular vein. A recovery period of at least 12 h was allowed after catheterization. Lipopolysaccharide (Escherichia coli serotype O111:B4, cat. no. L3012, Sigma Aldrich) was diluted with sterile saline to a final concentration of 1 µg/mL, aliquoted into sterile single-use vials, and stored at −20°C. Diluted LPS (0.05 µg of LPS/kg of BW) was delivered intravenously (i.v.) at time 0 (0700 h) using a 3-mL syringe via catheter, and subsequently the extension line was flushed with 10 mL of heparinized saline (1 mL of heparin 10,000 IU/mL in 1,000 mL of saline) to ensure all LPS reached general circulation and ensure functionality of the catheter. Catheterization was chosen to allow for easier and accurate administration of the LPS solution into the vein and avoid stressful situations at blood sampling that may alter some blood metabolites, such as cortisol. Feeding time for MR was at 0700 and 1430 h. The morning feeding of MR on the day of the LPS challenge was skipped in all male calves, although they had ad libitum access to starter at all times.

Of the 52 males enrolled in the experiment, 10 died before the LPS challenge (2 from the CN group and 7 from the NN group), and reasons for death were similar to those in females. The first males enrolled in the LPS challenge received 0.1 µg/kg BW; however, those calves...
reacted so severely that veterinarian intervention was needed (n = 1, 1, and 2 for NN, NC, and CC, respectively) and were not included in the final data set. The remaining bulls received 0.05 µg/kg BW of LPS, and in total 38 bulls (NN, n = 9; NC, n = 8; CN, n = 10; CC, n = 11) completed the 72-h sampling period after the LPS challenge and were used for statistical analysis of the data gathered during the LPS challenge.

**Rectal Temperature, Heart Rate, and Respiration Rate**

Immediately before LPS challenge (0 h) and at 1, 2, 3, 4, 6, 8, 10, 12, 24, 30, 36, 48, 60, and 72 h relative to LPS challenge, rectal temperature (RT), heart rate (HR), and respiratory rate (RR) were assessed using a thermometer and stethoscope.

**Calf Activity**

To assess calf activity, standing time was monitored continuously on a subset of male calves (6, 6, 6, and 9 for the NN, NC, CN, and CC groups, respectively) beginning at the time of the LPS challenge, using electronic accelerometers (Hobo Pendant G data logger, Onset Computer Corp.) placed on the rear leg (as validated by Bonk et al., 2013) at least 12 h before the challenge. These data were used to calculate hourly standing time for 10 h following the challenge. Further, standing bout frequency and duration were characterized for the 24-h period following the challenge.

**Sample Collection and Laboratory Analyses for Male Calves**

To assess blood metabolites, about 10 mL of blood samples were collected via the catheter immediately before LPS challenge and at 1, 2, 3, 4, 6, 8, 10, 12, 24, 30, 36, 48, 60, and 72 h relative to LPS challenge into evacuated tubes containing lithium heparin as the anticoagulant, inverted carefully several times, and immediately placed on ice. Within 60 min of collection, chilled samples were centrifuged at 1,125 × g at 4°C for 20 min, plasma was harvested, and multiple aliquots of about 0.6 mL were frozen at −20°C until analysis. Unless otherwise stated, plasma samples were run in duplicate, and samples from the 4 treatments were included in each plate per run, along with a known control plasma sample.

**Metabolites and Minerals**

A Technicon Autoanalyzer (Technicon Instruments Corp.) was used to measure plasma concentrations of glucose (Bran and Luebbe Industrial Method 339-19; Gochman and Schmitz, 1972) and BUN (Bran and Luebbe Industrial Method 339-01; Marsh et al., 1965). Intra- and interassay CV were 3.8 and 3.3% for glucose and 3.7 and 4.6% for BUN, respectively. Concentrations of fatty acids (NEFA-C kit, Wako Diagnostics Inc.; as modified by Johnson and Peters, 1993) and BHB (Wako Autokit 3-HB; Wako Diagnostics Inc.) were determined in duplicate. Inter- and intra-assay CV were 3.2 and 8.8% and 2.7 and 9.8% for fatty acids and BHB, respectively. For plasma concentrations of TAG (Stanbio Triglycerides LiquiColor Procedure No. 2100, Stanbio Laboratory), intra- and interassay CV were 4.0 and 8.4%, respectively. Plasma total Ca and Zn were determined using an atomic absorption spectrophotometer. Inter- and intra-assay CV were 1.4 and 6.7% for total Ca and 2.4 and 8.7% for Zn, respectively.

**Cytokines and Hormones**

Plasma concentrations of haptoglobin were measured using a colorimetric procedure that measures haptoglobin-hemoglobin complexing by estimating differences in peroxidase activity (Makimura and Suzuki, 1982). The absolute absorbance values at 450 nm were multiplied by 100 and used for statistical analysis. Intra- and interassay CV were 2.1 and 12.3%, respectively. Plasma concentrations of SAA were measured using a commercial solid-phase sandwich ELISA phase SAA assay (TP-802, Tri-Delta Diagnostics Inc.) with samples diluted 1:500 in 1× diluent buffer to fit within the standard curve of the assay. The intra- and interassay CV were 7.5 and 11.7%, respectively. Cortisol was determined using automated chemiluminescent analyzer (Immulate 1000, Siemens Medical Solutions Diagnostics Inc.) at 0, 1, 2, 3, 4, 6, 8, 12, and 24 h relative to LPS challenge. Cortisol was run in single with duplication every 10 samples. The intra- and interassay CV were 2.0 and 5.2%, respectively. Plasma TNFα was measured at 0, 1, 2, 3, 4, and 6 h relative to LPS challenge using a commercially available ELISA kit (Vet Sets ELISA Development Kit, Kingfisher Biotech Inc.). The inter- and intra-assay CV for TNFα were 3.4 and 16.1%, respectively. Interleukin-6 was also measured at 0, 1, 2, 3, 4, and 6 h relative to LPS challenge using a commercially available ELISA kit (DY8190, R&D Systems). The inter- and intra-assay CV for IL-6 were 3.8 and 8.0%, respectively.

**Hematology**

For the assessment of blood hematology, whole blood was collected via venipuncture of the jugular vein from bulls into evacuated tubes containing K2EDTA at 0, 1,
Statistical Analyses

Calf was the experimental unit of analysis, considered random in mixed models with repeated measures. Data were analyzed using the MIXED procedure of SAS version 9.4 (SAS/STAT, SAS Institute Inc.). The 59 heifers and 52 bulls contributed data used to analyze gestation length, colostrum composition, IgG concentration fed to heifers and bulls, serum concentration of total protein and IgG, AEA of IgG, and death. Sex was included in the model for these analyses. All data generated after colostrum feeding were analyzed separately.

The REPEATED statement was used for dependent variables measured over time. Model included the fixed effects of dam treatment (NN + NC vs. CN + CC), colostrum treatment (NN + CN vs. NC + CC), interaction between dam and colostrum treatment (NN + CC vs. NC + CN), time of measurement, and all 2- and 3-way interactions. Calf was nested within treatment and was the error term for testing the effects of treatment. Most analyses used the first-order autoregressive structure for equally spaced measurements or spatial power for unequally spaced measurements; however, other covariance structures were tested (compound symmetry, unstructured, first-order antedependence). The covariance structure with the lowest Akaike’s information criterion was selected for each variable. When an interaction of treatment with time was detected as significant, treatment means at time points of measure were partitioned using the SLICE command of SAS. For data collected at single time points only, the MIXED procedure of SAS was used without the REPEATED statement. The Kenward-Roger method was used to calculate the approximate denominator degrees of freedom for the F-tests in the statistical models. Continuous data were tested for distribution of the residuals after fitting the statistical models using Shapiro-Wilk and homogeneity of variance by plotting residuals against predicted values. Non-normally distributed data were subjected to Box-Cox transformation using the TRANSREG procedure of SAS to achieve normality before analyses. The least squares means were back-transformed and standard error of the means calculated as outlined by Jørgensen and Pedersen (1998). Daily health scores were analyzed as categorical data using a Poisson distribution, but most (i.e., cough, ear, eye, and nasal) models did not converge because of zero-inflated data. Instead, weekly averages of health scores were calculated for each calf and analyzed as continuous data with PROC MIXED. Binary data were analyzed by logistic regression using the GLIMMIX procedure of SAS. Time to event, such as starter DMI >1.4 kg/d, was analyzed with Cox’s proportional hazard regression model using the PHREG procedure of SAS. The median days to event were calculated using PROC LIFETEST of SAS. The statistical model included the effects of dam treatment, colostrum treatment, and the interaction. Statistical significance was declared at $P \leq 0.05$, and tendency was declared at $0.05 < P \leq 0.10$.

RESULTS

Gestation Length, Birth Weight, Colostrum Quality, and Efficiency of Immunoglobulin Absorption

Treatment did not affect birth BW (mean range of 40.9–44.1 kg, Table 1). Gestation length was 2 d longer for calves fed RPC colostrum compared with control (CON) colostrum (Table 1). Gestation length also was 2 d longer for males compared with females.

Calves were fed colostrum with a mean of 95 min after birth, and the time did not differ among treatments (Table 1). The mean concentrations of fat (4.90%), protein (13.5%), lactose (3.25%), and SCS (6.44) in colostrum fed to calves did not differ across treatments (Table 1). Mean intake of IgG did not differ across treatment (408 g); however, bulls had greater intake of IgG compared with heifers ($P < 0.05$; 435 vs. 383 g, respectively). Based upon the serum concentration of total IgG measured between 24 and 30 h after colostrum feeding, 6 calves (n = 2 for NN, n = 1 for NC, and n = 3 for CC) failed to attain adequate passive transfer after colostrum feeding (serum total IgG <10 g/L). An interaction ($P = 0.07$) between prenatal and colostrum treatments was observed for serum IgG, because RPC colostrum increased serum IgG in calves from CON cows but did not affect serum IgG in calves from RPC cows (Table 1). Feeding RPC colostrum increased ($P = 0.01$) AEA compared with CON colostrum (23.0 vs. 27.4%). We also observed an interaction ($P < 0.10$) between prenatal and colostrum treatments for apparent efficiency of IgG absorption and serum concentrations of IgG because NN resulted in the lowest AEA (21.8%) and serum IgG (20.3 g/L) compared with the rest of the treatments (26.3% and 25.1 g/L, Table 1). Neither serum IgG nor AEA were affected by prenatal exposure to choline during the last 21 d of gestation (Table 1).

Growth, Feed Intake, and Health from Birth to Weaning of Female Calves

Prenatal RPC increased ($P < 0.01$) intake of MR by females during the first 21 d of life compared with
prenatal CON (748 vs. 704 g/d; Table 2, Figure 1). Collectively, prenatal RPC compared with prenatal CON increased \((P = 0.02)\) intake of MR from birth to 49 d of age (748 vs. 731 g/d, respectively; Table 2). Prenatal RPC also tended to increase \((P = 0.08)\) intake of starter DM by females during the first 21 d compared with prenatal CON (53.6 vs. 40.4 g/d). Likewise, feeding RPC colostrum increased intake of starter DM by females more rapidly \((P = 0.02)\) compared with CON colostrum (Table 2; Supplemental Figure S1, https://original-ufdc.uflib.ufl.edu/l/IR00011924/00001). The same was not true for intake of starter (Table 2) from birth to 56 d of age, as starter DMI was affected by neither prenatal RPC and colostrum source was detected for any of the growth variables measured.

Female calf eye, ear, cough, fecal, and nasal scores were <1 (Supplemental Table S2, https://original-ufdc.uflib.ufl.edu/l/IR00011924/00001). Only cough score was affected by treatment (prenatal by colostrum interaction, \(P = 0.04\)). A high proportion (82%) of females were affected by scours (fecal score ≥3). However, the proportion of females treated for scours and the mean age at first treatment for scours were not affected by treatments (80.4% and 8.7 d, respectively). Incidence of pneumonia, navel infection, or septicemia were not affected by treatments. Mean daily RT was not different among treatment groups. However, prenatal RPC tended to reduce \((P = 0.07)\) incidence of fever (RT ≥39.5°C) in females during the first 21 d of age compared with prenatal CON (31 vs. 58%, respectively; Supplemental Table S2). Beyond 21 d of age, no effect of treatment on fever incidence was detected (Supplemental Table S2).

### Blood Metabolites of Female Calves

Plasma concentrations of BUN, glucose, IGF-1, and TAG changed over time, as expected (Supplemental Table S3, https://original-ufdc.uflib.ufl.edu/l/IR00011924/00001). Glucose, IGF-1, and TAG did not differ among treatment groups (Supplemental Table S3).
S3). Plasma concentration of BUN was lower \((P = 0.05)\) in females that were fed colostrum from control cows compared with those fed colostrum from RPC cows \((6.2 \text{ vs. } 6.8 \text{ mg/dL, control vs. RPC, respectively; Supplemental Table S3})\).

Plasma concentrations of haptoglobin were greatest at 7 d of age \((time; P = 0.01)\) but did not differ among treatment groups. Likewise, plasma concentrations of SAA and fibrinogen increased from birth to 7 d of age before decreasing thereafter in all groups \((time; P < 0.01; Supplemental Table S3; Figure 2)\). However, the increase of SAA on d 7 was substantially lower in CC heifers compared with the other 3 treatments \((prenatal by colostrum by day interaction; P < 0.01; Supplemental Table S3; Figure 2)\). Overall, CC females had lower concentrations of SAA than the other 3 treatments \((prenatal RPC by colostrum interaction, P = 0.05, Supplemental Table S3; Figure 2)\). Furthermore, CC females tended to have a lower mean concentration of fibrinogen compared with CN females, but fibrinogen levels of NN and NC females were not different \((prenatal RPC by colostrum interaction, P = 0.09; Supplemental Table S3; Figure 2)\).

Hematology of Female Calves

Prenatal RPC in females resulted in greater levels of hematocrit \((P < 0.02)\) and numbers of erythrocytes \((P < 0.01)\), neutrophils \((P = 0.07)\), leukocytes \((P = 0.10)\), and lymphocytes \((P = 0.10)\) compared with prenatal CON \((Figure 3)\). Likewise, prenatal RPC tended to reduce counts of reticulocytes \((P = 0.06)\) but resulted in greater percentages of eosinophils and basophils \((P = 0.09)\) compared with prenatal CON \((Figure 3)\). In contrast, platelet counts were not affected by treatments \((P > 0.10)\). Percentages of neutrophils, lymphocytes, or monocytes \(\text{expressed as a percentage of leukocytes}\) were not affected by treatments \((P > 0.10)\; \text{Supplemental Table S4, https://original-ufdc.uflib.ufl.edu/l/IR00011924/00001; Figure 3}\).

Cell-Mediated Hypersensitivity to Epidermal Injection of PHA

At 28.8 and 56.3 d of age, mean change in skin-fold thickness due to injection of PHA differed across hours following the challenge, with the response becoming
less with increasing time (hours) following exposure to PHA (time effect; \( P < 0.01 \); Supplemental Figure S3A and B, respectively, https://original-ufdc.uflib.ufl.edu/l/IR00011924/00001). As expected, pre-injection means of skin-fold thickness did not differ across treatments. Neither prenatal RPC nor colostrum treatment affected antigen-specific recall responses at 28.8 d of age (0.96, 1.24, 1.51, and 1.19 mm for NN, NC, CN, and CC, respectively; Supplemental Figure S3A). However, by 56.3 d of age, prenatal RPC attenuated responses to PHA challenge compared with prenatal CON (0.98 vs. 1.41 mm, respectively; \( P = 0.01 \)). This increased skin thickness was specifically detected at 6 h (1.54 vs. 2.28 mm) after the PHA injection (prenatal by time; \( P = 0.04 \); Supplemental Figure S3B). Skin-fold thicknesses at 24 (0.90 vs. 1.16 mm) and 48 h (0.51 vs. 0.78 mm) after challenge were not different among treatments.

Post-challenge mean DMI of MR (803 g/d) did not differ across treatments; however, if calves were born from RPC-supplemented dams (CC calves), mean intake of starter after LPS was greater compared with calves from control dams (274 vs. 177 g/d; \( P \leq 0.05 \)). As a consequence, total DMI also was increased by prenatal RPC treatment compared with prenatal control (1,082 vs. 982 g/d; \( P \leq 0.05 \); Table 4; Figure 4). Mean BW on the day of the LPS challenge did not differ due to treatments (52.0 kg; \( P \geq 0.25 \); Table 4); however, calves in the prenatal RPC treatment tended to weigh more at 13 d after the LPS challenge compared with calves in prenatal control treatment (63.4 vs. 57.7 kg; \( P = 0.06 \); Table 4). Colostrum source, whether from dams fed RPC or not, and interaction between prenatal and colostrum treatments did not affect DMI or weight gain before or after LPS administration (Table 4).

### Feed Intake and Body Weight of Male Calves Challenged with LPS

Dry matter intake of MR and starter before LPS challenge did not differ among treatments (Table 4). Rectal temperature, RR, and HR increased \( (P < 0.01) \) rapidly after LPS injection (Figure 5). Calves ex-
experienced additional increases in RT after consumption of MR in the afternoon of the day of LPS injection and after subsequent consumptions of MR (Figure 5A). Over the 72 h after LPS injection, mean RT was lower (39.04 vs. 39.22°C; \( P = 0.03 \)) in prenatal RPC calves compared with prenatal control calves (Supplemental Table S5, https://original-ufdc.uflib.ufl.edu/l/IR00011924/00001; Figure 5A). From 0 to 6 h after MR challenge, the increment in rectal temperatures of prenatal RPC calves was less drastic than the increment in prenatal control calves (39.2 vs. 39.5°C; \( P < 0.01 \); Figure 5A). Colostrum treatment and interactions between prenatal and colostrum treatments did not affect rectal temperatures after LPS injection (Supplemental Table S5). Similar to temperature, RR after LPS remained lower (\( P = 0.04 \)) in prenatal RPC calves compared with prenatal control calves (Supplemental Table S6, https://original-ufdc.uflib.ufl.edu/l/IR00011924/00001; Figure 5B). Mean HR from 0 to 72 h post-LPS injection did not differ among treatments (Supplemental Table S6); however, HR at 0 and 1 h after LPS was lower in prenatal RPC calves compared with prenatal control calves (prenatal by time interaction; \( P < 0.01 \); Figure 5C).

Hourly patterns of standing activity reflected a prenatal RPC by time interaction (\( P < 0.01 \)), with prenatal RPC calves displaying more activity in the hour immediately following the challenge (hour 1; \( P < 0.01 \); Supplemental Table S6; Supplemental Figure S4, https://original-ufdc.uflib.ufl.edu/l/IR00011924/00001), whereas prenatal control calves were less active immediately after the challenge but increased activity coinciding with afternoon MR delivery (hour 8; \( P < 0.01 \); Supplemental Figure S4). Calves born to dams supplemented with RPC tended to have more frequent standing bouts over the 24-h period following the challenge (\( P = 0.08 \); Supplemental Table S6), suggesting they were slightly more active in general. Activity patterns following LPS challenge were not influenced by colostrum treatment or interaction between prenatal and colostrum treatment (Supplemental Table S6).

**Plasma Metabolites, Minerals, and Cytokines of Male Calves Challenged with LPS**

Concentrations of calcium, glucose, and zinc in plasma decreased after LPS injection, whereas BHB, BUN, fatty acids, and TAG increased after LPS (Figures 6 and 7). Similar to physiological and behavioral responses to LPS, prenatal RPC calves showed less pronounced changes in plasma glucose, BHB, fatty acids, and TAG over time compared with prenatal control calves (prenatal RPC by time interaction; \( P < 0.01 \); Supplemental Table S7, https://original-ufdc.uflib.ufl.edu/l/IR00011924/00001; Figure 6). Analysis of plasma TAG as a percentage of initial concentrations also revealed that prenatal RPC calves had a less pronounced change in plasma TAG after LPS compared
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Figure 3. Hematology results of preweaning female Holstein calves born from dams supplemented with either 0 or 12.9 g/d of rumen-protected choline (RPC) during late gestation (prenatal RPC effect). Solid line with black square represents the main effect of prenatal supplementation (+ prenatal RPC) or no supplementation of choline (− prenatal RPC). Blood samples were collected at birth (0) and on d 7, 14, and 21 after calving. Differences between dietary treatments were detected at days with *($P \leq 0.05$). Data are presented as LSM ± SEM (error bars).

with prenatal control calves (106 vs. 155%; $P = 0.02$; Figure 6F). Plasma BUN tended to be lower (6.1 vs. 6.9 mg/dL; $P = 0.07$) in prenatal RPC calves compared with prenatal control calves (Supplemental Table S7; Figure 6). Plasma calcium tended to be greater (10.1 vs. 9.6 mg/dL; $P = 0.08$), whereas plasma zinc tended to be lower (0.79 vs. 0.91 mg/L; $P = 0.08$) in prenatal RPC calves compared with prenatal control calves (Supplemental Table S7). Except for plasma TAG, which was lower in calves fed RPC colostrum compared with control colostrum (16.3 vs. 22.2 mg/dL; $P = 0.001$), the colostrum treatment and interactions between prenatal and colostrum treatments did not affect concentrations of metabolites or minerals in plasma (Supplemental Table S7).

Concentrations of cortisol, haptoglobin, IL-6, SAA, and TNF-α increased ($P < 0.05$) after LPS injection (Supplemental Table S7; Figures 7 and 8). Except for IL-6, the response to LPS was greater in prenatal control calves compared with prenatal RPC calves. Concentrations of cortisol in plasma were lower (16 vs. 19 ng/mL; $P = 0.03$) in prenatal RPC calves compared with prenatal control calves from 0 to 72 h after LPS injection (Supplemental Table S7; Figure 7A). Concentrations of haptoglobin after LPS were not different between treatments (Supplemental Table S7; Figure 7C); however, as a percentage of the initial haptoglobin concentration, prenatal RPC calves had an attenuated change in haptoglobin compared with prenatal control calves (121 vs. 181% of initial; $P = 0.08$; Figure 7D). In contrast to the other cytokines, the peak IL-6 concentration at 3 h was greater (408 vs. 189 pg/mL; $P < 0.05$) in prenatal RPC calves compared with CON calves (Figure 8A). Plasma SAA, in contrast, tended to be less (4.6 vs. 4.8 mg/dL; $P = 0.09$) in prenatal RPC calves compared with prenatal CON calves (Fig-
Plasma TNF-α, which increased sharply after LPS, also was lower (13.1 vs. 18.5 ng/mL; \( P = 0.04 \)) overall in prenatal RPC calves compared with prenatal CON calves in the 6 h after LPS injection (Figure 7E). Colostrum and interactions between prenatal and colostrum treatments did not affect cytokine responses to LPS (Supplemental Table S7).

### Hematology of Male Calves Challenged with LPS

Hematocrit and concentrations of erythrocytes, reticulocytes, platelets, and leukocytes in blood decreased after LPS injection (Supplemental Table S8, Supplemental Figure S5, [https://original-ufdc.uflib.ufl.edu/l/IR00011924/00001](https://original-ufdc.uflib.ufl.edu/l/IR00011924/00001)). Treatments had very few effects on hematology responses to LPS, except that neutrophils, as a percentage of leukocytes, were greater (40 vs. 35%; \( P = 0.03 \)) in prenatal RPC calves compared with prenatal CON calves after LPS injection (Supplemental Table S8). Likewise, basophils and eosinophils as percentages of leukocytes tended to be greater (0.8 vs. 0.4%; \( P = 0.06 \)) in prenatal RPC calves compared with CON calves. Concentration of hemoglobin did not change during the 12 h after LPS injection (Supplemental Table S8; Supplemental Figure S5), but an interaction between prenatal RPC by time was detected (\( P = 0.01 \); Supplemental Table S8), although the SLICE command did not detect significant differences at any time (\( P > 0.22 \)).

### Table 4. Dry matter intakes and BW of calves before and after LPS challenge

<table>
<thead>
<tr>
<th>Measure</th>
<th>Treatment</th>
<th>SEM</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calves enrolled, n</td>
<td>NN</td>
<td>9</td>
<td>—</td>
</tr>
<tr>
<td>Age at LPS challenge, d</td>
<td>NN</td>
<td>23.8</td>
<td>0.05</td>
</tr>
<tr>
<td>Age at completion, d</td>
<td>NN</td>
<td>34.9</td>
<td>0.06</td>
</tr>
<tr>
<td>DMI before LPS challenge</td>
<td>NN</td>
<td>758</td>
<td>0.87</td>
</tr>
<tr>
<td>MR intake, g</td>
<td>NN</td>
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<td>0.11</td>
</tr>
<tr>
<td>Starter intake, g</td>
<td>NN</td>
<td>805</td>
<td>0.15</td>
</tr>
<tr>
<td>Total DMI, g</td>
<td>NN</td>
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<td>0.05</td>
</tr>
<tr>
<td>DMI after LPS challenge</td>
<td>NN</td>
<td>807</td>
<td>0.45</td>
</tr>
<tr>
<td>MR intake, g</td>
<td>NN</td>
<td>200</td>
<td>0.93</td>
</tr>
<tr>
<td>Starter intake, g</td>
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</tr>
<tr>
<td>Birth</td>
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<td>0.48</td>
</tr>
<tr>
<td>LPS challenge</td>
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</tr>
<tr>
<td>Completion</td>
<td>NN</td>
<td>59.1</td>
<td>0.06</td>
</tr>
<tr>
<td>ADG, kg/d</td>
<td>NN</td>
<td>0.79</td>
<td>0.94</td>
</tr>
</tbody>
</table>

1Calves were born to cows fed prepartum diet without (N) or with (C) rumen-protected choline. At birth calves were assigned colostrum from N or C cows in a 2 × 2 factorial arrangement resulting in 4 treatments: calves born to and fed colostrum from N cows (NN); calves born from N cows and fed colostrum from C cows (NC); calves born from C cows and fed colostrum from N cows (CN); and calves born to and fed colostrum from C cows (CC).

2PT = prenatal treatment (NN + NC vs. CN + CC); CT = colostrum treatment (NN + CN vs. NC + CC); PT × CT = interaction between PT and CT (NN + CC vs. NC + CN).

3Day of age at which calves were challenged with LPS.

4Day of age at which calves were removed from experiment and sold.

5Average DMI [milk replacer (MR), starter, total] from birth to day of LPS administration.

6Age at LPS challenge was used as covariate.

7Average DMI (MR, starter, total) from 0 to 8 d after LPS administration.

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**Figure 4.** Effect of rumen-protected choline (RPC) supplementation during the last 3 wk of gestation on total DMI of preweaning male Holstein calves after dosing LPS (0.05 µg/kg BW, *Escherichia coli* serotype O111:B4). Prenatal RPC supplementation (+ Prenatal RPC); no prenatal RPC supplementation (− Prenatal RPC). Prenatal RPC effect (\( P = 0.05 \)), interaction between Prenatal RPC and time (\( P = 0.03 \)). At each time point (day), differences between treatments were found as follows: *\( P \leq 0.05 \), †\( 0.05 > P \leq 0.10 \). Values represent LSM and SEM (error bars).
DISCUSSION

Supplementing RPC in late gestation has positive effects on lactation performance of cows (Zenobi et al., 2018a; Arshad et al., 2020; Bollatti et al., 2020). Here we show that, in addition to positive effects on lactation performance, feeding RPC in late gestation improved AEA of IgG from colostrum, improved MR and starter intakes in the first 21 d of life, and attenuated inflammatory responses of the offspring. These data help explain the results reported by Zenobi et al. (2018a), where female calves born from dams supplemented with RPC during the last 3 wk of gestation tended to have greater ADG from calving to 56 and 350 d of age (0.81 vs. 0.85 kg/d) compared with females born from cows not fed RPC. These data provide strong evidence that RPC supplementation to pregnant nonlactating dairy cows can help meet choline requirements of the dam and offspring during pregnancy and improve not only cow performance but also calf performance.

Transfer of Passive Immunity

Greater AEA from colostrum of cows fed RPC in late gestation is one of the major findings of this experiment that has potential implications for calf health. Although the nutrient content of colostrum (concentrations of fat, total protein, and lactose, and SCS) and consequently the quantity of these nutrients consumed did not differ among treatments, we found that feeding colostrum harvested from cows supplemented with RPC increased serum IgG compared with calves fed control colostrum. Intake of IgG from colostrum is a major determinant for neonatal morbidity, mortality, and calf immune competence (Quigley and Drewry, 1998). Although our sample size was limited, we also observed positive benefits of feeding RPC in late gestation on incidence of fever and survival of offspring (bulls and heifers). Concentrations of IgG and AEA may vary due to calf age and total amount of IgG consumed. Those 2 factors were eliminated in the present study because all calves were fed similar amounts of IgG within 2 h of life. Results suggest that the 2 colostrum sources contained either different constituents or the same constituents in different proportions, which influenced gut absorption of IgG. Recently, it was shown that feeding RPC could potentially alter choline metabolites in the colostrum of RPC-fed cows compared with control (Swartz et al., 2022).

Growth Performance of Female Calves

We hypothesized that maternal choline supplementation would increase BW gain of female calves on the basis of our previous data (Zenobi et al., 2018a). Our group reported that heifers born from dams supplemented with RPC during the last 3 wk of gestation tended to have greater ADG from calving to 56 and 350 d of age (0.81 vs. 0.85 kg/d). In the present study, ADG in the first 28 d tended to be greater in prenatal RPC heifers. Here we observed that, as early as the first 21 d of life, DMI of offspring of RPC-supplemented cows was greater than those of CON cows. The benefit of greater
feeding intake during early life was revealed by the age at which heifers met the criteria to be weaned (intake of 1.4 kg/d of starter, as-fed basis, for 3 consecutive days; AFIA, 2017). Here we found that 31% of heifers in the NN treatment group did not reach the target for weaning by 56 d of age, whereas all heifers assigned to the other 3 treatments consumed 1.4 kg/d of starter for 3 consecutive days before 56 d of age.

**Immune Competence of Female Calves**

The group of heifers born from RPC-supplemented dams had greater concentrations of blood leukocytes as well as neutrophils, the latter being the first line of defense against infections (Nathan, 2006). Deficiency of folic acid (a methyl donor, like choline, and highly interrelated with choline metabolism) has been induced experimentally pre- and postnatal in many animal species, with consistent findings of macrocytic anemia (a reduced number of red blood cells) and leukopenia (a reduced number of white blood cells; Hoffbrand, 1978; Kumar and Axelrod, 1978). The lower concentrations of white blood cells and red blood cells support the hypothesis that a broad segment of the immunologic system is affected in utero by an insufficient supply of choline or choline biomolecules, and that defects created by this lack of exposure in utero is not corrected solely by postnatal supplementation of choline via colostrum. Certainly, inference of immune competence cannot be determined from leukocyte counts, so we cannot conclude that maternal RPC benefits immunity on the sole basis of leukocyte counts.

Heifers born from dams supplemented with RPC during late gestation showed a reduction in skin-fold thickness to PHA at 56 d of age, especially at 6 h after stimulation. Skin-fold changes evaluates delayed-type
hypersensitivity by measuring the ability of immune cells to infiltrate and accumulate into regions of antigen deposition. The response to PHA is strictly a cell-mediated response, not an antibody-mediated response (Bernhagen et al., 1996). The use of PHA to induce a delayed-type hypersensitivity response in cows resulted in the primary infiltration of eosinophils, macrophages, and neutrophils, but not lymphocytes (Hernández et al., 2005). Mice fed a restrictive diet experienced a significant delay in maturation of the immune system, as shown by the greater response to PHA later in life compared with well-nourished animals (Walford et al., 1973). Similarly, we speculate that choline supply may be limiting in CON animals under the conditions of the present experiment, resulting in delayed development of antigen-specific immune cells and more rapid accumulation of myeloid cells at the skin-test site.

**Attenuation of Inflammatory Response to LPS Challenge**

Results from the LPS challenge performed in this study revealed an effect of maternal RPC supplementation on the immune competence of the offspring. In particular, calves born from RPC-fed dams had lower RT and less-severe systemic inflammation. These responses included reduced hypoglycemia, hypocalcemia, hyperketonemia, and hyperlipidemia, as well as lower plasma concentrations of BUN, SAA, cortisol, and TNFα. In addition, greater DM intake and BW after the administration of LPS are evidence of a greater capability of calves born from RPC-fed dams to respond to and recover from this stressful situation. In alignment with these findings, some parameters of immune competence assessed in the present study, including incidence of

**Figure 7.** Effect of rumen-protected choline (RPC) supplementation during the last 3 wk of gestation on (A) total calcium, (B) zinc, (C) haptoglobin, (D) haptoglobin as percentage of initial concentration, (E) serum amyloid A (SAA), and (F) cortisol of preweaning male Holstein calves after dosing LPS (0.05 µg/kg BW, *Escherichia coli* serotype O111:B4). OD = optical density. Prenatal RPC supplementation (+ Prenatal RPC); no prenatal RPC supplementation (− Prenatal RPC). (A) Prenatal RPC effect (*P* = 0.08), interaction between prenatal RPC and time (*P* = 0.92); (B) prenatal RPC effect (*P* = 0.07), interaction between prenatal RPC and time (*P* = 0.38); (C) prenatal RPC effect (*P* = 0.13), interaction between prenatal RPC and time (*P* = 0.12); (D) prenatal RPC effect (*P* = 0.08), interaction between prenatal RPC and time (*P* = 0.34); (E) prenatal RPC effect (*P* = 0.33), interaction between prenatal RPC and time (*P* = 0.09); (F) prenatal RPC effect (*P* = 0.03), interaction between prenatal RPC and time (*P* = 0.54). At each time point (h), differences between treatments were found as follows: *P* ≤ 0.05, †0.05 > *P* ≤ 0.10. Values represent LSM and SEM (error bars).
fever, plasma concentrations of SAA, and fibrinogen of female calves, support the notion that maternal RPC supplementation during late gestation exerts an effect on immune competence of the offspring.

It is challenging and intriguing to elucidate the possible explanations by which pre- and postnatal choline supplementation exerted these positive results. One possibility is that RPC supplementation during late gestation supports final maturation of organs and the immune system, as has been suggested for rats (Rappay et al., 1971) and rabbits (Hallman et al., 1997). Indeed, the results reported here revealed that late-gestation supplementation with RPC, regardless of colostrum source, conferred on Holstein calves an enhanced ability to cope with an LPS challenge during postnatal life. Similarly, pregnant rats fed diets limited in lipotropes, including choline, methionine, and vitamin B12, produced offspring that were less able to survive intentional bacterial infections compared with pups from dams fed diets adequate in lipotropes; they also had lower amounts of IgG and white blood cells in peripheral blood (Newberne et al., 1970). Similar results were observed by Williams et al. (1979), including long-term effects on the immune response to different challenges in adulthood. In our experiment, although not all of our hematological comparisons reached statistical significance, several of them did, and all the patterns over time were similar. We believe these results signify that gestation in an RPC-supplemented dam enhanced the maturity of the immune system, which has been identified as the crucial component for survivability of newborn calves (Gruse et al., 2016).

Maternal RPC may exert effects on offspring in several ways. One possibility is that heifers born from dams supplemented with RPC undergo optimized development and maturation of different organs, such as the gastrointestinal tract, the lungs, or even the immune system. The intestinal brush border membrane is mainly (64%) composed of phospholipids, with equal proportions of phosphatidylcholine and its precursor, phosphatidylethanolamine (Christon et al., 1991). Secretions from the mucous membranes of the gastrointestinal tract are even richer in phosphatidylcholine (Rubio et al., 1995; Mahmood et al., 2002). The structural integrity of the brush border and intestinal mucosa are important components of innate barrier function in the intestine. Indeed, supplementation of phosphatidylcholine improved the intestinal barrier’s defense against a bacterial challenge (Olson et al., 2014). Therefore, increasing prenatal consumption of choline by the dam when choline is in high demand by the fetus may benefit the integrity and functionality of the gastrointestinal tract in the newborn calf. Supplementation of RPC increased circulating choline metabolites in pregnant, nonlactating Holstein cows (Zenobi et al., 2018b), which may augment delivery of these essential compounds to the fetus. In fact, intake of choline metabolites via swallowing of amniotic fluid has been documented in sheep (Trahair et al., 1986; Avila and Harding, 1991) and rabbits (Hallman et al., 1997). In the latter, over 70% of the double-labeled dipalmitoyl-phosphatidylcholine injected into the amniotic cavity was found in the fetal gastrointestinal tract within 44 h. Physiologically, concentration of phosphatidylcholine in amniotic fluid increases exponentially starting 4 to 6 wk before calving (Warren et al., 1974) and the concentration of phosphatidylcholine

Figure 8. Effect of rumen-protected choline (RPC) supplementation during the last 3 wk of gestation on (A) IL-6, and (B) tumor necrotic factor-α (TNF-α) of preweaning male Holstein calves after dosing LPS (0.05 µg/kg BW, Escherichia coli serotype O111:B4). Prenatal RPC supplementation (+ Prenatal RPC); no prenatal RPC supplementation (− Prenatal RPC). (A) Prenatal RPC effect (P = 0.44), interaction between prenatal RPC and time (P < 0.01); (B) prenatal RPC effect (P = 0.04), interaction between prenatal RPC and time (P = 0.40). At each time point (h), differences between treatments were found as follows: *P ≤ 0.05, †0.05 > P ≤ 0.10. Values represent LSM and SEM (error bars).
in amniotic fluid have been positively correlated with postpartum maturation of human lungs (Warren et al., 1973). It is possible that high-producing dairy cows do not experience as much of the physiological increase in concentrations of choline metabolites in plasma, thus limiting the availability of such compounds to the fetal tissues that depend on these types of molecules for final maturation.

Alternatively, prenatal choline supplementation may program the offspring by introducing epigenome modifications (Mehedint et al., 2010b). Choline intake can modulate DNA and histone methylation and methyl binding proteins through its role as a methyl donor (Kovacheva et al., 2007; Davison et al., 2009). Additionally, studies in rodents have demonstrated that maternal dietary choline deficiency altered hepatic and brain DNA and histone methylation of the offspring (Niculescu et al., 2006; Kovacheva et al., 2007; Davison et al., 2009; Mehedint et al., 2010a). Furthermore, varied maternal choline intake (930 vs. 480 mg of choline per day) throughout the third trimester of pregnancy altered DNA methylation and expression patterns of cortisol-regulating genes in both the placenta and cord blood (Jiang et al., 2014). Taken together, these pieces of evidence support the concept that choline plays a role in prenatal programming of the human offspring, raising the question of whether this is also true for dairy cows.

**CONCLUSIONS**

This experiment illustrates that diet fed during the last 3 wk of gestation affects early performance and immunity of newborn calves. Notably, feeding colostrum from RPC-fed dams increased AEA of calves. Female calves born from dams supplemented with RPC consumed more MR and starter during the first 21 d of life, resulting in greater BW gain during the first 4 wk of life. Pre- and postnatal supplementation of RPC resulted in greater likelihood of female calves to be ready for weaning by 56 d of age. Female calves born to RPC-fed dams had fewer cases of fever, corresponding to the attenuated inflammatory responses of male calves in an LPS challenge model. These novel findings suggest a role for maternal choline supplementation in supporting development of neonatal calves. If these results are confirmed in future studies, the case for choline as an essential nutrient for transition cows and their calves will be strengthened.

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