Effects of supplementing colostrum beyond the first day of life on growth and health parameters of preweaned Holstein heifers

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ABSTRACT

The preweaning period for a dairy calf is characterized by high morbidity and mortality rates, leading to financial losses for producers. Identifying strategies to improve the health and welfare of calves while reducing antimicrobial use continues to be crucial to the success of the dairy industry. The objective of this study was to determine the effects of feeding colostrum replacer (CR) to dairy heifer calves beyond day one of life on growth, serum IgG, the incidence of diarrhea and bovine respiratory disease (BRD), and the risk of mortality in the preweaning period. At birth, Holstein heifer calves (n = 200; 50/treatment) weighing 40.7 ± 0.35 kg (mean ± SE) were fed 3.2 L of CR (205 g IgG/feeding) at 0 h and 12 h of life. Calves were then randomly assigned to 1 of 4 treatments: 450 g of milk replacer (MR) from d 2 to d 14 (control, CON), 380 g CR + 225 g MR from d 2 to d 3, then 450 g MR from d 4 to d 14 (transition, TRAN), 45 g CR + 450 g MR from d 2 to d 14 (extended, EXT); or 380 g CR + 225 g MR from d 2 to d 3, then 45 g CR + 450 g MR from d 4 to d 14 (transition + extended, TRAN+EXT). Each treatment was reconstituted to 3 L and fed twice daily. All CR treatments were fed using bovine derived CR containing 27% IgG. From d 15 to d 41, all calves were fed 600 g MR reconstituted to 4 L twice daily. Body weight was recorded at birth and every 7 d until study completion on d 49. Blood samples were taken daily until d 7 to evaluate serum IgG and then every 7 d until d 49. A health assessment was performed daily to evaluate calves for BRD and diarrhea. Data were analyzed using mixed linear regression, mixed logistic regression, and survival analysis models in SAS 9.4. Serum IgG concentrations were not affected by treatment for the study period. The EXT and TRAN+EXT groups had greater average daily gain (ADG) from d 7 – d 14 (0.14 kg/day) and the TRAN group had greater ADG from d 14 – d 21 (0.11 kg/d), compared with CON. There was no association of treatment with the odds or the duration of a diarrhea bout. However, provision of CR to the TRAN and EXT calves was associated with a reduced hazard of diarrhea compared with CON calves. Furthermore, TRAN and EXT calves have a lower hazard of mortality compared with CON calves, with TRAN and EXT calves had a 2.8- and 3.8-times lower hazard of mortality, respectively. Our findings suggest that the supplementation of CR to dairy calves positively impacts ADG, and reduces the hazard of diarrhea and mortality during the preweaning period. Future research should look to further refine the supplementation strategy of CR to calves and explore the mechanism of action.

Keywords: dairy calf, colostrum, transition milk, diarrhea

INTRODUCTION

The preweaning period is crucial for dairy calves as it has implications on their future productivity (Volkmann et al., 2019). However, the preweaning period continues to be characterized by high morbidity (38%) and mortality rates (5%) in dairy calves (Urie et al., 2018). Thus, research investigating ways to improve the growth and health of dairy calves in the preweaning period is crucial (Urie et al., 2018). Disease, primarily diarrhea and bovine respiratory disease, is the leading cause of mortality in dairy calves (McGuirk, 2008; Urie et al., 2018). Additionally, calf disease decreases preweaning growth, first lactation milk yield, and increases the age at first calving (Heinrichs and Heinrichs, 2011; Volkmann et al., 2019; Abuelo et al., 2021). Similarly, increased growth in heifers during the preweaning period can increase first lactation yield and lifetime milk yield, and decrease age at first calving, compared with calves with reduced growth in the preweaning period,
and which may allow a cow to become profitable earlier in life (Krpálková et al., 2014a; Krpálková et al., 2014b; Volkmann et al., 2019). However, it is important to note that beyond the preweaning period growth must be controlled to avoid over-conditioning and decreased fertility, and beyond first lactation fertility and health are the largest determinants of the productivity of a cow (Krpálková et al., 2014a; Krpálková et al., 2014b; Volkmann et al., 2019). Therefore, producers will benefit from novel strategies to improve the health and growth of heifers early in life, usually beginning in the first hours of life with colostrum feeding (Puppel et al., 2019).

Colostrum, the first secretion produced by the mammary gland of the dam following parturition, supplies immunity to calves through the successful passive transfer of immunoglobulins, primarily immunoglobulin G (IgG; Puppel et al., 2019). Bovine colostrum has a different composition to mature milk and is rich in hundreds of bioactives and growth factors that benefit the growth and development of the calf (e.g., oligosaccharides, IgGs, lactoferrin Puppel et al., 2019; Fischer-Tlustos et al., 2021). In a traditional dairy calf rearing system, maternal colostrum or colostrum replacer (CR) is fed to calves during the first day of life to achieve transfer of passive immunity (Urie et al., 2018). The calf is usually fed fresh milk or milk replacer (MR) for the remainder of the preweaning period (Urie et al., 2018). However, this is in stark contrast to a calf was reared by the dam who would consume transition milk (TM) for 2 to 6 milkings post-parturition (Blum and Hammon, 2000).

Transition milk contains higher levels of bioactives, including IgGs and oligosaccharides compared with mature milk (Fischer-Tlustos et al., 2020). Certain studies have attempted to mimic TM by feeding calves a 1:1 mixture of pasteurized milk and colostrum from d 2 to d 7, which has been shown to improve IgG persistency and gastrointestinal development compared with feeding solely milk or MR (Hare et al., 2020; Pyo et al., 2020). Other studies have evaluated feeding smaller amounts of dried whole bovine colostrum (e.g., 70 to 100 g/d; Berge et al., 2009; Chamorro et al., 2017) or pasteurized pooled colostrum (Kargar et al., 2020) for a longer period of time (e.g., 1 to 14 d) as a preventative strategy to avoid disease. The extended preventative colostrum feeding program has been shown to increase average daily gains (ADG), decrease the occurrence and severity of bovine respiratory disease (BRD) and diarrhea events, and decrease mortality during the preweaning period compared with calves who did not receive the extended colostrum supplementation (Berge et al., 2009; Chamorro et al., 2017; Kargar et al., 2020). The mechanisms by which colostrum improves health and ADG in dairy calves needs to be investigated. Furthermore, previous studies are not consistent in terms of the quantity and quality of colostrum supplemented to calves, as well as the length of the supplementation period. There have been few studies directly comparing the effects of different colostrum supplementation protocols after day one of life, and while effectiveness has been observed with varying strategies, differences between quantities and lengths of supplementation strategies are not well studied.

Therefore, this study aimed to determine the effects of feeding colostrum to dairy heifer calves beyond day one of life on growth, serum IgG, the incidence of diarrhea and BRD, and the risk of mortality in the first 49 d of life. It was hypothesized that feeding increased amounts of colostrum in the first 2 weeks of life will improve growth and IgG status, decrease the probability of diarrhea and BRD, and increase survival likelihood of preweaned calves compared with calves receiving only milk replacer.

**MATERIALS AND METHODS**

This experiment was conducted following the guidelines of the Canadian Council of Animal Care (CCAC, 1993) at a commercial dairy farm in Southern Ontario, Canada, milking 1600 cows. This farm was chosen due to the high volume of cows allowing for all calves to be enrolled in a short period and for their existing relationship with the University of Guelph. The animal use protocol was approved by the Animal Care Committee at the University of Guelph (Animal Use Protocol #4488).

**Experimental Design and Animals**

All Holstein heifer calves born from June 2021 to August 2021 were enrolled at birth in this randomized control trial. Calves were separated from their dams within 1 h post-parturition to prevent colostrum suckling. Calves were then weighed (Global Industrial Digital Floor Scale, 1200 lbs, Global Equipment Company Inc., ON, Canada), navel-dipped with iodine, vigor scored, fed a 3.2 L of bovine-derived CR meal containing 205 g of IgG (29% IgG and 22% fat, DM basis; Saskatoon Colostrum Company Ltd., SK, Canada) within 1 h of birth via esophageal tube, and placed into individual hutches to be transferred to the calf barn. Calf vigor was scored using a 0-to-3-point scale for meconium staining, responsiveness, heart rate, respiratory rate, tongue swelling, and mucous membrane color, as described by Robichaud et al. (2017), where the greatest possible score was 21 points, and a lower score indicates greater vigor. All calves received a sec-
and 3.2 L bovine-derived CR meal at 12 h postnatal containing 205 g of IgG via esophageal tube.

**Treatments and Feeding**

Calves (n = 200) were randomly allocated using the RAND function in Microsoft Excel. Treatments as fed were: (1) 450 g of MR from d 2 to d 14 (control, CON), (2) 380 g of CR + 225 g of MR from d 2 to d 3, then 450 g of MR from d 4 to d 14 (transition, TRAN), (3) 45 g of CR + 450 g of MR from d 2 to d 14 (extended, EXT); or (4) 380 g of CR + 225 g of MR from d 2 to d 3, then 45 g of CR + 450 g of MR from d 4 to d 14 (transition + extended, TRAN+EXT). The TRAN diet was based on a study by Kargar et al. (2020), who found a 50:50 mixture of milk and colostrum fed for 3 d increased intestinal development; the EXT diet was based on a study by Kargar et al. (2020), who found that supplementing with 7% or 14% pasteurized colostrum for 14 d led to improved health and growth. The treatments were mixed in warm water (40°C) to reach a final volume of 3 L and fed by bottle twice daily at 0700 h and 1600 h. All CR treatments were fed using bovine-derived CR containing 29% IgG (DM basis; Saskatoon Colostrum Company Ltd.). The commercial MR was comprised of 27% protein, 19% fat, and 43% lactose (DM basis; Grober Nutrition, ON, Canada). From d 15 to d 41, all calves were fed 600 g of MR reconstituted to 4 L twice daily. Calves began the weaning process on d 42 and followed a subsequent step-down process: 3 L of MR twice daily (d 42 to d 43), 2 L MR of twice daily (d 44 to d 45), 1 L of MR twice daily (d 46 to d 47), 1 L/d of MR (d 48), and complete weaning on d 49. Calves had ad libitum access to fresh water via bottles from d 0 to d 21, and in pails from d 22 to d 49. Calves were given ad libitum access to calf starter (21.9% protein, 3.1% fat, 34.1% starch, DM basis, custom mix, Talbot Elevators Ltd., ON, Canada) via pail beginning on d 3. Starter intakes were calculated by orts and weighed twice weekly. Forage was not provided.

Analysis of the protein, fat, and lactose levels of the treatments was performed by Lactanet Canada (ON, Canada). Osmolality was measured in triplicate using an osmometer (Advanced Micro Osmometer Model 3300, Advanced Instruments Inc.) and BRIX of each diet was measured in triplicate using a digital BRIX refractometer (PAL-1 Refractometer, Atago). Starter samples were taken bi-weekly throughout the study period and sent to A&L Canada Laboratories Inc. (London, ON) for wet chemistry analysis. Crude protein was determined by combustion (LECO FP628 nitrogen analyzer), crude fat was measured using petroleum ether as a high temperature solvent to extract fat and oil, and starch was determined using heat stable amylase and amyloglucosidase via the Megazyme K-TSTA method.

To change from feedings based on time of birth (0 h and 12 h) to set-time feedings, calves fed between 3 h to 6 h after their 12 h meal were fed a 1.5 L meal to reduce the risk of refusal. Feedings for d 2 to d 3 were pre-weighed using a precision lab scale into color-coded individual bags, to allow those feeding to be blinded to the treatment and mixed on a per calf basis, then fed via bottle. On d 1 to 3, calves were fed via esophageal tube if they did not consume at least half the offered meal and milk intake was recorded. From d 4 until weaning, feedings were mixed in large batches by treatment, and prepared by research staff that did not perform health exams.

**Body Weight and Frame Measurements**

Body weights were measured and recorded at birth before colostrum feeding, and every 7 d for all calves (d 0, d 7, d 14, d 21, d 28, d 35, d 42, and d 49) using a digital weigh scale following the morning feeding. Frame sizes were also measured and recorded for all calves at h 12, d 28 and d 49. Ensuring that calves were standing square on even ground, measurements were taken using a tape measure for hip height (distance from top of hip to bottom of back hoof), withers height (distance from top of withers to bottom of front hoof), heart girth (chest circumference taken behind the withers), chest girth (chest circumference taken at the widest point of the torso), and body length (distance from withers to tail head).

**Disease Prevention Protocol**

All calves received halofuginone (0.5 mg, 2 mL/10 kg of BW; Halagon, Vetoquinol, QC, Canada) orally at birth and daily from d 1 to d 7 to delay the onset of diarrhea caused by *Cryptosporidium parvum*. Calves were vaccinated on d 1 of life with 2 intranasal vaccines covering bovine respiratory syncytial virus, infectious bovine rhinotracheitis virus, parainfluenza 3 virus, *Mannheimia haemolytica*, and *Pasteurella multocida* (INFORCE 3; Zoetis, QC, Canada; and Once PMH; Merck Animal Health, WI, USA) and were provided with an intranasal vaccine, covering BRSV, PI-3, and IBR, and an injectable vaccine, covering BRSV, BVD, PI-3, IBR, *Mannheimia haemolytica*, and *Pasteurella multocida*, on d 49 (INFORCE 3; Zoetis, QC, Canada; and Vista Once SQ; Merck Animal Health, WI, USA).
Antimicrobial Intervention, and Endpoints

Health Exam Protocol, Disease Diagnosis, Antimicrobial Intervention, and Endpoints

Health exams were performed by one of 6 trained investigators blinded to the treatments once daily on every calf enrolled in this study after the morning feeding from d 1 to d 49. The health exam included fecal consistency scoring (Renaud et al., 2020) and respiratory scoring for outward signs of BRD (Love et al., 2014). The interobserver agreement was measured ahead of the study’s onset using Fleiss’ kappa by having all observers go to a commercial calf-rearing facility on the same day to independently score 40 calves of unknown health status. The interobserver agreement was κ = 0.65.

A fecal consistency scoring system was used to assess for diarrhea in calves (0 = normal, firm not hard; 1 = soft, piles and spreads slightly, does not hold form; 2 = runny, spreads easily; and 3 = watery, devoid of any solid material, splatters; Renaud et al., 2020). A score of 0 or 1 was considered normal and a score of 2 or 3 was considered abnormal. A diarrhea bout was classified as when a calf had a fecal score of ≥2 for 2 consecutive days, or fecal score of 3 for one day, at which point they received an oral electrolyte solution (CalfLyte II, Vetoquinol, QC, Canada) a minimum of 3 h after feeding. Calves that refused >1.5 L of their milk meal were also administered oral electrolytes via bottle or esophageal tube if unwilling to suckle. Calves with fecal scores ≥2 with a temperature of ≥39.5°C received a single s.c. dose of meloxicam (20 mg meloxicam, 2.5 mL/100 kg BW; Metacam, Boehringer Ingelheim, Burlington, Canada). Calves with a skin tent duration of 3 to 4 s received 1 L of an electrolyte solution s.c. (Lactated Ringer’s Injection USP, Baxter, Mississauga, Canada), and calves with a skin tent duration of ≥5 s or who were unable to stand received 1 L of the same electrolyte solution i.v. (Smith, 2009).

Bovine respiratory disease scoring followed the scoring system of Love et al. (2014), where weighted scores for eye discharge (2 points), abnormal nasal discharge (4 points), spontaneous or induced cough (0 or 2 points), rapid or labored respiration rate (0 or 2 points) and the presence of droopy ears or head tilt (0 or 5 points) were cumulatively summed into a total respiratory score. Rectal temperatures were taken by a researcher if the total respiratory score was considered abnormal at ≥4 and a fever added 2 points to the cumulative score. Calves with fever and a BRD score of <5 received a single s.c. dose of meloxicam (20 mg/mL meloxicam, 2.5 mL/100 kg BW; Metacam, Boehringer Ingelheim, ON, Canada). A total respiratory score of ≥5 was classified as a BRD bout, and the calf received antimicrobial intervention according to the farm veterinary protocol. Calves with their first BRD bout on the day of initial diagnosis (d 0) received an injection of florfenicol and flunixin meglumine (300 mg, 16.5 mg, 6 mL/45 kg BW; Resflor, Merck Animal Health, WI, USA), with a second injection given at d 2 if the calf continued to have a respiratory score of ≥5. If a calf did not resolve their respiratory score and was still positive for BRD at d 4, or if the calf relapsed and experienced a second BRD bout (minimum of 2 consecutive days of respiratory score of ≤4 between bouts), the calf then received a single dose of marbofloxacin (160 mg, 2.5 mL/40 kg BW; Forcyl, Vetoquinol, QC, Canada). If the calf experienced a third bout of BRD, or if the BRD was not resolved (respiratory score of ≤4) 2 d after the last treatment, the calf then received a single dose of tulathromycin (100 mg, 2.5 mL/45 kg BW; Draxxin, Zoetis, QC, Canada).

If a calf did not improve after treatment, a veterinarian was consulted. If the veterinarian determined that the calf had reached a predetermined endpoint of not responding to treatment, had an acute injury, or was suffering and unlikely to recover, the calf was then euthanized. Euthanasia was performed using a captive bolt gun and pithing, and death was confirmed with the absence of a corneal reflex and heartbeat (CCAC, 1993).

Blood Sampling and Analysis

Serum blood samples were taken at birth before colostrum feeding for baseline measures, daily from d 1 to d 7, and on d 14, d 21, d 28, d 35, d 42, and d 49. Samples were taken from the jugular vein using 20G × 1 in vacutainer needles into 10 mL vacutainer tubes (BD, KY, USA). Serum samples were allowed to clot at room temperature before centrifuging at 3,000 x g for 15 min at 4°C. Serum was aliquoted into 1.5 mL microcentrifuge tubes in triplicate and frozen at −20°C. Serum samples were shipped to the Saskatoon Colos- trum Company Ltd. quality assurance laboratory to measure serum IgG via radial immunodiffusion analysis (Chelack et al., 1993; Chamorro et al., 2014). Serum IgG concentrations at 24 h of life were categorized as poor, fair, good, and excellent passive transfer of immunity at <10 mg/mL, 10 – 17.9 mg/mL, 18 – 24.9 mg/mL, and ≥25 mg/mL, respectively, as defined by Lombard et al. (2020). The time to reach maximum IgG concentration (IgGTmax) and maximum IgG concentration reached (IgGCmax) were also determined per calf.

Sample Size and Power Analysis

The required number of animals was determined using ADG data from previous studies conducted during
the preweaning period (van Niekerk et al., 2020; Buss et al., 2021). Using a standard deviation for ADG of 0.15 kg/d, we aimed to detect a difference of 0.1 kg/d ADG. Thus, at a power of 80% and an α of 0.05, we required 37 calves per treatment. We increased the sample size to 50 per treatment to account for loss due to mortality.

Statistical Analysis

All statistical analyses were completed using SAS software (version 9.4, SAS Institute Inc., UC, USA). Data was uploaded into the software from a Microsoft Excel spreadsheet (version 2211, Microsoft, WA, USA) and manually checked for non-biologically feasible values or entry errors. Descriptive statistics were generated and reviewed for outliers using box plots of the residuals, and normality was assessed using a Shapiro-Wilks test and visually inspecting the residual fit of modeled values. Values that were greater than 3 standard deviations from the mean were removed as outliers. All modeled residuals were assessed for homogeneity and met the equal variance assumption. The covariance structure chosen for linear mixed regression models with repeated measures was selected based on the model with the lowest Akaike’s information criterion. Statistical differences were determined at $P \leq 0.05$.

Diet Composition, Calf Immune Parameters and Growth

Linear mixed regression models were used to assess for mean differences in the calf diet by study treatment for the mean total fat content, crude protein content, lactose content, total osmolality, and metabolizable energy with treatment as a fixed effect. The association of the study treatments with total dry matter intake and starter intake over the study period (49 d), as well as one-time immune parameters, including, IgGT_max and IgGC_max were analyzed with mixed linear regression models with treatment as a fixed effect and calf as a random effect. We explored the association of the study treatments with the repeated growth parameter ADG with mixed linear regression models, birthweight was a quantitative covariate, time was a repeated measure (0 to 49 d), and calf was a random effect with a first-order autoregressive covariance structure. Simple weekly effects were also analyzed. Finally, we investigated the association of the study treatments with the repeated calf immune parameters total IgG, and BRIX with mixed linear regression models with birthweight as a quantitative covariate, time as a repeated measure (0 to 49 d), and calf as the subject with a compound symmetry covariance structure.

Calf Health Outcomes

The association of study treatment on the probability of incurring diarrhea, BRD, and mortality were analyzed using mixed logistic regression models with treatment and day as fixed effects, week of birth as a covariate, and calf as a random effect. Kaplan-Meier survival curves and cox-proportional hazard models were also calculated for the risk of mortality and diarrhea.

RESULTS

Descriptive Statistics

A total of 200 heifers were enrolled, and calves weighed 40.7 ± 0.35 kg at birth and had a baseline IgG of 0.9 ± 0.36 mg/mL and vigor score of 4.0 ± 0.27, with no differences between treatment groups. Most calves (97% - 194/200) experienced at least one diarrhea bout during this study. Similarly, 97% (194/200) of calves experienced at least one bout of BRD during this study. With respect to mortality, 12% (24/200; 10 CON, 4 TRAN, 3 EXT, and 7 TRAN+EXT) of calves reached a previously established endpoint and were euthanized. The main reason of euthanasia was for BRD (22/25 calves), with 2 calves being euthanized due to leg injuries. Environmental conditions were taken from the nearest weather station (London, ON); temperature ranged from 5.3 – 35.4°C (mean ± SE, 21.0 ± 0.33°C) humidity from 31 – 100% (73.0 ± 1.26%), and humidex from 11 – 40 (25.2 ± 0.64) throughout the study period.

Diet Composition

The analyses of the diet composition for mean differences by treatment are presented in Table 1. The CON and EXT diet did not differ ($P > 0.53$) in fat, protein, or lactose content. The TRAN diet was greater in fat and protein, and lower in lactose, compared with the CON and EXT diets. The colostrum replacer fed at 0 h and 12 h was higher in fat, protein, and metabolizable energy than the CON, TRAN, and EXT diets by design. Metabolizable energy was higher in the TRAN and EXT diets, compared with the CON diet. Intake of MR, CR and grain did not differ (Table 2) across treatment groups. Metabolizable energy intake (MEI) during the treatment period (d 1 – d 14) was greater (Table 2) by study design in TRAN and TRAN+EXT calves compared with the CON calves.
The study treatments did not impact ADG across the entire study period (Figure 1). However, treatment effects were observed within individual time points. Specifically, TRAN calves had a greater ADG from d 14 to 21 ($P = 0.05$, Figure 1) compared with CON calves. Across treatments, ADG increased (Figure 1) by time.

Effect of Colostrum Study Treatments on Growth Performance

The study treatments did not impact ADG across the entire study period (Figure 1). However, treatment effects were observed within individual time points. Specifically, TRAN calves had a greater ADG from d 14 to 21 ($P = 0.05$, Figure 1) compared with CON calves. Across treatments, ADG increased (Figure 1) by time.

Effect of Colostrum Supplementation on Diarrhea, Bovine Respiratory Disease and Mortality

Health results are reported in the following order, unless otherwise stated: TRAN, EXT, and TRAN+EXT, with CON as the referent. No treatment effects were observed on the days with diarrhea ($P = 0.81, 0.15$, and $0.81$) or number of bouts of diarrhea ($P = 0.92, 0.09$, and $0.16$). The TRAN and EXT diets reduced the hazard of diarrhea in the first 21 d of life, where CON calves were at a 1.8 (95% CI: 2.00 – 16.00; $P = 0.01$) and 1.6 (95% CI 2.00 – 14.00; $P = 0.03$) greater hazard of diarrhea, respectively (Figure 2). The TRAN+EXT diet did not affect the risk of diarrhea ($P = 0.15$; Figure 2). There was no association of treatment on the days

Table 1. The composition of milk replacer, CR, transition diet, and extended diet fed to calves (n = 200) to investigate if feeding extended CR improved calf health and performance up to weaning (49 d)

<table>
<thead>
<tr>
<th>Item</th>
<th>CON</th>
<th>TRAN</th>
<th>EXT</th>
<th>CR</th>
<th>SEM</th>
<th>$P$ - Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat (%)</td>
<td>1.85a</td>
<td>2.96b</td>
<td>2.17a</td>
<td>3.84c</td>
<td>0.089</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>3.88a</td>
<td>9.74b</td>
<td>4.80a</td>
<td>11.33c</td>
<td>0.247</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Lactose (%)</td>
<td>6.42a</td>
<td>5.08b</td>
<td>6.72a</td>
<td>2.73c</td>
<td>0.153</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Brix (%)</td>
<td>13.03a</td>
<td>20.67b</td>
<td>14.77a</td>
<td>28.97c</td>
<td>0.742</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Osmolality (mOsm)</td>
<td>396.0a</td>
<td>478.0b</td>
<td>402.0a</td>
<td>499.5c</td>
<td>3.68</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>IgG (%)</td>
<td>0.0a</td>
<td>17.0b</td>
<td>2.5a</td>
<td>27.0c</td>
<td>6.35</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>IgG Delivered (g)</td>
<td>0.0a</td>
<td>102.5b</td>
<td>12.2a</td>
<td>205.0c</td>
<td>0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Calculated ME (Mcal/kg)</td>
<td>0.60a</td>
<td>0.96b</td>
<td>0.69a</td>
<td>1.03c</td>
<td>0.02</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Means within a row with different superscripts differ ($P \leq 0.05$).

Table 2. The daily grain intake, dry matter (DM) diet intake, milk replacer (MR) intake, and metabolizable energy intake (MEI) of 200 Holstein heifer calves (n = 50/trt) for the treatment period (d 1 – d 14), the preweaning period (d 15 – d 42), and the weaning transition (d 43 – d 49) to investigate if feeding an extended colostrum diet improved calf health and performance

<table>
<thead>
<tr>
<th>Item</th>
<th>CON</th>
<th>TRAN</th>
<th>EXT</th>
<th>TRAN+EXT</th>
<th>SEM</th>
<th>$P$ - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment Period</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grain Intake (kg/day)</td>
<td>0.56</td>
<td>0.55</td>
<td>0.44</td>
<td>0.37</td>
<td>0.16</td>
<td>0.60</td>
</tr>
<tr>
<td>DM MR Intake (kg/day)</td>
<td>0.53</td>
<td>0.54</td>
<td>0.54</td>
<td>0.54</td>
<td>0.224</td>
<td>0.07</td>
</tr>
<tr>
<td>MEI (Mcal/day)</td>
<td>3.20</td>
<td>3.24</td>
<td>3.24</td>
<td>3.24</td>
<td>0.022</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Means within a row with different superscripts differ ($P \leq 0.05$).

Diets, as fed: control (CON) 100% milk replacer (MR) d 2 – d 49, transition (TRAN) received 50% colostrum replacer (CR) + 50% MR d 2 – d 3 and 100% MR d 4 – d 49, extended (EXT) received 91% MR + 9% CR d 2 – d 14 and 100% MR d 15 – d 49, and transition + extended (TRAN+EXT) received 50% CR + 50% MR d 2 – d 3, 91% MR + 9% CR d 4 – d 14, and 100% MR d 15 – d 49.
with BRD ($P = 0.11, 0.40, \text{and } 0.12$), or the number of BRD bouts ($P = 0.40, 0.16, \text{and } 0.47$).

The CR supplementation had a positive effect on survival, where CON calves were at a 3.8 (95% CI: 0.99 – 14.91; $P = 0.05$) greater hazard of mortality, compared with EXT calves (Figure 3). The TRAN and TRAN+EXT diets did not affect hazard of mortality ($P = 0.10$ and 0.40, respectively, 95% CI 0.55 – 4.52; Figure 3).

**Effect of Colostrum Supplementation on Serum IgG**

Serum IgG concentrations were not affected (Figure 4) by treatment. Similarly, serum IgG$_{\text{Cmax}}$ (28.9 ± 0.50 mg/mL) and IgG$_{\text{Tmax}}$ (24h) were not affected by treatment ($P > 0.42$). All calves received successful passive transfer of immunity ($\geq 10$ mg/mL serum IgG at 24 h of life). No calves in any treatment were categorized as poor passive transfer of immunity, and distribution of calves among fair, good, and excellent passive transfer of immunity categories did not differ (Table 3) by treatment. However, there was a time effect (Figure 4) on serum IgG concentrations.

**DISCUSSION**

Previous studies have demonstrated inconsistent benefits from supplementing colostrum to calves past the first day of life. However, our findings suggest that bovine-derived CR supplementation after day one of life positively affected growth performance, and reduced risk of diarrhea and mortality in preweaned dairy heifers compared with control calves.

In the current study, we observed no effect of treatment on DMI, MR intake, or starter intake in the calves. However, as the study design intended, there was an effect of treatment on MEI, with the EXT and TRAN+EXT treatments having greater MEI due to the CR being greater in energy content than the MR. There was also an increase in ADG seen at wk 3 of life in the TRAN group, though this positive benefit was not evident by the end of the study period. These findings disagree with some published work on colostrum supplementation past the first day of life. For example, Kargar et al. (2020) found that partially replacing the milk meal with 0.70 kg/day of whole colostrum (0.18 kg/day DM basis) for 14 d increased BW and ADG, up to 81 d of life. However, similar to our findings, Berge...
et al. (2009) found that supplementing 0.14 kg/day of bovine-derived CR from d 1 – d 14 increased ADG compared with control calves up to d 28 of life, but this increase was not maintained throughout the preweaning period. A suggestion as to why our findings disagreed with Kargar et al. (2020) is the quantity of colostrum supplied. In our study, the TRAN diet supplied high levels of bovine-derived CR to the calves (0.76 kg/day), but for only 2 d, whereas the EXT diet supplied lower quantities of bovine-derived CR (0.09 kg/day) over a longer period of time compared with the aforementioned studies, which observed positive impacts of feeding colostrum on growth. Supporting this, a study by Cantor et al. (2021) fed low levels of CR at 0.13 kg/day for 3 d, and did not find any impacts of supplementation on growth factors in the calves compared with MR controls. Therefore, it is likely that the quantity of CR fed daily, and the duration of CR feeding is why we did not observe a positive effect of treatment on ADG across the study period. It is also possible that the source of colostrum plays a role in the effects seen, as Kargar et al. (2020) fed a pasteurized pooled colostrum, whereas Berge et al. (2009) fed a whole-dried colostrum, as in this study, and Cantor et al. (2021) fed a fractionated dried colostrum. It should also be noted that the calves were weaned from 1.2 kg MR/day to 0 kg MR/day in 7 d. This aggressive weaning protocol led to low ADG at wk 7 of life, which may have impacted the results. Future research should evaluate the effects of the quantity of colostrum supplemented and supplement timeframe on growth parameters in calves.

When we evaluated the association of treatment on diarrhea, we found that feeding both the TRAN and the EXT diets decreased the risk of diarrhea compared with the control. These findings are in line with many other studies evaluating extended colostrum feeding, with recent studies showing that feeding colostrum for 14 d reduces days with abnormal fecal scores (Berge et al., 2009; Chamorro et al., 2017; Kargar et al., 2020). In addition, Snodgrass et al. (1982) observed that the supplementation of 10% whole colostrum delayed the onset of diarrhea in calves compared with a negative control. This effect has not been observed in studies feeding TM; however, most studies looking at TM have not followed the calves beyond the first few days of life (Hare et al., 2020; Pyo et al., 2020). This suggests that while the effect of extended colostrum on diarrhea is understood, the effect of TM on diarrhea must be further investigated. It has been suggested that increased metabolizable energy intake can impact IgA concentrations, hematocrit levels, hemoglobin, and other markers of immune response in calves (Chen et al., 2021); thus,
the effect of the TRAN diet on diarrhea may be caused by the increase in energy. However, it may also be related to the increase in bioactive compounds (i.e., IgA, lactoferrin, oligosaccharides etc.) delivered in the meal, as the EXT diet saw a similar effect yet did not supply significantly greater metabolizable energy compared with the control. The fat source in the diet may have also played a role, as the CR contained colostral fat whereas the MR contained a mixture of lard and coconut fat. A study looking at fat source in MR found that the milk fat MR reduced diarrhea when compared with lard or vegetal fats (Hill et al., 2007); thus, the colostral fat delivered in the TRAN and EXT diets may have played a role in the reduced hazard of diarrhea. It is not yet clear why the same effect was not observed in the TRAN+EXT diet, but may be related to the amount of total solids delivered. Studies exploring the effect of total solids delivered in MR have reported an increase in fecal scores and diarrhea occurrences, which is attributed to an increase in osmolality of the diet, causing osmotic diarrhea (Jenny et al., 1978; Shiasi Sardoabi et al., 2021). Thus, the increase in solids delivered in both the TRAN and EXT phases of the TRAN+EXT diet may have prevented the TRAN+EXT diet from showing a positive effect on hazard of diarrhea.

In the current study, we did not observe an effect of treatment on the duration of BRD or age at BRD onset. It is, however, important to note that majority of calves had a BRD bout in this study, and this may have been related to the humidex values observed throughout the study period (Supplementary Figure 1). Not only has elevated humidex been shown to increase the occurrence and severity of BRD in calves, but it can also lead to heat stress, which causes similar signs of increased respiratory rate and body temperature, thus possibly leading to misdiagnoses as BRD (Dubrovsky et al., 2019). This is a limitation of our study, and we suggest that future studies should use lung ultrasonography to confirm BRD. Many studies looking at extended colostrum feeding did not report respiratory health information, possibly due to a lack of significance or literature supporting the need to measure this information in relation to colostrum feeding. However, Berge et al. (2009) measured respiratory morbidity using an ordinal severity scale for the first 28 d of life and found no effect of colostrum supplementation for the first 14 d of life compared with control calves. Contrary to Berge et al. (2009), Chamorro et al. (2017) using nasal discharge and depression scores, Kargar et al. (2020) using veterinary diagnoses, and Cantor et al. (2021) using lung ultrasonography found a reduction in the occurrence of pneumonia from feeding colostrum for 14 d when compared with a milk replacer or whole milk control. However, these studies used different methods of identifying

Figure 3. Effect of the transition, extended, and transition with extended colostrum supplementation diets compared with a control diet on probability of a calf surviving the preweaning period using Kaplan-Meier survival estimates. Holstein heifer calves (n = 50/trt) received 1 of 4 treatments (as fed): 1) control (CON): 100% milk replacer (MR) from d 2 – d 49; 2) transition (TRAN): 50% colostrum replacer (CR) + 50% MR d 2 – d 3 and 100% MR d 4 – d 49; 3) extended (EXT): 91% MR + 9% CR d 2 – d 14 and 100% MR d 15 – d 49; or 4) transition + extended (TRAN+EXT): 50% CR + 50% MR d 2 – d 3, 91% MR + 9% CR d 4 – d 14, and 100% MR d 15 – d 49. Compared with the CON group, EXT calves were at a lower hazard of mortality (P = 0.05).
BRD when compared with each other or the present study, making it difficult to make a direct comparison. The present study may not have seen a difference in BRD due to the high incidence of BRD, impacting the ability to observe differences among treatments. Future research should investigate the mechanisms behind how low-level colostrum supplementation reduces the risk of BRD in calves.

Regarding mortality, the EXT diet reduced mortality compared with the CON diet. This finding is supported by a study completed by Quigley and Wolfe (2003) where spray-dried animal plasma was fed for the preweaning period as an IgG supplement; however, these findings contradict many subsequent extended colostrum feeding trials that found no effect of treatment on mortality (Berge et al., 2009; Chamorro et al., 2017; Kargar et al., 2020). A potential explanation for the different mortality results found in this study could be the high levels of mortality observed in the trial, which aligns more closely with Quigley and Wolfe (2003) than the more recent studies. It is also interesting that the EXT diet, which showed lower probabilities of mortality in the calves, had a lower hazard of diarrhea. As wk 2 and 3 of life were the time points with the greatest disease challenge for calves, it raises the question of whether the increased MEI supplied to the calves receiving CR

Table 3. Percentage of calves with poor, fair, good, and excellent serum IgG levels at 24h of life, as defined by Lombard et al. (2020), when fed transition, extended, and transition with extended colostrum supplementation diets compared with a control diet in 200 Holstein heifer calves (n = 50/trt)

<table>
<thead>
<tr>
<th>Serum IgG Category</th>
<th>CON</th>
<th>TRAN</th>
<th>EXT</th>
<th>TRAN+EXT</th>
<th>P - Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor (&lt;10 mg/mL)</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0.59</td>
</tr>
<tr>
<td>Fair (10 - 17.9 mg/mL)</td>
<td>12.0%</td>
<td>8.0%</td>
<td>6.0%</td>
<td>8.0%</td>
<td>0.59</td>
</tr>
<tr>
<td>Good (18 - 24.9 mg/mL)</td>
<td>32.0%</td>
<td>28.0%</td>
<td>38.0%</td>
<td>32.0%</td>
<td>0.59</td>
</tr>
<tr>
<td>Excellent (≥25 mg/mL)</td>
<td>56.0%</td>
<td>64.0%</td>
<td>56.0%</td>
<td>60.0%</td>
<td>0.59</td>
</tr>
</tbody>
</table>

1Diets, as fed: control (CON) received 100% milk replacer (MR) from d 2 – d 49, transition (TRAN) received 50% colostrum replacer (CR) + 50% MR d 2 – d 3 and 100% MR d 4 – d 49, extended (EXT) received 91% MR + 9% CR d 2 – d 14 and 100% MR d 15 – d 49, and transition + extended (TRAN+EXT) received 50% CR + 50% MR d 2 – d 3, 91% MR + 9% CR d 4 – d 14, and 100% MR d 15 – d 49.

Figure 4. Effect of the transition, extended, and transition with extended colostrum supplementation diets compared with a control diet on serum IgG decay (least squares means ± SEM) from d 0 – 49. Holstein heifer calves (n = 50/trt) received 1 of 4 treatments (as fed): 1) control (CON): 100% milk replacer (MR) from d 2 – d 49; 2) transition (TRAN): 50% colostrum replacer (CR) + 50% MR d 2 – d 3 and 100% MR d 4 – d 49; 3) extended (EXT): 91% MR + 9% CR d 2 – d 14 and 100% MR d 15 – d 49; or 4) transition + extended (TRAN+EXT): 50% CR + 50% MR d 2 – d 3, 91% MR + 9% CR d 4 – d 14, and 100% MR d 15 – d 49.
reduced the likelihood of death by delivering more nutrients, providing a protective effect to the calf through increased energy and body reserves during this challenging period. Transition milk has not been suggested to reduce hazard of mortality in previous studies (Conneely et al., 2014; Hare et al., 2020; Pyo et al., 2020). It is unclear why the same reduction in mortality was not observed in the TRAN+EXT diet as in the EXT diet, however, it may have been related to the hazard of diarrhea being unaffected by the TRAN+EXT diet, leading to the calves undergoing a greater challenge. This could have led to TRAN+EXT diets being more vulnerable, compared with the EXT calves who saw a reduction in hazard of diarrhea.

When evaluating the effect of treatment on calf serum IgG, concentrations were unaffected by the treatments. The current literature investigating extended colostrum feeding does not commonly report serum IgG concentrations over time; however, our findings are consistent with the theory that a calf’s ability to absorb IgG from colostrum reduces over time (Stott et al., 1979). As the EXT diet began at 24 h of life, and only 14 g of IgG were delivered per meal in the EXT diet, it is not unsurprising that further absorption in colostrum IgG was not observed. However, there is no clear consensus in the literature on whether feeding TM impacts serum IgG concentrations. Hare et al. (2020) found that feeding TM up to d 3 of life improved the maximum serum IgG concentration reached and serum IgG persistence in the first 72 h. However, like our findings, Conneely et al. (2014) found no association between feeding 2 or 4 TM meals with serum IgG concentrations. A possible explanation for these contradictions is the quantity of colostrum delivered and the timing of the first TM meal. All calves received 2 colostrum meals in the current study, equalling 410 g of IgG in the first 12 h of life. Conneely et al. (2014) suggested that excessive feeding of colostrum can diminish the absorptive efficiency of the calf’s gut, thus the calf may not have absorbed the extra IgG supplied by the transition meal. Timing may have also played a role, as the first transition meal was offered at 24 h of life, contrary to Hare et al. (2020), who fed the first transition meal at 12 h of life. It is possible that at 24 h the calf’s gut was no longer able to absorb IgG as the absorption of IgG is only possible for a limited time post-parturition (Stott et al., 1979; Fischer et al., 2018).

CONCLUSION

Overall, these findings suggest that feeding colostrum to dairy heifers beyond day one of life, either in high concentrations for a short period or in lower concentrations for an extended period, can positively impact ADG, risk of diarrhea and reduce mortality. However, there is still a lack of understanding regarding which feeding strategy is most beneficial to calf growth and development, in addition to the specific mechanisms responsible for the observed effects – both of which require further investigation.

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