Evaluating the effectiveness of colostrum as a therapy for diarrhea in preweaned calves

H. S. M. Carter, M. A. Steele, J. H. C. Costa, and D. L. Renaud

1Department of Population Medicine, University of Guelph, Guelph, ON, Canada, N1G 2W1
2Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada, N1G 2W
3Department of Animal and Food Science, University of Kentucky, Lexington 40506

ABSTRACT

Diarrhea is the primary cause of morbidity and mortality in dairy calves. Many cases of diarrhea in calves are treated with antimicrobials, increasing the risk of antimicrobial resistance, therefore, creating a need for alternative therapies. The objective of this study was to evaluate the effects of feeding spray-dried maternal derived bovine colostrum replacer at the onset of diarrhea on calf growth and duration and severity of the disease in preweaning dairy calves. At a calf-raising facility in southern Ontario, calves were scored for fecal consistency twice daily on a scale of 0 to 3 and enrolled into the trial when they had 2 consecutive fecal scores of 2 (runny or spreads readily) or one fecal score of 3 (liquid consistency, splatters). Calves were then randomly allocated to receive one of the following 3 treatments: (1) control (CON; n = 35): 8 feedings over 4 d of 2.5 L of milk replacer at a concentration of 130 g/L (26% crude protein and 17% fat); (2) short-term colostrum supplementation (STC; n = 35): 4 feedings over the first 2 d of 2.5 L of a mixture of milk replacer at 65 g/L and bovine colostrum replacer at 65 g/L (26% IgG and 14.5% fat) followed by 4 feedings over 2 d of 2.5 L of milk replacer at a concentration of 130 g/L; or (3) long-term colostrum supplementation (LTC; n = 38): 8 feedings over 4 d of 2.5 L of a mixture of milk replacer at 65 g/L and bovine colostrum replacer at 65 g/L. Serum IgG was determined at arrival to the facility and body weight, days to enrollment since facility arrival, and severity of diarrhea were recorded at enrollment. Daily health exams evaluating fecal consistency were performed for 28 consecutive days after enrollment and body weight was measured at d 0, 1, 2, 3, 4, 7, 14, 21, 28, 42 and 56 after enrollment. The median days to resolution of a case of diarrhea was 3.5 d (range: 0.5–11.5 d), 2.75 d (range: 0.5–11.0 d), and 2.75 d (range: 0.5–7.0 d) in CON, STC, and LTC, respectively. Using a Cox proportional hazards model, it was found that calves in LTC group had faster resolution of diarrhea compared with calves in the CON group. In addition, there was an association between both days to enrollment since facility arrival and body weight and resolution of diarrhea, where calves who were at the facility longer before enrollment and heavier at the onset of diarrhea, resolved diarrhea quicker. In addition, calves with a fecal score of 3 at enrollment took longer to resolve their case of diarrhea. With respect to body weight, a linear regression model was built and found that over the 56 d following enrollment calves in the LTC treatment grew 98 g/d more than calves in the CON group. These results suggest that bovine colostrum may be an effective therapy for diarrhea in preweaning calves.

Key words: morbidity, treatment, preweaning

INTRODUCTION

Diarrhea has been reported to cause 56% of the disease and 32% of the deaths in preweaning dairy calves (Urie et al., 2018). This disease poses significant welfare and health concerns, such as dehydration, anorexia, reduced immune function, and death, as well as reduced levels of growth and an increased risk of developing respiratory disease (Schinwald et al., 2022). In addition, diarrhea in preweaning calves can result in long-term economic and production consequences such as poorer reproduction and lower levels of milk production in their first lactation (Bartels et al., 2010; Abuelo et al., 2021). Beyond consequences surrounding productivity, there is concern surrounding the overuse of antimicrobials in cases of diarrhea. Specifically, studies suggest that three-fourths of gastrointestinal diseases in calves are treated with antimicrobials; however, less than half of the producers have designed a protocol to determine when to administer antimicrobial treatments (Urie et al., 2018; Uyama et al., 2022). Exposure to antimicrobials in early life can also lead to long-lasting immunocompromising effects due to alterations to the gut microbiota, resulting in dysfunction of the gastro-
intestinal tract (GIT; Helander and Fändriks, 2014; Oultram et al., 2015; Van Vleck Pereira et al., 2016). Additionally, the heavy reliance on antimicrobials can lead to the development of resistant pathogens, such as strains of Salmonella and Escherichia coli (Malmuthuge et al., 2015; Scott et al., 2018). Therefore, investigation into effective alternatives to antimicrobials is vital to aid in diminishing health and societal concerns related to antimicrobial usage.

Bovine colostrum may be a potential therapy for diarrhea and alternative to antimicrobials. Both colostrum and spray-dried colostrum replacer contain high concentrations of bioactive factors (i.e., antibodies, hormones, growth factors, oligosaccharides, and fatty acids) and nutrients naturally tailored to optimize calf health and immunity (Godden et al., 2019; Carter et al., 2021; Fischer-Thustos et al., 2021). Colostrum’s beneficial qualities have been used for centuries to provide supplemental nutrition and as a therapy for numerous illnesses, including diarrhea in humans (Kim et al., 2009; Rathe et al., 2014; Ulfman et al., 2018). Recently, the use of colostrum in calves has been explored beyond the first feeding after calving and many advantages have been identified (Berge et al., 2009b; Chamorro et al., 2017; Kargar et al., 2020; Cantor et al., 2021). Specifically, these experiments suggest that there may be benefits to using bovine colostrum as a prophylactic to prevent diarrhea in calves by feeding colostrum after the first day of life. Several benefits were identified, including improved growth rates of diseased calves, reduced incidence and duration of other diseases, including bovine respiratory disease and diarrhea, as well as reduced antimicrobial usage and mortality. Despite these positive benefits of supplementing colostrum beyond the first feeding after calving, evidence supporting the use of maternal derived bovine colostrum and colostrum replacer as a therapy for diarrhea in calves is lacking.

The primary objective of this randomized controlled trial was to evaluate the effect of bovine colostrum on diarrhea resolution in young dairy calves. A secondary objective was to determine the effect of colostrum in diarrheic calves on growth over the 56-d experimental period. We hypothesized that feeding calves bovine colostrum at the onset of diarrhea would decrease the length and severity of the disease while improving growth.

### MATERIALS AND METHODS

This randomized control trial was conducted at a commercial calf-raising facility in southwestern Ontario from June 7 to July 19, 2021. This facility was chosen based on its capability to conduct this study and proximity to the University of Guelph. The study was approved by the University of Guelph Animal Care Committee (AUP:4551). This manuscript is reported following the Reporting Guidelines for Randomized Controlled Trials for Livestock and Food Safety (REFLECT) statement (Sargeant et al., 2010).

### Housing and Feeding

Calves arrived at the participating farm at approximately 3 to 7 d of age. However, the exact age was not known as data collection began at arrival to the facility. Calves were sourced from auctions, drovers, and local dairy producers. Two batches of 80 calves were used; they arrived 21 d apart and were housed in rooms with 80 individual 1 m² pens with slatted partitions and rubber flooring. From d 0 to 20, calves were fed 2.5 L of milk replacer at a concentration of 130 g/L (Table 1; custom mix, Mapleview Agri Ltd.). Between d 21 and 27, the milk allowance was increased to 3 L of milk replacer twice daily. On d 28, the milk allowance was increased again to 3.5 L of milk replacer 2 twice daily. From d 35 to 41, milk allowance was decreased to 3 L of milk replacer twice daily. From d 42 to 49, milk allowance was decreased again, with calves receiving 2 L of milk replacer twice daily, and from d 49 to 55 calves were fed 2 L of milk replacer only once per day. Refusals were measured and recorded by weight. Calves had ad libitum access to texturized calf starter (18% CP, 26.5% starch, 17.3% NDF, 3.6% crude fat, and 5.6% ash; custom mix, Wallenstein Feed and Supply).

### Table 1. As-fed macronutrient composition of milk replacer and bovine colostrum replacer fed to calves enrolled into 1 of 3 treatments at the onset of diarrhea¹

| Component | Milk replacer (MR) | Colostrum replacer (CR) | Mixture (MR and CR) |
|-----------|-------------------|------------------------|---------------------|
| Moisture (%) | 3 | 5.8 | 4.4 |
| CP (%) | 26 | 56.9 | 41.5 |
| IgG (%) | 26 | 33 |
| Fat (%) | 20 | 14.5 | 17.3 |
| Lactose2 (%) | 44 | 11 | 27.5 |
| ME3 (Mcal/kg) | 4.71 | 4.66 | 4.70 |

¹Control (n = 35): 8 feedings over 4 d of 2.5 L of MR at a concentration of 130 g/L; short-term colostrum supplementation (n = 35): 4 feedings over the first 2 d of 2.5 L of a mixture of MR at 65 g/L and colostrum replacer at 65 g/L followed by 4 feedings over 2 d of 2.5 L of MR at a concentration of 130 g/L; or long-term colostrum supplementation (n = 38): 8 feedings over 4 d of 2.5 L of a mixture of MR at 65 g/L and colostrum replacer at 65 g/L.

²Lactose was calculated with the following equation: lactose = 100 − CP − fat − ash (Quigley, 2007).

³ME was calculated using the following equation: gross energy (Mcal/kg) = 0.057 × CP% + 0.092 × fat% + 0.0395 × lactose%. ME (Mcal/kg) = gross energy × 0.97 × 0.96 (Quigley, 2007).
chopped straw, and water throughout the entirety of the study.

Upon arrival to the calf-rearing facility, calves were weighed using a Tru-Test digital weight scale (Mineral Wells), and blood samples were taken via jugular venipuncture with a 10-mL sterile vacutainer tube (BD Vacutainer Serum Blood Collection Tubes; Becton, Dickinson and Co.) and put on ice for 2 h until centrifugation occurred. Blood serum was separated by centrifugation (3,000 × g at 4°C for 15 min) and frozen at −20°C until serum IgG concentration determination using radial immunodiffusion (Shivley et al., 2018).

Blood samples were randomly allocated to receive one of three treatments: (1) control (CON), (2) short-term colostrum supplementation (STC), and (3) long-term colostrum supplementation (LTC). Calves were assigned to each treatment by observers according to the RAND command in Microsoft Excel (Version 16.21.1, Microsoft Corp.) generated by the research team. Moreover, calves were fed a dose of 2 L at 115 g/L of electrolytes when a milk refusal of over 25% was recorded or when signs of dehydration (sunken eyes, prolonged skin tent, or change in attitude) appeared.

Once enrolled, calves were followed for 56 d. Fecal consistency score was recorded for each calf twice daily with a resolution of diarrhea defined as having 2 consecutive fecal scores of ≤1. Weights were recorded on 0, 1, 2, 3, 7, 14, 21, 28, 42, and 56 d after enrollment using a Tru-Test digital weigh scale. Additionally, antimicrobial treatments, mortality, and milk refusals were recorded throughout the study. With respect to antimicrobial therapy for diarrhea, if a calf did not consume all milk offered or had a depressed attitude, it was administered trimethoprim sulphadioxide (Borgal; MSD Animal Health) intramuscularly at a dose of 3 mL/45 kg of BW and provided with 1 L of Lact-R (Bimeda-MTC) subcutaneously. If a calf showed signs of respiratory disease, it was treated subcutaneously using florfenicol (Nufloc; Merck) at a dose of 6 mL/45 kg of BW. If a relapse occurred, ceftiofur (Excenel; Zoetis Inc.) was administered for 4 consecutive days at a dose of 2 mL/45 kg.

Fecal samples were taken at enrollment, split into triplicates, and stored at −20°C until they were sent for viral, bacterial, and parasitological analysis at a commercial laboratory (Animal Health Laboratory, Guelph, ON, Canada). Fecal samples were analyzed for rotavirus, coronavirus, and Cryptosporidium parvum using real-time PCR testing. For PCR testing, each fecal sample was swabbed and resuspended in PBS to make a 10% suspension. The suspension was vortexed and centrifuged before extraction. All samples were extracted using the MagNA Pure 96 (Roche) and the PCR was run on the Light Cycler 480 (Roche). The feces were swabbed and plated on MacConkey agar and Hektoen agar and incubated at 35°C in atmospheric conditions for a total of 48 h to evaluate bacterial isolation. Plates were read at both 24 and 48 h for E. coli growth.

**Sample Size Calculation**

The sample size was calculated based on the anticipated time to resolution of diarrhea of 3 d (SD = 2.5) in the LTC group compared with 5 d (SD = 2.5) in the CON group (Katsoulos et al., 2017; Renaud et al., 2019). Using a 95% confidence interval and a power of 80%, it was determined that 78 calves (26 per treatment) would be required for this study.

**Statistical Analysis**

All statistical analyses were conducted in Stata 17 (StataCorp LP). Data were imported from Microsoft
Excel (version 16.21.1, Microsoft Corp.) into Stata17 and checked for completeness. A causal diagram was created to evaluate relationships between the explanatory variables and the outcome of interest (Figure 1). Descriptive statistics were generated on all explanatory variables in the data set using a one-way ANOVA or Kruskal-Wallis test to describe continuous normally distributed or non-normally distributed variables, respectively. A \( \chi^2 \) test was used for categorical variables.

Cox proportional hazards models were built to investigate the number of days to resolution of an abnormal fecal consistency score as well as antimicrobial treatments. Resolution of diarrhea was defined as having 2 consecutive fecal scores of \( \leq 1 \). A mixed linear regression model was created to evaluate the effect of treatment on ADG \([(BW \text{ at } d \ 56 - \text{weight at enrollment})/56]\) over the experimental period, whereas a repeated measures linear regression model was used to assess growth at the different time points weight was collected. In addition, a repeated measure logistic regression model was created to evaluate the effect treatment had on refusals, whereas an exact logistic regression model was used to analyze mortality. In all statistical models, room was used as a random effect to control for variation that existed between the rooms of 80 calves.

The assumption of linearity of continuous explanatory variables was assessed in each model by plotting Lowess smoothing curves. If the predictor failed to meet the assumptions of linearity, the variable was categorized. Specifically, serum IgG concentration at arrival to the facility was categorized based on a scale developed by Lombard et al. (2020; \( \leq 10, 10.0-17.9, 18.0-24.9, \) or \( \geq 25 \) g/L representing poor, fair, good, and excellent transfer of passive immunity, respectively), and BW at enrollment and days to enrollment were categorized into quartiles. Univariable regression models were built using a \( P \)-value \( (P < 0.2) \) to screen for variables that were associated with the outcome. Pearson and Spearman correlation coefficients were used to assess collinearity between variables. If either of the coefficients exceeded 0.8, the variable was classified as collinear and 2 models were built for collinear variables, with the variable from the model with the lowest Akaike information criterion and Bayesian information criterion being retained. A manual backward selection process was used where all risk factors that had univariate associations \( (P < 0.2) \) were offered into multivariable models. All variables that were removed from the model were assessed for confounding by evaluating the effect of the removed variables on the remaining variables. A variable was deemed as a confounder if it was not an intervening variable based on the causal diagram and if the coefficient of a variable with statistical significance changed by at least 20%. Biologically plausible interaction terms were assessed, and variables remained in the final model if they were statistically significant \( (P < 0.05) \).

The proportional hazards assumption of the Cox proportional hazards models was tested using Shoenfeld residuals. In all models except the Cox proportional hazards model, we assessed the homogeneity of random effects at each level by graphing the best linear unbiased estimators against their predicted values. We also assessed the distribution of random effects at each level.
RESULTS

Descriptive Results

Of the 160 calves brought to the facility, a total of 108 male Holstein dairy calves were enrolled in the study with 35 calves enrolled in both the CON and STC treatment group and 38 calves enrolled in the LTC treatment group. Flow through the study is shown in Figure 2. The majority of the calves were sourced directly from producers within a 30 km radius of the calf-rearing facility (38%) and auction facilities (37%), with the remaining quarter of the calves being sourced from drovers (25%). The mean serum IgG concentration at arrival to the facility was 19.6 g/L ± 11.33 g/L (mean ± SD) and ranged from 0.7 to 58.2 g/L. Approximately one-third of the calves had a fair (10–17.9 g/L) concentration of serum IgG (32%), followed by excellent (>25 g/L; 29%), poor (<10 g/L; 21%) and good (18–24.9 g/L) levels of IgG (18%). The average BW at enrollment was 52 ± 4.8 kg and ranged from 41.7 to 64 kg. The average days to enrollment was 4.9 ± 1.75 d and ranged from 2 to 8 d. Half of the calves were enrolled with 2 consecutive fecal scores of 2 (49%) and half were enrolled with one fecal score of 3 (51%). Rotavirus (40%) was the most common pathogen detected at enrollment, followed by Cryptosporidium parvum (26%), coronavirus (21%), and E. coli (13%). Half of the calves had 2 pathogens identified in their fecal samples (48%), the remainder had 3 pathogens (30%), one pathogen (14%), and no pathogens (8%) identified. There were no statistically significant differences between groups with respect to BW at enrollment ($P = 0.09$), days to enrollment ($P = 0.94$), IgG concentrations ($P = 0.21$), pathogen load at enrollment ($P = 0.35$), severity of diarrhea ($P = 0.98$), or the facility where the calf was sourced ($P = 0.72$).

Refusals

A total of 122 refusals of ≥25% of a feeding (14% of all feedings) were recorded during the first 8 feedings after enrollment. Most refusals occurred in the CON group (38%), followed by the LTC group (35%), with the fewest in the STC group (27%). Refusals occurred mostly in the first (16%) and second (20%) feeding after enrollment and decreased thereafter. No differences were found in refusals between the STC ($P = 0.92$) group and LTC ($P = 0.86$) group compared with the control group (Figure 3).
Resolution of Diarrhea

The median days for resolution of an abnormal fecal score was 3.5 d (range = 0.5–11.5), 2.75 d (range = 0.5–11), and 2.75 d (range = 0.5–7) in the CON, STC, and LTC groups, respectively. Using a Cox proportional hazards model, treatment group ($P = 0.02$), serum IgG concentration ($P = 0.19$), BW at enrollment ($P = 0.05$), days to enrollment following facility arrival ($P = 0.001$), severity of diarrhea ($P = 0.11$), and the number of pathogens detected at enrollment in fecal samples ($P = 0.18$) were significant in univariable analysis. In the final multivariable model, treatment group, severity of diarrhea, BW at enrollment, days to enrollment following arrival, and the number of pathogens identified were significant (Table 2). Specifically, calves enrolled into the LTC colostrum group resolved faster compared with calves in the CON (Table 2). In addition, calves enrolled with a fecal score of 3 took longer to resolve their diarrhea, whereas calves with a higher BW at enrollment, calves enrolled more than 4 d after arrival, and those infected by only one pathogen compared with multiple pathogens resolved their diarrhea faster (Table 2). A Kaplan-Meier survival function visually illustrates the difference between treatment groups in terms of the days to resolution of an abnormal fecal consistency score (Figure 4).

Antimicrobial Therapy for Diarrhea

A total of 44/108 (41%) calves were treated for diarrhea with antimicrobials. Calves in the STC group received the most treatments (17/44, 39%) followed by the CON group (15/44, 34%), and the LTC group (12/44, 27%). A Kaplan-Meier failure function was used to visually identify the proportion of calves treated for diarrhea with antimicrobials (Figure 5a). Body weight at enrollment had a significant effect on antimicrobial treatments for diarrhea at the univariable level and was added at the multivariable level. However, in the final model, solely BW at enrollment was significant and treatment group was forced into the model. Specifically, with respect to BW at enrollment, the hazard of treatment using antimicrobials for diarrhea was significantly lower for calves weighing greater than 55.8 kg at enrollment [hazard ratio (HR) = 0.09, $P = 0.03$, 95% CI = 0.01–0.79] compared with calves weighing $\leq$49.1 kg. No differences were found concerning the hazard of...
antimicrobial therapy for diarrhea between STC (HR = 1.5, \( P = 0.40, 95\% \ CI = 0.58–3.89\)) and LTC (HR = 0.97, \( P = 0.96, 95\% \ CI = 0.37–2.55\)) groups compared with the CON group.

### Antimicrobial Therapy for Respiratory Disease

Throughout the study, 27 (25\%) calves were treated by observers for respiratory disease using antimicrobials, with 9 (33\%) calves treated in the CON group, 9 (33\%) calves in the STC group, and 9 (33\%) calves treated in the LTC group. A Kaplan-Meier failure function was generated to illustrate the proportion of calves treated for respiratory diseases with antimicrobials (Figure 5b). At the univariable level, severity of diarrhea had a statistically significant effect on antimicrobial treatments for respiratory disease. In the final Cox proportional hazards model, treatment group was forced into the model, however, severity was the sole variable found to be associated with treatment for respiratory disease. Calves enrolled with one fecal score of 3 had a significantly lower hazard for treatment of respiratory disease with antimicrobials compared with calves enrolled with 2 consecutive fecal scores of 2 (HR = 4.27, \( P = 0.01, 95\% \ CI = 1.43–12.8\)). No differences were found between the LTC (HR = 0.74, \( P = 0.60, 95\% \ CI = 0.25–2.21\)) and STC (HR = 0.98, \( P = 0.97, 95\% \ CI = 0.34–2.79\)) groups compared with the CON group with respect to hazard of treatment of respiratory disease using antimicrobials.

### Mortality

During the study, 8 calves died (7\%), the majority in the CON group (5/8, 63\%), followed by the STC group (3/8, 37\%), and no deaths in the LTC group. A Kaplan-Meier failure function was used to illustrate the proportion of calf mortality after enrollment separated by treatment group (Figure 5c). At the univariable level, there were no significant explanatory variables for mortality using an exact logistic regression model. Specifically for the treatment group, no differences were found between the STC group (odds ratio = 0.55, \( P = 0.66, 95\% \ CI = 0.08–3.11\)) and LTC group (odds ratio = 0.16, \( P = 0.16, 95\% \ CI = 0.003–1.60\)) compared with the CON group at the univariable level.

### Growth Following Enrollment

The mean ADG from enrollment to d 56 following enrollment was 0.82 ± 0.22 kg/d, with the CON group having an ADG of 0.80 ± 0.18 kg/d, STC group having a mean ADG of 0.74 ± 0.24 kg/d, and the LTC group having a mean ADG of 0.90 ± 0.19 kg/d. Body weight at enrollment and treatment group were unconditionally associated with ADG and were included in the mixed linear regression model. In the final model, it was found that BW at enrollment and treatment group were statistically significant. Specifically, calves with a BW of 49 to 51.7 kg at enrollment gained less than calves weighing 48.9 kg or less, whereas calves in the

### Growth Following Enrollment

The mean ADG from enrollment to d 56 following enrollment was 0.82 ± 0.22 kg/d, with the CON group having an ADG of 0.80 ± 0.18 kg/d, STC group having a mean ADG of 0.74 ± 0.24 kg/d, and the LTC group having a mean ADG of 0.90 ± 0.19 kg/d. Body weight at enrollment and treatment group were unconditionally associated with ADG and were included in the mixed linear regression model. In the final model, it was found that BW at enrollment and treatment group were statistically significant. Specifically, calves with a BW of 49 to 51.7 kg at enrollment gained less than calves weighing 48.9 kg or less, whereas calves in the
LTC group gained 98 g/d more than the CON group ($P = 0.04$; CI = 0.01–0.19; Table 3).

A repeated measure linear regression model was used to highlight differences in BW over the different time points BW was collected. In the final model, it was found that BW at enrollment ($P = 0.01$), time point ($P = 0.01$), and an interaction between treatment group and time ($P = 0.01$) were significant. A plot was generated to illustrate the change in BW using weight at enrollment as a covariate (Figure 6). The LTC group had a significantly higher body weight at d 42 and 56, weighing 4.21 kg ($P = 0.002$) and 6.03 kg ($P < 0.001$) more than the CON group, respectively. However, the STC group weighed 3.18 kg ($P = 0.02$) less than the CON group at 42 d.

**DISCUSSION**

Diarrhea is the most common disease in young dairy calves. With its high prevalence, this disease can have substantial economic consequences due to associated therapeutic costs and long-term production repercussions (Abuelo et al., 2021). Additionally, the antimicrobials used to treat diarrhea can trigger long-lasting effects that negatively affect calf immunity through the modification of the indigenous GIT microbiota (Hlander and Fändriks, 2014; Oultram et al., 2015). In this study, we found that using spray-dried maternal derived bovine colostrum replacer as a therapy for diarrhea in preweaning calves could be beneficial as it reduced the time to resolution of an abnormal fecal score and improved growth after a bout of diarrhea.

A calf’s gut is sterile in utero, as their protective environment in utero inhibits the opportunity to develop an adaptive immune system; therefore, it is vital that they are supplied with immunoglobulins to ensure overall health and survival before closure of the gut (Chase et al., 2008; Godden et al., 2019). Although immunoglobulins are most effective during the first day of life, supplementation further into the preweaning period can still provide benefits in the GIT by binding to pathogens in the intestinal mucosal membrane, thus reducing their intrusion capabilities (Hilpert et al., 1987; Rump et al., 1992). The supplementation of antibodies has been observed to reduce diarrhea symptoms in calves demonstrating their direct association with disease resolution (Besser et al., 1987; Kuroki et al., 1997; Parreño et al., 2010). The isolation of specific immunoglobulins from chicken egg yolk or bovine colostrum such as IgY, as well as therapy using hyperimmunized bovine colostrum and chicken egg yolk, fed orally, has shown to reduce diarrhea and mortality rates, as well as improve weight gain (Kuroki et al., 1997; Ikemori et al., 1997; Shimizu et al., 1988). Additionally, injectable colostral
whey with high concentrations of IgG rotavirus antibody titer reduced the effects of a rotavirus challenge in neonatal calves (Besser et al., 1987). Similarly, the oral supplementation of hyperimmunized bovine colostrum has been reported to improve clinical symptoms and virus shedding in newborn calves challenged with rotavirus (Parreño et al., 2010). The aforementioned studies provide evidence that immunoglobulins may play a major role in the reduction of the time to resolution of diarrhea in calves after closure of the gut.

In addition to immunoglobulins, colostrum is rich in several other components with antimicrobial properties, including lactoferrin, lactoperoxidase, and lysozymes (Pakkanen and Aalto, 1997). These bioactives are able to inhibit microbial growth, reduce binding capacity of pathogens, and facilitate cell lysis of invading bacteria (Batish et al., 1988; Pakkanen and Aalto, 1997). Moreover, several hormones and growth factors in colostrum, such as insulin and IGF, are involved in epithelial cell growth and repair, thus improving gut health (Pakkanen and Aalto, 1997; Steinhoff-Wagner et al., 2011). These components demonstrate that colostrum has protective characteristics and development enhancing capabilities on the GIT (Shen et al., 2015; Pyo et al., 2020). Colostrum also contains casein and α-LA, peptides with the ability to improve tissue repair and the GIT mucosal integrity, as well as reduce gut inflammation (Matsumoto et al., 2001; Stelwagen et al., 2009; Playford and Weiser, 2021). Furthermore, the concentrations of oligosaccharides and specific fatty acids are vast compared with milk and boast immune regulating and prophylactic capabilities that may improve GIT health, systemic inflammation, and oxidative status (Fischer-Tlustos et al., 2020; Opgenorth et al., 2020a,b). Altogether, the immune enhancing and reparative abilities of colostrum may have played a role in reducing the time to resolution of diarrhea leading to improved welfare and growth of the calves in this study.

Bovine colostrum has been evaluated as a therapeutic for diarrhea in several studies across many different species. It has been determined that the severity and duration of acute diarrhea in children can be reduced with colostrum therapy (Davidson et al., 1989; Rathe et al., 2014; Barakat et al., 2020). Similarly, bovine colostrum has been found to reduce diarrhea incidence and the growth of diarrhea causing pathogens in piglets (Sugiharto et al., 2015). Despite these studies, no studies have been conducted using bovine colostrum or colostrum replacer as a therapy for diarrhea in preweaning calves, although certain antimicrobial alternatives have been explored. Specifically, a study evaluating the benefits of a mixed species probiotic bolus at the onset of diarrhea found that it resolved diarrhea 0.8 d faster compared with a control (Renaud et al., 2019). In addition, a study evaluating the time to resolution of diarrhea using Greek oregano essential oil found resolution to be 1.32 d faster than the control (Katsoulas et al.)

Figure 5. Kaplan-Meier failure curve investigating (a) treatment of diarrhea with antimicrobials, (b) treatment of respiratory disease with antimicrobials, and (c) mortality after enrollment of individually housed male Holstein calves randomly allocated to 1 of 3 treatment groups at the onset of diarrhea: control (CON; n = 35): 8 feedings over 4 d of 2.5 L of milk replacer (MR) at a concentration of 130 g/L; short-term colostrum supplementation (STC; n = 35): 4 feedings over the first 2 d of 2.5 L of a mixture of MR at 65 g/L and colostrum replacer at 65 g/L followed by 4 feedings over 2 d of 2.5 L of MR at a concentration of 130 g/L; or long-term colostrum supplementation (LTC; n = 38): 8 feedings over 4 d of 2.5 L of a mixture of MR at 65 g/L and colostrum replacer at 65 g/L.
et al., 2017). In comparison, we found that the LTC group had faster resolution of diarrhea with a mean difference in resolution of 1.36 d between the LTC and CON group. Comparing these studies highlights that colostrum has potential benefits as a therapy for diarrhea in preweaning calves.

Beyond differences in time to resolution, the LTC treatment also had a positive effect on growth over the 56 d following enrollment, with a significantly higher BW at d 42 and 56. Calves who develop diarrhea in the preweaning period have been shown to have a reduced ADG (Donovan et al., 1998; Anderson et al., 2003; Abuelo et al., 2021) due to damage the intestinal epithelium resulting in reduced digestion and absorption capabilities in the GIT (Nydam and Mohammed, 2005). In addition, there are longer term effects to calves, with lower levels of growth leading to calves being bred later and having reduced production and economic value (Ettema and Santos, 2004). Therefore, the addition of spray-dried maternal derived colostrum replacer at the onset of diarrhea in preweanign calves has the potential to reduce the resulting production gap of a diseased calf. Colostrum’s ability to reduce the time to resolution of diarrhea, paired with its intestinal regenerative and growth characteristics, present an attractive combination of attributes to stimulate increased growth when used as a treatment in young calves. Similar trends have been observed with respect to growth in previous studies which used colostrum as a prophylactic supplement following birth (Berge et al., 2009a; Kargar et al., 2020). It is important to note that studies evaluating alternative therapeutics for diarrhea showed no evidence of improved growth between their treatments (Katsoulos et al., 2017; Renaud et al., 2019), highlighting the potential additional benefit of colostrum supplementation during a bout of diarrhea.

Although antimicrobial treatments and the level of mortality were numerically lower in the LTC group, no significant differences were identified compared with the CON group. Reliance on treatment protocols and decision making by on-farm personnel using a more liberal therapeutic approach may have posed an issue, although it is possible that with a higher power, a difference in antimicrobial treatments between treatment groups may have been detected. A larger sample size of animals may have offset the limitations posed by lack of protocol adherence, thus enhancing the differences between treatment groups and antimicrobial usage.

There are some limitations to consider when interpreting the results of this study. First, similar to other studies providing colostrum as a prophylactic or therapeutically, no significant differences were identified compared with the CON group. Reliance on treatment protocols and decision making by on-farm personnel using a more liberal therapeutic approach may have posed an issue, although it is possible that with a higher power, a difference in antimicrobial treatments between treatment groups may have been detected. A larger sample size of animals may have offset the limitations posed by lack of protocol adherence, thus enhancing the differences between treatment groups and antimicrobial usage.

Table 3. Results of a mixed linear regression model evaluating predictors affecting growth for 56 d following enrollment at the onset of diarrhea of young calves receiving 1 of 3 treatments

| Variable          | Group      | Coefficient (kg/d) | SE  | 95% CI          | P-value |
|-------------------|------------|--------------------|-----|-----------------|---------|
| Treatment group   | CON Referent | −0.48              | 0.05| −0.15 – −0.53   | 0.35    |
|                   | STC        | 0.98               | 0.05| 0.01 – 0.19     | 0.04    |
|                   | LTC        | −0.13              | 0.06| −0.26 – −0.19   | 0.02    |
| BW at enrollment  | 49–51.7 Referent | −0.17              | 0.06| −0.13 – −0.09   | 0.76    |
| (kg)              | ≥55.9      | 0.02               | 0.06| −0.09 – −0.14   | 0.71    |

1Control (CON; n = 35): 8 feedings over 4 d of 2.5 L of milk replacer (MR) at a concentration of 130 g/L; short-term colostrum supplementation (STC; n = 35): 4 feedings over the first 2 d of 2.5 L of a mixture of MR at 65 g/L and colostrum replacer at 65 g/L; long-term colostrum supplementation (LTC; n = 38): 8 feedings over 4 d of 2.5 L of a mixture of MR at 65 g/L and colostrum replacer at 65 g/L.
py after the first day of life, the colostrum replacer and milk replacer were not isocaloric and isonitrogenous (Berge et al., 2009a; Chamorro et al., 2017; Kargar et al., 2020; Cantor et al., 2021). In this study, all calves were fed on a low plane of nutrition and the calves who were fed milk replacer and the calves who were fed colostrum replacer received different nutrient and energy intakes. Although the feeding rate was equal across treatments and differences between the milk and colostrum replacer energy compositions are small (Table 1), the formulations are not completely comparable to one another. An increased plane of nutrition and as well as several nutrients and bioactives in colostrum have been found to have positive effects on immunity and gut health (Carter et al., 2021; Lorenz et al., 2021); therefore, it is unclear whether specific bioactives in or the macronutrient composition of colostrum resulted in faster resolution of diarrhea. Furthermore, this study did not focus on fluid therapy and did not perform dehydration scoring. Although there was no difference in refusals and all calves were given electrolytes upon enrollment, this could have been a potential variable to explore and should be recorded in future studies. An additional limitation is that in comparison to antimicrobial treatments, the use of this treatment strategy could be initially costly and may not yet be practical for producers at the dose of colostrum replacer used. The optimal dose, duration, and timing for this therapy is still unknown, therefore, future research should further explore these variables to enhance effectiveness of this treatment. By improving practicality and economic considerations while upholding the efficacy of the therapy, bovine colostrum could become a favored therapeutic for diarrhea in preweaning calves.

CONCLUSIONS

Supplementation of 8 feedings over 4 d of 2.5 L of a mixture of milk replacer at 65 g/L and bovine colostrum replacer at 65 g/L compared with 8 feedings over 4 d of 2.5 L of milk replacer at a concentration of 130 g/L was determined to resolve diarrhea sooner and improve growth levels in young dairy calves. This may provide life-long benefits for calves diagnosed with diarrhea in the preweaning period. Due to the preliminary nature of this research, future studies are necessary to determine the most practical and effective dose and duration of the therapy.

ACKNOWLEDGMENTS

Funding for this study was provided by the Ontario Ministry of Agriculture Food and Rural Affairs (Guelph, ON, Canada) and Saskatoon Colostrum Company (Saskatoon, SK, Canada). The authors have not stated any conflicts of interest.

REFERENCES

Abuelo, A., F. Cullens, and J. L. Brester. 2021. Effect of preweaning disease on the reproductive performance and first-lactation milk production of heifers in a large dairy herd. J. Dairy Sci. 104:7008–7017. https://doi.org/10.3168/jds.2020-19791.

Anderson, D. C., D. D. Kress, T. M. M. Bernardini, K. C. Davis, D. L. Boss, and D. E. Doornbos. 2003. The effect of scours on calf weaning weight. Prof. Anim. Sci. 19:399–403. https://doi.org/10.1017/S1080-7444(15)31455-8.

Barakat, S. H., M. A. Meheissen, O. M. Omar, and D. A. Elbana. 2020. Bovine colostrum in the treatment of acute diarrhea in children: A double-blinded randomized controlled trial. J. Trop. Pediatr. 66:46–55. https://doi.org/10.1093/troped/fmd029.

Bartels, C. J. M., M. Holzhauer, R. Jorritsma, W. A. J. M. Swart, and T. J. M. Lam. 2010. Prevalence, prediction and risk factors of enteropathogens in normal and non-normal faeces of young Dutch dairy calves. Prev. Vet. Med. 93:162–169. https://doi.org/10.1016/j.prevetmed.2009.09.020.

Batis, V. K., H. Chander, K. C. Zumdegni, K. L. Bhatia, and R. S. Singh. 1988. Antibacterial activity of lactoferrin against some common food-borne pathogenic organisms – ProQuest. Aust. J. Dairy Technol. 43:16–18.

Berge, A. C. B., T. E. Besser, D. A. Moore, and W. M. Sischo. 2009a. Evaluation of the effects of oral colostrum supplementation during the first fourteen days on the health and performance of preweaned calves. J. Dairy Sci. 92:286–295. https://doi.org/10.3168/jds.2008-1433.

Berge, A. C. B., D. A. Moore, T. E. Besser, and W. M. Sischo. 2009b. Targeting therapy to minimize antimicrobial use in preweaned calves: Effects on health, growth, and treatment costs. J. Dairy Sci. 92:4707–4714. https://doi.org/10.3168/jds.2009-2199.

Besser, T. E., T. C. McGuire, and C. C. Gay. 1987. The transfer of serum IgG1 antibody in the gastrointestinal tract in newborn calves. Vet. Immunol. Immunopathol. 17:51–56. https://doi.org/10.1016/0165-2411(87)90126-7.

Cantor, M. C., D. L. Renaud, and J. H. C. Costa. 2021. Nutraceutical intervention with colostrum replacer: Can we reduce disease hazard, ameliorate disease severity, and improve performance in preweaned dairy calves? J. Dairy Sci. 104:7168–7176. https://doi.org/10.3168/jds.2020-19654.

Carter, H. S. M., D. L. Renaud, M. A. Steele, A. J. Fischer-Tlustos, and J. H. C. Costa. 2021. A narrative review on the unexplored potential of colostrum as a preventative treatment and therapy for diarrhea in neonatal dairy calves. Animals (Basel) 11:2221. https://doi.org/10.3390/ani11082221.

Chamorro, M. F., N. Cernichiaro, and D. M. Haines. 2017. Evaluation of the effects of colostrum replacer supplementation of the milk replacer ration on the occurrence of disease, antibiotic therapy, and performance of pre-weaned dairy calves. J. Dairy Sci. 100:1378–1387. https://doi.org/10.3168/jds.2016-11652.

Chase, C. C. L., D. J. Hurley, and A. J. Reber. 2008. Neonatal immune development in the calf and its impact on vaccine response. Vet. Clin. North Am. Food Anim. Pract. 24:87–104. https://doi.org/10.1016/j.cvfa.2007.11.001.

Davidson, G. P., P. B. Whyte, E. Daniels, K. Franklin, H. Nunnan, P. I. McCloud, A. G. Moore, P. I. McCloud, and D. J. Moore. 1989. Passive immunisation of children with bovine colostrum containing antibodies to human rotavirus. Lancet 2:709–712. https://doi.org/10.1016/S0140-6736(89)90771-X.

Donovan, G. A., I. R. Dooho, D. M. Montgomery, and F. L. Bennett. 1998. Calf and disease factors affecting growth in female Holstein calves in Florida, USA. Prev. Vet. Med. 33:1–10. https://doi.org/10.1016/S0167-5877(97)00059-7.

Ettema, J. F., and J. E. P. Santos. 2004. Impact of age at calving on lactation, reproduction, health, and income in first-parity Hol
Ikemori, Y., M. Ohta, K. Umeda, F. C. Icatlo Jr., M. Kuroki, H. Kargar, S., M. Roshan, S. M. Ghoreishi, A. Akhlaghi, M. Kanani, L. L. Lorenz, I., R. Huber, and Malmuthuge, N., P. J. Griebel, and L. L. Guan. 2015. The gut microbiome and its potential role of colostal biactive components on neonatal calf development and metabolism. Can. J. Anim. Sci. 101:405–426. https://doi.org/10.1139/cjas-2020-0149.

Fischer-Thüstos, A. J., A. Lopez, K. S. Hare, K. M. Wood, and M. A. Steele. 2021. Effects of colostroan management on transfer of passive immunity and the potential role of colostal biactive components on neonatal calf development, nutrient metabolism, and growth. Can. J. Anim. Sci. 101:405–426. https://doi.org/10.1139/cjas-2020-0149.

Helander, H. F., and L. Fändriks. 2014. Surface area of the digestive tract-revisited. Scand. J. Gastroenterol. 49:681–689. https://doi.org/10.1111/sge.12393.

Karger, S., M. Roshan, S. M. Ghoreishi, A. Akhlaghi, M. Kanani, A. R. Abedi Shams-Abadi, and M. H. Ghaffari. 2020. Extended colostroan feeding for 2 weeks improves growth performance and reduces the susceptibility to diarrhea and pneumonia in neonatal Holstein dairy calves. J. Dairy Sci. 103:8130–8142. https://doi.org/10.3168/jds.2020-18355.

Bavarian dairy farms. Animals (Basel) 11:3251. https://doi.org/10.3390/ani11113251.

Malmuthuge, N., P. J. Griebel, and L. L. Guan. 2015. The gut microbiome and its potential role in the development and function of newborn calf gastrointestinal tract. Front. Vet. Sci. 2:268. https://doi.org/10.3389/fvets.2015.00268.

Matsumoto, H., Y. Shimokawa, Y. Ushida, T. Toida, and H. Hayasawa. 2001. New biological function of bovine α-lactalbumin: Protective effect against ethanol- and stress-induced gastric mucosal injury in rats. Biosci. Biotechnol. Biochem. 65:1104–1111. https://doi.org/10.1271/bbb.65.1104.

McGuirk, S. M. 2008. Disease management of dairy calves and heifers. Vet. Clin. North Am. Food Anim. Pract. 24:139–153. https://doi.org/10.1016/j.cvfa.2008.05.007.

Medrano-Galarza, C., S. J. LeBlanc, A. Jones-Bitton, T. J. DeVries, J. Rushen, A. Marie de Passillé, M. I. Endres, and D. B. Haley. 2018. Associations between management practices and within-pen prevalence of calf diarrhea and respiratory disease on dairy farms using automated milk feeders. J. Dairy Sci. 101:2293–2308. https://doi.org/10.3168/jds.2017-13733.

Nydam, D. V., and H. O. Mohammed. 2005. Quantitative risk assessment of Cryptosporidium species infection in dairy calves. J. Dairy Sci. 88:3932–3943. https://doi.org/10.3168/jds.S0022-0302(05)73079-4.

Opgenorth, J., L. M. Sordillo, A. L. Lock, J. C. Mandy, and J. M. VandeHaar. 2020a. Colostroan supplementation with n-3 fatty acids alters plasma polyunsaturated fatty acids and inflammatory mediators in newborn calves. J. Dairy Sci. 103:11676–11688. https://doi.org/10.3168/jds.2019-18045.

Oultram, J., E. Phipps, A. G. V. Teixeira, C. Foditsch, M. L. Bicalho, V. S. Machado, R. C. Bicalho, and G. Oikonomou. 2015. Effects of antibiotics (oxytetracycline, florfenicol or tulathromycin) on neonatal calves’ faecal microbiota and microbial diversity. Vet. Rec. 177:598. https://doi.org/10.1136/vr.103320.

Kargar, S., A. Steele. 2021. Effects of colostroan management on the control of neonatal diarrhea syndrome in calves. Res. Vet. Sci. 115:478–483. https://doi.org/10.1016/j.rvsc.2017.07.029.

Kim, J. H., W. S. Jung, N. J. Choi, D. O. Kim, D. H. Shin, and Y. J. Kim. 2009. Health-promoting effects of bovine colostrum in Type 2 diabetic patients can reduce blood glucose, cholesterol, triglyceride and ketones. J. Nutr. Biochem. 20:298–303. https://doi.org/10.1016/j.jnutbio.2008.04.002.

Kuroki, M., M. Ohta, Y. Ikemori, F. C. Icatlo Jr., C. Kobayashi, H. Yokoyama, and Y. Kodama. 1997. Field evaluation of chicken egg yolk immunoglobulins specific for bovine rotavirus in neonatal calves. Arch. Virol. 142:843–851. https://doi.org/10.1007/s007050050123.

Parreño, V., G. Marcoppíolo, C. Vega, L. Garaicochea, D. Rodriguez, L. Saif, and F. Fernández. 2010. Milk supplemented with immune colostrum: Protection against rotavirus diarrhea and modulatory effect on the systemic and mucosal antibody responses in calves experimentally challenged with bovine rotavirus. Vet. Immunol. Immunopathol. 136:12–27. https://doi.org/10.1016/j.vetimm.2010.01.003.

Quigley, J. 2007. Call Note #122 - Calculating ME in milk and milk replacers. Calfnotes.com. Accessed May 12, 2022. https://www.calfnotes.com/pdf/files/CN122.pdf.

Rathe, M., K. Müeller, P. T. Sangild, and S. Husb. 2014. Clinical applications of bovine colostrum therapy: A systematic review. Nutr. Rev. 72:237–254. https://doi.org/10.1111/nutre.12089.

Rao, L. D., L. Buss, J. N. Wilms, and M. A. Steele. 2020. Technical note: Is fecal consistency scoring an accurate measure of fecal dry matter in dairy calves? J. Dairy Sci. 103:10709–10714. https://doi.org/10.3168/jds.2020-19007.

Renauld, D. L., F. F. Kelton, J. S. Weese, C. Noble, and T. F. Duffield. 2019. Evaluation of a multispecies probiotic as a supportiv...
Schinwald, M., K. Creutzinger, A. Keunen, C. B. Winder, D. Haley, and D. L. Renaud. 2022. Predictors of diarrhea, mortality, and weight gain in male dairy calves. J. Dairy Sci. 105:5296–5309.

Scott, N. A., A. Andrusaite, P. Andersen, M. Lawson, C. Alcon-Giner, C. Leclaire, S. Caim, G. Le Gall, T. Shaw, J. P. R. Connolly, A. J. Roe, H. Wessel, A. Bravo-Blas, C. A. Thomason, V. Kustele, P. Wang, D. A. Peterson, A. Bancroft, X. Li, R. Gencis, A. McI. Mowat, L. J. Hall, M. A. Travis, S. W. F. Milling, and E. R. Mann. 2018. Antibiotics induce sustained dysregulation of intestinal T cell immunity by perturbing macrophage homeostasis. Sci. Transl. Med. 10eaao4755. https://doi.org/10.1126/scitranslmed.aao4755.

Shen, R. L., T. Thymann, M. V. Ostergaard, A. C. F. Stoy, L. Krych, Stelwagen, K., E. Carpen, P. Andersen, A. Hodgkinson, and T. T. J. Earleywine, J. D. Olson, and F. B. Garry. 2018. Preweaned heifer management on US dairy operations: Part V. Factors associated with morbidity and mortality in preweaned dairy heifer calves. J. Dairy Sci. 101:9229–9244. https://doi.org/10.3389/fnut.2018.00052.

Urma, T., D. F. Kelton, E. I. Morrison, E. de Jong, K. D. McCubbin, H. W. Barkema, S. Dufour, J. Sanchez, L. C. Heider, S. J. LeBlanc, C. B. Winder, J. T. McClure, and D. L. Renaud. 2022. Cross-sectional study of antimicrobial use and treatment decision for preweaning Canadian dairy calves. JDS Commun. https://doi.org/10.3389/jds.2021-0161.

Van Vleck Pereira, R., S. Lima, J. D. Siler, C. Foditsch, L. D. Warnick, and R. C. Bicalho. 2016. Ingestion of milk containing very low concentration of antimicrobials: Longitudinal effect on fecal microbiota composition in preweaned calves. PLoS One 11:e0147525. https://doi.org/10.1371/journal.pone.0147525.

ORCIDS

H. S. M. Carter: http://orcid.org/0000-0002-1050-4849
M. A. Steele: https://orcid.org/0000-0001-6941-6205
J. H. C. Costa: https://orcid.org/0000-0001-9311-4741
D. L. Renaud: https://orcid.org/0000-0002-3439-3987