Evaluation of bovine colostrum replacer supplementation to improve weaning transition in Holstein dairy calves

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ABSTRACT

The objective of this randomized controlled trial was to evaluate the efficacy of supplementing bovine colostrum replacer during weaning to reduce intestinal permeability and improve gain. For this experiment, 65 calves were enrolled and housed individually until 70 d of age. Calves were fed milk replacer (150 g/L) 3 times daily with 9 L/d, 10.5 L/d, 11.25 L/d, and 12 L/d offered from d 1–7, d 8–14, d 15–21, d 22–56, respectively. Calves were weaned over 8 d from d 57–64, receiving a total of 7.8 L in 2 meals/d from d 57–60 and 3.8 L/d in one feeding from d 61–64. At d 57, calves were blocked by birth weight and randomly assigned to one of 2 treatments, equal in metabolizable energy, which were fed once daily during weaning from d 57–64: control (CON; n = 31 calves): 3.8 L milk replacer (150 g/L) fed by nipple bottle, or colostrum supplementation (COL; n = 34): a mixture of 1 L bovine colostrum replacer (125 g/L) and 3 L milk replacer (150 g/L) with 3.8 L of the mixture fed by nipple bottle. Serum IgG was measured within 48 h of birth and body weight was taken at d 0, 57, 60, 64, 77, and 84. Starter intake and bovine respiratory disease (BRD) score were measured daily from d 50–70 and fecal consistency was examined daily from d 56–70. Serum β-hydroxybutyrate (BHB) and lung consolidation were evaluated at d 57, 64, and 70 and intestinal permeability was assessed by recovery of chromium-EDTA, lactulose, and D-mannitol from plasma after oral administration at d 56 and 65. There was no difference in body weight between treatment groups at the start of weaning, but COL were 2.79 kg (95% CI: 0.90–4.68) and 2.76 kg (95% CI: 0.86–4.65) heavier than CON at d 77 and 84, respectively. Additionally, COL tended to gain 100.00 g/d more than CON calves (95% CI: −10.41–207.13) from d 57–84. There were no differences in any of the other variables measured. Supplementation of bovine colostrum replacer during weaning may improve weight gain, but the mechanism of action is not clear.

Keywords: gain, performance, wean, supplement

INTRODUCTION

Weaning in dairy calves involves the transition from a liquid to solid diet, resulting in considerable modifications to the gastrointestinal tract, as both the primary location for digestion and the source of energy change (Baldwin et al., 2004). Moreover, dairy calves often fail to consume enough solid feed before weaning, especially if they are fed high volumes of milk, weaned before 8 weeks of age, or weaned abruptly (Eckert et al., 2015; Steele et al., 2017; Mirzaei et al., 2018). As a result, dairy calves often experience increased gastrointestinal epithelial permeability (Wood et al., 2015), reduced immune function of the gastrointestinal tract epithelium (Malmuthuge et al., 2013), and morphological changes to the villi and crypts of the intestine resulting in decreased absorptive capacity (Malmuthuge et al., 2013). These weaning-induced stressors are not limited to gastrointestinal challenges, but also manifest as increased respiratory disease. The incidence of respiratory disease has been found to be as high as 37.9% in recently weaned dairy calves, occurring as soon as 8 d post-weaning (Bach et al., 2011). Moreover, 20.2% calves have been found to have lung consolidation within the first week postweaning (Teixeira et al., 2017). As a result of these stressors, calves often experience reduced weight gain during weaning (Sweeney et al., 2010; Meale et al., 2015). As Soberon et al. (2012) demonstrated that every additional 100 g of ADG from weaning to breeding results in 820 kg more milk to the end of the third lactation, supporting the transition from liquid to solid feed to improve ADG may increase future productivity.
Supplemental bovine colostrum replacer might assist the transition at weaning. Colostrum contains a myriad of bioactive molecules, including growth factors and antioxidants, that influence immune, metabolic, and endocrine pathways (Blum and Hammon, 2000; Przybylska et al., 2007). In both human and animal models, supplementation of bovine colostrum replacer has improved gastrointestinal health and immunity (Struff et al., 2008; Marchbank et al., 2011; Eslamian et al., 2019), which may be a result of colostrum’s growth factor composition (Huguet et al., 2012). In weaned piglets, supplementation of bovine colostrum replacer reduced the negative effects of weaning (King et al., 2008; Huguet et al., 2012). Specifically, weaned pigs fed bovine colostrum replacer had increased intestinal lymphocyte density and villus height, and improved intestinal integrity after weaning, resulting in increased feed intake and gain, and gain to feed ratio (Huguet et al., 2007; King et al., 2008; Huguet et al., 2012). Intestinal permeability leads to poorer nutrient absorption (Gaebel et al., 1989) and thus improving intestinal integrity is important to growth. As dairy calves often experience reduced intestinal barrier function during weaning (Wood et al., 2015), there may be an opportunity to supplement their milk with bovine colostrum replacer during weaning to reduce intestinal permeability and subsequently improve weight gain, similar to what has been demonstrated in supplemented weaned pigs. The objective of this study was to evaluate the effect of supplementing bovine colostrum replacer during weaning on intestinal permeability and weight gain. We hypothesized that calves supplemented with bovine colostrum replacer during the weaning process would have reduced intestinal permeability and improved average daily gain over the weaning period compared with calves without supplementation.

**MATERIALS AND METHODS**

This randomized controlled trial was conducted at a single commercial dairy farm in southwestern Ontario from January 7 to April 26, 2023. This facility was chosen based on their farm size, willingness to participate, and meticulous calf health records. The study was approved by the University of Guelph Animal Care Committee (AUP 4833).

**Housing and Feeding**

All calves enrolled were female and fed 4 L of thawed bovine colostrum with at least 25% Brix by esophageal feeder within the first 12 h of life, with a second feeding of 4 L of colostrum fed by esophageal feeder within 12 h after the first feeding. Calves were administered 1 mL vitamin E and selenium complex intramuscularly (Selon-E, Vetoquinol, Quebec, Canada) immediately after birth, as part of the farm’s newborn calf protocol. Calves were moved to individual pens (2.0 m²) in an indoor facility shortly after birth where they remained until 70 d of age before moving into group pens. Calves were fed a commercial milk replacer (23.2% CP, 18.9% crude fat, and 49.7% lactose of DM) at 150 g/L (Table 1; Optivia, Trouw Nutrition, Puslinch, Ontario, Canada) by an Uddermatic A150t robotic rail feeding system (Uddermatic Inc., Alma, Ontario, Canada) and received 3 equal feedings of milk replacer daily at 0500 h, 1300 h, and 2100 h. Calves were fed milk replacer (150 g/L) 3 times daily at 9 L/d from d 1–7, 10.5 L/d from d 8–14, 11.25 L/d from d 15–21, and 12 L/d from d 22–56. Calves began weaning at d 57 and were weaned over 8 d in a step-down manner. From d 57–60, calves received 3.8 L by nipple bottle at 09:00 and 4 L at 21:00 by robotic feeder for a total of 7.8 L per day. From d 61–64, calves were fed 3.8 L at 0900 h by nipple bottle. Calves were also offered ad libitum pelleted calf starter (21% CP, 25% starch, 4.5% crude fiber, and 3.5% crude fat; Wallenstein Feed and Supply, Ltd., Wallenstein, Ontario, Canada) and water from one d of age.

**Treatment Assignment and Experimental Design**

At the beginning of weaning (57 d), calves were blocked by birth weight (<42 kg or ≥42 kg) and randomly assigned, using the RAND function in Microsoft Excel (Microsoft Corp., Redmond, WA), to one of 2 treatments fed once daily by nipple bottle at 0900 h during weaning from d 57–64. The treatments were control (CON; n = 31 calves): 3.8 L milk replacer (150 g/L) fed by nipple bottle with no bovine colostrum replacer supplementation (Table 1) or bovine colostrum replacer supplementation (COL; n = 34): a mixture of 1 L bovine colostrum replacer at 125 g/L (16.4 g IgG; Calif’s Choice Total HiCal, Saskatoon Colostrum Company, Saskatoon, Saskatchewan, Canada) and 3 L milk replacer at 150 g/L, with 3.8 L of the mixture fed by nipple bottle (Table 1). This concentration of bovine colostrum replacer was selected, as similar concentrations have demonstrated therapeutic efficacy in reducing diarrhea (Carter et al., 2022), and 3.8 L of the mixture was fed as that was the maximum capacity of the nipple bottles. To ensure homogeneity of the COL treatment, the total required volumes of bovine colostrum replacer and milk replacer were blended in a larger pail and then dispensed into each 3.8 L nipple bottle, to deliver a total of 119 g of bovine colostrum replacer per calf. Any surplus mixture was discarded. Both treatments were similar in metabolizable energy, with CON calves receiving a total of 29.26 Mcal and
COL calves receiving a total of 29.29 Mcal over the 8-d treatment period from liquid feed (Table 1). Those responsible for mixing and feeding were not blinded to treatment groups due to the feeding concentrations of milk replacer and bovine colostrum replacer differing; however, those assessing outcomes were blinded.

After weaning, all calves remained in individual pens until 70 d of age then moved to a group pen until 84 d of age. Each group pen consisted of both CON and COL calves, with group sizes ranging from 10 to 12 animals, with 5.3–6.4 m² of space per calf.

### Body Weight, Starter Intake, and Health Measurements

Body weight (BW) was recorded using an electronic scale (Model VS-2000, A and A Scales LLC., Prospect Park, NJ) at birth, at the start of weaning (57 d of age), at 60, at the completion of weaning (64 d), and at 70, 77, and 84 d.

Calves’ lungs were scored 3 times by a single, trained veterinarian technician on weekdays: LUS57: within 2 d before the start of weaning (55–57 d); LUS64: within 2 d after the completion of weaning (64–66 d), and LUS70: one week after LUS64 (70–72 d). Lungs were scanned using the method described by Ollivet et al. (2015) with a portable linear ultrasound probe (Easi-Scan, IMV Imaging, Rochester, MN) set at a depth of 8 cm and a frequency of 8.5 mHz. Lung tissue was considered consolidated if hypoechoic areas were present eliminating the bright white band at the pleural interface and reverberation aspect found in normal lung tissue (Blond and Buczinski, 2009). For any lung tissue that had hypoechoic areas, the extent of lung consolidation was determined by using the 1-cm gridlines of the ultrasound, summing the affected areas of all lung lobes. Lungs were scored on a 0–5 scale with a lung score of 0 = normal, no or few comet-tails and <1 cm consolidation; 1 = lesion patches totalling ≥1 cm but <2 cm; 2 = lesion patches ≥2 cm but <3 cm; 3 = lesion patches totalling ≥3 cm but <4 cm; 4 = lesion patches ≥4 cm but <5 cm; and 5 = lesion patches totalling ≥5 cm of consolidation.

Disease events, recorded by farm personnel (VetLogix, Cambridge, Ontario, Canada), were collected to assess the incidence of disease from birth until 84 d. Calves were assigned a score from 0 to 15 based on presence or absence of spontaneous cough, nasal discharge, ocular discharge, rectal temperature, rapid/difficult breathing, and ear droop/head tilt. Calves with a score of ≥5 were considered to have respiratory disease (Love et al., 2014). Calves were identified as having respiratory disease if their respiratory rate was >45 breaths per minute and rectal temperature ≥39.5°C. Calves with respiratory disease were treated with ampicillin (Polyflex; Boehringer Ingelheim Animal Health, 6 mg/kg BW IM once daily) for 3–5 d and meloxicam (Metacam 20 mg/mL; Boehringer Ingelheim Animal Health, 2.5 mL/100 kg of BW SC).

Fecal consistency of calves was also scored once daily from d 56–70 by a single trained observer according to the system developed by Larson et al. (1977), where 1 = normal (feces were slightly deformed after reaching the

### Table 1. As-fed macronutrient composition of milk replacer (MR), bovine colostrum replacer (CR), and a mixture fed to calves enrolled in one of two treatments at the onset of weaning

<table>
<thead>
<tr>
<th>Component</th>
<th>Milk replacer (MR)</th>
<th>Bovine colostrum replacer (CR)</th>
<th>Mixture (MR and CR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>5.0</td>
<td>6.0</td>
<td>5.28</td>
</tr>
<tr>
<td>CP (%)</td>
<td>22.0</td>
<td>43.6</td>
<td>28.05</td>
</tr>
<tr>
<td>IgG (%)</td>
<td>—</td>
<td>14.8</td>
<td>4.14</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>18.0</td>
<td>25.4</td>
<td>19.96</td>
</tr>
<tr>
<td>Lactose (%)</td>
<td>47.2</td>
<td>21.0</td>
<td>39.86</td>
</tr>
<tr>
<td>ME (Mcal/kg)</td>
<td>4.91</td>
<td>5.82</td>
<td>5.11</td>
</tr>
<tr>
<td>ME (Mcal/L, as fed)</td>
<td>0.64</td>
<td>—</td>
<td>0.65</td>
</tr>
</tbody>
</table>

1CON (n = 31): Days 57–60 of age received two feedings of MR at 150 g/L per day, 3.8 L by nipple bottle at 09:00 and 4 L at 21:00 by robotic feeder for a total of 7.8 L per day and from 61 to 64 d of age received one feeding of 3.8 L of 150 g/L MR daily by nipple bottle at 09:00, or COL (n = 34): Days 57–60 of age received two feedings per day, at 09:00 fed 3.8 L of a mixture of 3 parts MR (150 g/L) and 1 part CR (125 g/L) fed by a nipple bottle and 4 L of MR (150 g/L) at 21:00 by robotic feeder, and from 61 to 64 d of age received one 3.8 L feeding of a mixture of 3 parts MR (150 g/L) and 1 part CR (125 g/L) by nipple bottle at 09:00.

2Lactose was calculated as: Lactose = 100 – moisture – ash – fat – protein (Quigley, 2007).

3ME (Mcal/kg) was calculated as: ME = [(0.057 x CP % + 0.092 x fat % + 0.0395 x lactose %) x 0.93] / (1 – moisture) (Quigley, 2007).

4ME (Mcal/L as fed) was calculated as ME = (Mcal/kg) * (concentration %) where concentration % = [(grams of powder) / (grams of powder + 1 L water)] *100%.

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Blood Sample Collection and Analysis

Whole blood from calves 24–48 h old was collected by jugular venipuncture using a 20-gauge 25 mm needle into a 10 mL tube without anti-coagulant (BD Vacutainer serum, Becton, Dickson, and Company, NJ) for serum total protein analysis using an optical total protein refractometer and serum IgG measurement at the Saskatoon Colostrum Company Ltd. (Saskatoon, SK) by radial immunodiffusion (Tyler et al., 1996). Using identical methods, whole blood was also collected at the beginning of weaning (57 d of age), end of weaning (64 d), and one week after weaning (70 d) for measurement of β-hydroxybutyrate (BHB) using photometry at the Animal Health Laboratory (Guelph, Ontario, Canada). All samples were stored at room temperature for one hour to allow blood to clot and were then centrifuged at 1,500 × g for 15 min. Serum was stored at −20°C until analysis.

To assess intestinal permeability, the day before the start of weaning (d 56) and the day after weaning was completed (d 65), 15 calves from each treatment with approximately equal representation from each block (<42 kg or ≥42 kg birth weight) were orally dosed with chromium-EDTA (Cr-EDTA; 0.1 g/kg BW), lactulose (0.4 g/kg BW), and d-mannitol (0.12 g/kg BW) 2 h after their morning milk feeding using a 50 mL catheter tip syringe (50 mL BD catheter tip syringe, Becton, Dickson, and Company, NJ). Approximately 10 mL of whole blood was collected via jugular venipuncture using a 20-gauge 1” needle into a 10 mL heparinized vacuum tube (BD Vacutainer lithium and sodium heparin tube, Becton, Dickson, and Company, NJ) 120 min after the marker was administered, which was determined to be the optimal sampling time by Pisoni et al. (2022). After centrifugation, plasma was stored at −20°C until analysis of intestinal permeability by measuring the Cr-EDTA, lactulose, and d-mannitol markers. Before Cr-EDTA analysis, plasma samples were diluted 10-fold with 4% (wt/vol) 1-butanol, 0.01% (wt/vol) EDTA, 0.01% (wt/vol) Triton X-100 and 1% (wt/vol) tetramethylammonium hydroxide (TMAH) made up with high purity water (>18 MΩ). Total plasma chromium concentration was analyzed at the Trent University Water Quality Centre (Peterborough, Ontario, Canada) by inductively coupled plasma mass spectrometry (Agilent 8800 ICP-QQQ-MS, Agilent, California, USA) using H2 reaction gas in MS/MS mode. Plasma lactulose and d-mannitol samples were purified and run in duplicate as previously described by Welboren et al. (2021). In brief, ammonium formate in 90% acetonitrile with 4 μL of mannitol and lactulose internal standards were added to each 53.3 μL plasma sample. Vials were mixed and centrifuged at 10,000 × g at 4°C for 10 min to separate the soluble and insoluble fractions and the soluble supernatant was collected. Samples were analyzed using mass spectrometry (Thermo Vanquish Duo, tandem UHPLC system coupled to TSQ Altis, triple quadrupole mass spectrometer; Thermo Fisher Scientific, Massachusetts, USA). The mean value of the duplicate samples was used for analysis and the intra- and inter-assay coefficients of variation (CV) were all lower than 10%.

Sample Size Calculation

A power analysis (power = 0.80 and α = 0.05) was used to estimate the sample size targeting an expected improvement of 75 g/d in average daily gain between the onset of weaning at 57 d of age to the completion at 64 d of age between treatment groups, with an expected standard deviation of 105 g/d. Based on the sample size calculation, a total of 64 calves (32 per treatment) were required. For our second objective, the mean and standard deviation of serum Cr-EDTA concentrations (0.22 ppm, 0.31 ppm; SD = 0.06 ppm) from Amado et al. (2019) were used in a power analysis (power = 0.80 and α = 0.05) to estimate the number of calves required to detect a difference of 0.09 ppm in the recovery of gastrointestinal permeability markers. Based on the sample size calculation, a total of 18 calves (9 per treatment group) were required.
Statistical Analysis

Statistical analyses were conducted in Stata 17.0 (StataCorp LP, College Station, TX). Data were manually examined for errors and completeness in Microsoft Excel (Microsoft Corporation 2022, Redmond, WA) and observations that were biologically implausible or incomplete were excluded. Descriptive statistics were generated for all explanatory variables. Further comparisons between treatment groups and explanatory variables were performed using $t$-tests for normally distributed continuous variables and Mann-Whitney tests for non-normally distributed continuous variables. Continuous variables were assessed for normal distribution using the Shapiro-Wilk test. Pearson’s chi-squared tests were used to compare differences between treatment groups and categorical explanatory variables unless there was a low frequency of observations (<5 observations per category), in which case Fisher’s exact tests were used. Serum IgG was categorized as described by Lombard et al. (2020).

Lung ultrasound score, BRD score, and fecal score were transformed from an ordinal scale to a binary outcome due to the lack of variability. Calves with lung consolidation totalling $3 \text{ cm}^2$ or greater were considered positive for lung consolidation (Dunn et al., 2018), BRD scores of $\geq 5$ were considered as being positive for BRD (Love et al., 2014), and fecal scores $\geq 3$ were considered to be diarrhea (Larson et al., 1977; Renaud et al., 2020). Due to a low overall frequency of calves positive for lung consolidation, differences in lung consolidation between treatments at each measured time point were determined using Fisher’s exact test. Univariable logistic regression models were used to assess for differences in the binary outcome variables positive BRD score (BRD score of $\geq 5$) and abnormal fecal score (fecal score $\geq 3$) at any of the measured time points, and occurrence of a health event. A linear regression model was used to assess differences in ADG over the duration of the trial ($d \geq 5$–84). Repeated measures linear regression models were used to assess differences in body weight, starter intake, plasma Cr-EDTA, plasma lactulose, plasma $\delta$-mannitol, lactulose to $\delta$-mannitol ratio, and serum BHB at the different time points before, during, and after weaning. Plasma concentration of Cr-EDTA, $\delta$-mannitol, lactulose, and lactulose-to-$\delta$-mannitol ratio were logarithmically transformed (natural logarithm) to obtain normal distributions, and the results were subsequently back transformed. All repeated measure models included the fixed effects of treatment, day, and treatment $\times$ day interaction, and the random effect of calf nested within block (calf birthweight).

For univariable analysis, any variable with $P < 0.20$ was offered to multivariable models. Variables screened in univariable analysis for each outcome evaluated included block, treatment, and having a positive BRD score, abnormal fecal score, or health event before the trial ($d \leq 57$). The multivariable models were built through a backward stepwise elimination process, with variables with $P \leq 0.05$ retained in the final model, along with variables identified as confounders if their removal led to a $>25\%$ change in the coefficient of another significant variable. Logistic regression models were assessed using the Pearson goodness-of-fit test, as only categorical variables were included in the final model, whereas fit of all repeated measure models were examined by visual evaluation of the homoscedasticity and normality of the residuals and best linear unbiased predictors. Additionally, fit of linear regression models was assessed by examining the normality and homoscedasticity of the residuals. Outliers were evaluated and if they were not deemed erroneous, they were retained in the data set. Differences were considered significant at $P < 0.05$, and trends were declared at $0.05 \leq P \leq 0.10$ for all models.

RESULTS

Descriptive Results

No calves died during the trial so all enrolled calves were included in the analyses, with 31 calves in CON and 34 calves in COL. The mean serum IgG concentration, serum total protein, and birthweight did not differ between groups (Table 2). Based on serum IgG, 55/65 (84.6%) calves were in the excellent category [CON: 28/31 (43.0%); COL: 27/34 (41.5%)]; 8/65 (12.3%) calves in the good category [CON = 5/34 (7.7%); 2/65 (3.1%) in the fair category [CON = 0/31 (0%); COL = 2/34 (6.1%)]; and none in the poor category, which did not differ by treatment group ($P = 0.87$).

Intakes and BHB

There were no liquid feed refusals during the trial. Calves increased starter intake from $d$ 58, but there was no difference in starter intake between treatments or any detected treatment by day interaction (Figure 1). There was also no detected treatment effect on serum BHB concentration or any treatment by day interaction (Table 3).

Lung Consolidation and BRD Score

There was no detected association between treatment and lung consolidation at $d$ 57 [CON = 1/31 (3.2%); COL = 1/34 (2.9%); $P = 0.73$], $d$ 64 [CON = 1/31


There was no difference between treatment groups in positive BRD score (BRD score ≥5) from d 57–70 \([\text{odds ratio (OR)} = 1.05, 95\% \text{ CI: 0.35 to 3.13, } P = 0.93]\). Overall, 16.1\% \((n = 5/31)\) of CON calves had a positive BRD score from d 50–56 and 29.0\% \((n = 9/31)\) from d 57–70. Similarly, 17.6\% \((n = 6/34)\) of COL calves had a positive BRD score from d 50–56 and 32.4\% \((n = 11/34)\) from d 57–70.

**Fecal Score**

There was no detected difference between treatment groups in abnormal fecal score \((\text{fecal score} \geq 3)\) from d 57–70 \([\text{OR} = 0.85, 95\% \text{ CI: 0.29–2.50; } P = 0.76]\). Overall, 71.0\% \((n = 22/31)\) of CON calves had an abnormal fecal score from d 57–70. Similarly, 67.6\% \((n = 23/34)\) of COL calves had an abnormal fecal score from d 57–70.

**Morbidity**

From d 57–84, 16.1\% \((n = 5/31)\) in CON and 14.7\% \((n = 5/34)\) in COL were treated for BRD \([\text{OR} = 0.84, 95\% \text{ CI: 0.21–3.30; } P = 0.80]\). There were no diarrhea treatments in either CON or COL calves from d 57–84.

**Intestinal Permeability**

There were no detected effects of treatment on plasma Cr-EDTA, lactulose, or D-mannitol concentrations or the lactulose to D-mannitol ratio (Table 4).

**Gain**

There was no difference in body weight between treatment groups at the start of weaning on d 57 (Table 2). There was a treatment by day interaction for BW, where COL were 2.79 kg and 2.76 kg heavier than CON at d 77 and 84, respectively (Figure 2). From d 57–84, CON calves had an ADG of 0.96 kg/d ± 0.22 kg whereas COL calves gained 1.06 kg/d ± 0.22 kg. In the univariable analysis, body weight at 57 d, treatment, and block were associated with ADG. In the final model, treatment was the only variable associated with ADG; however, block was also retained, as it was a confounding variable. In the final linear regression model,

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**Table 2.** Characteristics of 65 calves randomly assigned to 1 of 2 treatments

<table>
<thead>
<tr>
<th>Variable</th>
<th>CON Mean ± SE</th>
<th>COL Mean ± SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum IgG (g/L)</td>
<td>36.8 ± 2.0</td>
<td>36.1 ± 1.9</td>
<td>0.80</td>
</tr>
<tr>
<td>Serum total protein (g/dL)</td>
<td>6.4 ± 0.1</td>
<td>6.5 ± 0.1</td>
<td>0.87</td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>42.0 ± 0.8</td>
<td>42.7 ± 0.8</td>
<td>0.51</td>
</tr>
<tr>
<td>Body weight 57 d (kg)</td>
<td>90.8 ± 0.7</td>
<td>90.8 ± 0.6</td>
<td>0.97</td>
</tr>
</tbody>
</table>

\(^{1}\)Control (CON; \(n = 31\)): no colostrum supplementation during the weaning period; colostrum (COL; \(n = 34\)): once daily supplementation of 119 g bovine colostrum replacer during the weaning period.

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**Table 3.** Effect of bovine colostrum replacer supplementation on serum BHB\(^{1}\) in 65 calves randomly assigned to 1 of 2 treatments

<table>
<thead>
<tr>
<th>Variable</th>
<th>CON Mean (95% CI)</th>
<th>COL Mean (95% CI)</th>
<th>Trt P-value</th>
<th>Day P-value</th>
<th>Trt x Day P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum BHB (μmol/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 57</td>
<td>60.34 (40.17–80.52)</td>
<td>59.68 (40.43–78.94)</td>
<td>0.37</td>
<td>&lt;0.001</td>
<td>0.25</td>
</tr>
<tr>
<td>d 64</td>
<td>136.44 (116.27–156.61)</td>
<td>163.01 (143.75–182.26)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 70</td>
<td>225.63 (205.46–245.81)</td>
<td>223.91 (204.36–243.47)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^{1}\)Serum BHB was measured on d 57, 64, and 70.

\(^{2}\)Control (COL; \(n = 31\)): no colostrum supplementation during the weaning period; colostrum (COL; \(n = 34\)): once daily supplementation of 119 g bovine colostrum replacer during the weaning period.
there was a tendency for COL calves to have 0.10 kg/d ± 0.05 kg greater ADG from d 57–84 ($P = 0.08$).

**DISCUSSION**

Supplementing calves with bovine colostrum replacer during the weaning process improved weight gain. However, this effect was only observed at 2 and 3 weeks after weaning, once calves entered group pens. We detected no difference in gain while calves were supplemented with bovine colostrum replacer during weaning, nor during the week following weaning when calves were individually housed. While gain benefits have been observed in weanling piglets supplemented with bovine colostrum replacer (Pluske et al., 1999; Boudry et al., 2008; Huguet et al., 2012), the timing of the gain benefits differed. In the piglets, there was improved gain during supplementation with bovine colostrum replacer, with Huguet et al. (2012) observing improved ADG for 5 weeks after the cessation of the experimental diets. However, this effect was only seen if the piglets received bovine colostrum replacer supplementation for at least 7 d. A potential reason for the delayed gain effect is that moving individually reared animals into group housing is a stressor that often results in increased oxidative stress (Rubio et al., 2021), reduced starter intake, and decreased ADG (De Paula Vieira et al., 2010). In response to oxidative stress from weaning, the activity of superoxide dismutase and glutathione peroxidase is increased (Majlesi et al., 2021). Bovine colostrum contains a variety of enzymatic and non-enzymatic antioxidants, including superoxide dismutase and glutathione peroxidase (Przybylska et al., 2007). Thus, bovine colostrum replacer supplementation may have mitigated the stress of moving calves into group housing by increasing their total antioxidant defenses. Additionally, the growth factors also present in bovine colostrum (Blum and Hammon, 2000) may have stimulated intestinal tissue growth to improve absorptive capacity (Xu et al., 2002; Pyo et al., 2020), which could also explain the lag in observed gain benefits. Nonetheless, although bovine colostrum replacer

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### Table 4. Effect of bovine colostrum replacer supplementation on intestinal permeability1 in 65 calves randomly assigned to 1 of 2 treatments2

<table>
<thead>
<tr>
<th>Variable</th>
<th>CON</th>
<th>COL</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (95% CI)</td>
<td>Mean (95% CI)</td>
<td>Trt</td>
</tr>
<tr>
<td>Cr-EDTA (ppm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 56</td>
<td>28.50 (24.53–33.12)</td>
<td>26.58 (23.10–30.57)</td>
<td>0.58</td>
</tr>
<tr>
<td>d 65</td>
<td>29.96 (25.79–34.81)</td>
<td>29.67 (25.79–34.12)</td>
<td></td>
</tr>
<tr>
<td>Lactulose (μg/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 56</td>
<td>6.11 (5.26–7.17)</td>
<td>5.75 (5.00–6.89)</td>
<td>0.94</td>
</tr>
<tr>
<td>d 65</td>
<td>6.89 (5.93–8.08)</td>
<td>7.24 (6.23–8.33)</td>
<td></td>
</tr>
<tr>
<td>Mannitol (μg/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 56</td>
<td>4.95 (4.48–5.53)</td>
<td>4.95 (4.48–5.42)</td>
<td>0.80</td>
</tr>
<tr>
<td>d 65</td>
<td>6.11 (5.53–6.82)</td>
<td>6.36 (5.75–7.03)</td>
<td></td>
</tr>
<tr>
<td>LMR3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d 56</td>
<td>1.23 (1.13–1.34)</td>
<td>1.17 (1.08–1.27)</td>
<td>0.72</td>
</tr>
<tr>
<td>d 65</td>
<td>1.13 (1.03–1.22)</td>
<td>1.14 (1.04–1.24)</td>
<td></td>
</tr>
</tbody>
</table>

1Intestinal permeability was determined by measuring recovery of Cr-EDTA, lactulose, and mannitol from plasma after oral administration of markers on the day before weaning (d 56) and the day following the completion of weaning (d 65).

2Control (CON; n = 31): no colostrum supplementation during the weaning period; colostrum (COL; n = 34): once daily supplementation of 119 g bovine colostrum replacer during the weaning period.

3Lactulose-to-mannitol ratio (LMR)
supplementation may have mitigated these stressors, the exact mechanisms are not understood.

Given that weaning has been shown to compromise total-tract barrier function (Wood et al., 2015), we speculated that improved intestinal barrier function would lead to improved weight gain, as increases in intestinal permeability reduce nutrient absorption (Gaebel et al., 1989). Piglets supplemented with bovine colostrum replacer had improved intestinal integrity after weaning (Huguet et al., 2007; King et al., 2008). Supplementing weaning piglets with bovine colostrum replacer has been shown to stimulate migration of duodenal epithelial cells for mucosal restoration (Huguet et al., 2007), as well as reduce the gene expression of inflammatory cytokines to minimize intestinal inflammation and permeability (Lo Verso et al., 2020). However, we did not detect a difference in intestinal permeability between groups. Nonetheless, weaning does not only compromise intestinal barrier function, but also induces anatomical and functional disorders of the intestines (Pié et al., 2004; Malmuthuge et al., 2013). Therefore, the mechanism for increased gain in colostrum-supplemented calves may be improved intestinal absorptive capacity. In weaned piglets, supplementation of bovine colostrum replacer increased villus perimeter (Huguet et al., 2007) and height (King et al., 2008), which directly improves absorptive capacity (Puske et al., 1997) as well as growth (Wang et al., 2019). Similarly with calves, colostrum supplementation has improved the functional capacity of the intestines by increasing villus height and mucosal surface area (Pyo et al., 2020). However, it is unclear whether the weight gain improvement observed here was the result of improved feed intake, improved nutrient absorption from increased absorptive capacity, or both, as we did not monitor starter intakes beyond d 70, nor did we dissect calves to examine the morphology of their intestines.

We did not observe health benefits by supplementing calves with bovine colostrum replacer during weaning. This contrasts previous studies in which bovine colostrum replacer supplementation improved fecal scores in weaning piglets (Huguet et al., 2012) and in calves with diarrhea (Carter et al., 2022). Also, in contrast to our findings, Cantor et al. (2021) demonstrated that bovine colostrum replacer could reduce the likelihood of BRD and lung consolidation when fed as therapy to calves with negative deviations in milk intake or drinking speed. However, our inability to detect health benefits in colostrum-supplemented calves may be due to the low incidence of diarrhea and respiratory disease. Our sample size was not based on detecting differences in disease risks. Additionally, in contrast to our study, Cantor et al. (2021) and Huguet et al. (2012) both used de-fatted bovine colostrum replacer sources, which may have altered the colostral bioactive profile and/or concentrations of bioactives, and thus the health responses. Moreover, Cantor et al. (2021) and Carter et al. (2022) supplemented calves during a time when maternal immunity was waning, but the components of the active immune system were not yet fully functional (Chase et al., 2008), which may explain why colostrum intervention ameliorated illness in those studies but not ours. Nonetheless, the lack of treatment effects on health outcomes suggests that the observed weight gain improvement was not due to reductions in the incidence of diarrhea, BRD, or lung consolidation, all of which are known to reduce ADG (Soberon et al., 2012; Dunn et al., 2018; Abuelo et al., 2021). Moreover, despite the lack of health benefits from bovine colostrum supplementation, the gain benefits may have important economic considerations. The investment of supplementing calves with 119 g of bovine colostrum replacer over the 8-d weaning period was $48.50 per calf; however, the improvement of 100 g/d ADG could potentially contribute to 820 kg more milk to the end of the third lactation (Soberon et al., 2012), equating to $762 additional revenue based on Canada’s milk price of $93.00 per standard hectolitre (Canadian Dairy Commission, 2023) or $361 based on a $20.00 per 45.4 kg (100 pounds) US milk price (National Agricultural Statistics Service, 2024). Additionally, the return on investment may be greater if the minimum efficacious dose is lower than the 119 g dose used in this study.

Current recommendations are to wean calves gradually in a stepwise manner to minimize growth losses (Sweeney et al., 2010; Steele et al., 2017). However, this study had a relatively short and abrupt weaning process of 8 d, which despite current recommendations, is still practiced by a meaningful number of dairy farms (Medrano-Galarza et al., 2017; Barry et al., 2020). As such, the results of this study may not be applicable if calves were weaned over a longer time. Although we controlled for energy intake in our study, we did not control for crude protein or fat, which may have contributed to the improved gain in colostrum-supplemented calves. However, in previous studies examining the effect of increased fat or protein on weight gain, when metabolizable energy intakes were equal, the observed effects occurred during the feeding period (Echeverry-Munera et al., 2021; Kim et al., 2022), whereas our gain effects were delayed until at least 2 weeks after the supplementation ended. As this was the first investigation of bovine colostrum replacer to reduce weaning stress, the optimal dose is unknown and should be explored. Moreover, we were not able to determine the mechanism of action for improved gain in colostrum-supplemented calves and, thus, should be investigated in future research.
CONCLUSIONS
Calves that were supplemented with 119 g of bovine colostrum replacer once daily during an 8-d weaning period had improved weight gain, but only 2 and 3 weeks after weaning. Nonetheless, supplementation of bovine colostrum during weaning may help improve bodyweight gain during weaning. We did not detect effects of bovine colostrum replacer supplementation on disease occurrence or intestinal permeability; however, as calves in this study were generally healthy, this may have limited our ability to detect differences in intestinal permeability and health outcomes. Thus, the mechanism for this weight gain advantage in colostrum-supplemented calves remains to be elucidated.

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