The effect of colostrum storage conditions on dairy heifer calf serum immunoglobulin G concentration and preweaning health and growth rate

C. Cummins,*† D. P. Berry,† J. P. Murphy,† I. Lorenz,* and E. Kennedy†¹

*School of Veterinary Medicine, University College Dublin, Dublin 4, Ireland

†Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland

ABSTRACT

The objective of the present study was to compare serum IgG concentration, weight gain, and health characteristics in Irish spring-born dairy calves fed colostrum stored using a range of conditions. Immediately after birth, 75 dairy heifer calves were assigned to 1 of 5 experimental colostrum treatments: (1) fresh pasteurized colostrum, fed immediately after pasteurization; (2) fresh colostrum, fed immediately after collection but not pasteurized; (3) colostrum stored unpasteurized at 4°C in a temperature-controlled unit for 2 d before being fed to calves; (4) colostrum stored unpasteurized at 13°C in a temperature-controlled unit for 2 d before being fed to calves; and (5) colostrum stored unpasteurized at 22°C in a temperature-controlled unit for 2 d before being fed to calves. All colostrum had IgG concentrations >50 g/L and was fed to calves promptly after birth. Blood samples were obtained from calves via the jugular vein at 0 h (before colostrum feeding) and at 24 h of age to determine the rate of passive transfer of IgG; individual calf live-weights were recorded to monitor weight gain (kg/d) from birth to weaning. Colostrum stored in warmer conditions (i.e., 22°C) had >42 times more bacteria present and a pH that was 0.85 units lower and resulted in a serum IgG concentration that was almost 2 times lower compared with colostrum that was pasteurized, untreated, or stored at 4°C for 2 d. Colostrum stored at 4°C for 2 d had more bacteria present than pasteurized and fresh colostrum but did not result in reduced calf serum IgG concentrations. Average daily weight gain from birth to weaning did not differ among treatments. Even if colostrum has sufficient IgG (>50 g/L) but cannot be fed to calves when freshly collected, storage at ≤4°C for 2 d is advisable to ensure adequate passive transfer when it is consumed by the calf.

Key words: colostrum storage, colostrum feeding, calf health, immunoglobulin G

INTRODUCTION

Colostrum is one of the most important sources of essential nutrients to improve the likelihood of neonatal survival (Furman-Fratczak et al., 2011). In particular, colostrum contains immunoglobulins, specifically IgG, which, when absorbed, protects the calf from infectious disease (Besser et al., 1991; Godden, 2008; Furman-Fratczak et al., 2011). Calves may be defined as having failure of passive transfer if they do not have a sufficient concentration of IgG in their serum (>10 g/L) when sampled at 24 h of age (Besser et al., 1991; Furman-Fratczak et al., 2011). Failure of passive transfer in calves is associated with a greater mortality rate, compromised preweaning health and weight gain (Robison et al., 1988; Wells et al., 1996; Donovan et al., 1998), and reduced productivity as an adult cow (Furman-Fratczak et al., 2011).

To minimize failure of passive transfer in newborn calves, a successful colostrum management plan needs to be in place, which requires producers to consistently provide neonatal calves with a sufficient volume of clean [i.e., total bacterial counts (TBC) <100,000 cfu/mL], high-quality (>50 g/L IgG) colostrum within the first hour of life (Godden, 2008). This requirement equates to no less than a total of 150 to 200 g of IgG for an average 35-kg calf to ensure optimal passive transfer of immunity (Chigerwe et al., 2008). On some farms, particularly in year-round calving systems, colostrum is routinely stored before feeding (USDA, 2007). On other farms, an occasional shortage in colostrum supply is inevitable and stored colostrum is used. Such shortages may be frequent if producers discard colostrum from cows that test positive for pathogens such as *Mycobacterium avium* ssp. *paratuberculosis* and *Mycoplasma bovis* mastitis, or from cows that are clinically ill at calving (McGuirk and Collins, 2004). Storage of colostrum refers to retaining colostrum for a length of time in a refrigerator or a freezer or at ambient temperatures.

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¹Corresponding author: Emer.Kennedy@teagasc.ie

Cummins et al. (2016a) reported that 90% of Irish dairy producers store colostrum, while colostrum is routinely stored on 89% of large dairy farms in North America. Colostrum has traditionally been stored in a freezer to prevent alterations to its composition, such as IgG concentration and bacterial level (Carlson and Muller, 1977; Schipper et al., 1981). However, other immune properties can be affected, including leukocytes (Donovan et al., 2007), which are also important in building an immune response in a calf to fight infection (Reidel-Caspari, 2001). Moreover, defrosting can take more than an hour, and the process can denature IgG, rendering it suboptimal for absorption (Jones et al., 1987). More than 20% of Irish dairy producers store colostrum at room temperature or in a refrigerator for up to 1 wk (Cummins et al., 2016a). Although such storage conditions do not have repercussions for colostral IgG concentration (Cummins et al., 2016b), increased bacterial growth and reduced pH have been documented in colostrum stored at room temperature or in a refrigerator for up to 96 h (Stewart et al., 2005; Cummins et al., 2016b). Previous research documented a reduction in immunoglobulin absorption in calves fed colostrum of relatively low pH (pH = 4.65; Foley et al., 1978), while James et al. (1981) suggested that a greater bacterial concentration in the calf's gut may adversely affect the passive transfer of IgG. Furthermore, numerous studies have demonstrated that heat treatment that decreases TBC in colostrum results in improved immunity and weight gain in dairy calves (Johnson et al., 2007; Elizondo-Salazar and Heinrichs, 2009; Godden et al., 2012; Gelsinger et al., 2015).

Most research to date investigating the effect of stored colostrum on calf health has been conducted in geographical locations operating year-round calving systems (Jenny et al., 1977; Foley et al., 1978; Morrill et al., 2012), and these studies do not directly reflect on-farm management practices commonplace in seasonal calving systems, which have intense periods with multiple calf births daily and high demands for colostrum (Cummins et al., 2016a). Year-round calving systems have continuous births throughout the year, but on any given day, a much lower percentage of the dairy herd calves than in a seasonal system. The hypothesis of the present study is that feeding colostrum stored in warmer conditions and therefore containing greater amounts of bacteria affects the rate of passive transfer of IgG and subsequent health in dairy calves. Therefore, the objective was to compare serum IgG concentration, weight gain, and health characteristics of spring-born (February and March) Irish dairy calves fed colostrum that underwent a range of processing and storage conditions. Additionally, colostral IgG concentrations, TBC, and pH were examined.

MATERIALS AND METHODS

This study was approved by the Teagasc Animal Ethics Committee (TAEC36/2013); all procedures were licensed and carried out in accordance with the Health Products Regulatory Authority of Ireland (AE19132/P015).

Seventy-five dairy heifer calves (15 calves per treatment) born between February 3 and March 25, 2014, were enrolled in the experiment. Sample size was calculated for serum IgG using an expected mean of 40 g/L (SD = 2.5 g/L) with an expected difference between the means of 2.7 g/L. At 0.8 power, 15 calves were required per treatment. When a similar calculation was completed for weight gain, only 11 calves were required per treatment. Consequently, 15 calves per treatment were used so that differences in IgG could be detected. Animal measurements were undertaken up to September 2014 at the Teagasc Moorepark Research farm located in Co. Cork, southern Ireland (52°9'N, 8°16'W). Five experimental treatments were applied to colostrum before it was fed to calves.

Cow Precalving Management

In total, 49 cows were used to provide colostrum to feed all 75 calves; 60% of these cows were Holstein-Friesian (**HF**) and 30% were Jersey × Holstein-Friesian (**JEX**). Dry-cow management was similar for all cows included in the study. Cows were dried off when producing <8 kg of milk/d or within 60 d of calving. Alternatively, cows were dried off if their BCS was <2.75 units (scale of 1–5, where 1 = emaciated and 5 = extremely fat; Edmonson et al., 1989), before meeting one of the aforementioned criteria.

All dry cows were fed according to BCS at drying off. Cows with a poorer BCS were removed from the main group of dry cows, and only colostrum from the main group was used to ensure uniformity between samples. Mineral supplementation began 2 mo before calving using a powder (Immuboo X-Mag Vit E Elite; Nutribio, Cork, Ireland) mixed through the silage. The composition of the mix was magnesium oxide, sodium chloride, magnesium phosphate, molasses (sugarcane), and magnesium sulfate. Body condition score was monitored throughout the dry period with the aim of calving cows being at a target BCS of 3.25 ± 0.5 units. Cows that were outside of this target in the 4 wk leading up to calving and at calving were not included in the study because the diet of these cows would be different. Cow health management included routine vaccination against bovine viral diarrhea, leptospirosis, infectious bovine rhinotracheitis, rotavirus, and salmonellosis.

All dry cows were housed in a cubicle shed until a few days before expected calving, when they were transferred to a straw-bedded group. For parturition, cows were moved to individual straw-bedded pens, where each cow was monitored by competent and trained personnel.

Calf Management

Of the 75 heifer calves enrolled in the study, 45 were HF and the remaining 30 calves were JEX. Immediately after birth, all calves were removed from their dam to ensure they had minimal contact with the cow and did not suckle. For identification purposes, as required by the Department of Agriculture, national individual animal identity tags were applied to both ears. Each calf was weighed (TruTest XR 3000, Tru-test Limited, Auckland, New Zealand), a blood sample was obtained (detailed below), and the navel sprayed with 10% iodine solution. Before being fed colostrum, calves were randomly assigned to 1 of 5 experimental treatment groups, stratified based on a rolling average according to date of birth, breed composition, and birth weight. The mean values of the aforementioned blocking factors were updated as each calf was enrolled to the study, ensuring all treatments remained similar and comparable.

Experimental Treatments

Colostrum (i.e., the first milking collected immediately postpartum) was subjected to 1 of 5 treatments before being fed to a calf. These experimental treatments were (1) fresh pasteurized colostrum (pasteurized and fed to calves immediately after collection; **PST**); (2) fresh colostrum (fed immediately after collection but not pasteurized; **FR**); (3) colostrum stored at 4°C in a temperature-controlled unit for 2 d before being fed to calves (**ST4**); (4) colostrum stored at 13°C in a temperature-controlled unit (Binder GmbH, Tuttingen, Germany) for 2 d before being fed to calves (**ST13**); and (5) colostrum stored at 22°C in a temperature controlled unit for 2 d before being fed to calves (**ST22**). Treatments ST4, ST13, and ST22 were not pasteurized and were stored in sterilized 2-L milk bottles. Colostrum in the PST treatment was pasteurized at 60°C for 60 min (Donahue et al., 2012) in a Janschitz eco mini FJ15 pasteurizer (Franz Janschitz GmbH, Althofen, Austria).

All calves received colostrum from the relevant treatment group at a rate of 8.5% of the calf's birth BW (Conneely et al., 2014) via oro-esophageal tube within 2 h of birth. The calf was subsequently given 4 feedings of unpasteurized transition milk from the calf's own dam or a single cow via nipple and bottle beginning at

the next standard feeding times (0800 h and 1500 h), unless the calf was born less than 3 h before the next standard feeding time. A sample of both colostrum and transition milk that was fed to each calf within the first 24 h of birth was collected and stored at -20°C in a 120-mL polystyrene container for later quantification of IgG, TBC, and pH.

At birth, each calf was placed in an individual pen measuring 0.8 × 1.2 m, where it remained for 3 d. After d 3, each calf was transferred to a group pen (5 × 7 m) of 15 calves, according to date of birth; calves from different treatments were housed together. The maximum age difference between calves in each group was 2 wk. At approximately 5 wk of age, calves were moved to pasture where they grazed in groups of 15 calves per 0.6-ha paddock.

All calves were offered 15% of their birth weight in milk replacer (26% CP; Volac, Killeshandra, Co. Cavan, Ireland), twice daily for 4 wk, after which they were transferred to once-a-day milk feeding. From d 4 of age, all calves had free access to water. During the housing period, calves received ad libitum hay and concentrates, and after turnout, they had ad libitum access to grass and concentrates.

Treatment effect on calf weight gain was monitored through weekly weighing until weaning, and every 2 wk thereafter, up to 6 mo (TruTest XR 3000). Calves were weaned according to their respective BW; HF calves were weaned at 90 kg and JEX calves were weaned at 85 kg. Once a calf had reached its target weaning weight, it was removed from the group into a new "weaning" group and was gradually weaned over the course of 1 wk. Subsequently, calves were moved into one large "weaned" group in which they remained for the duration of the monitoring period (i.e., up to 6 mo).

All calves were inspected twice daily for general demeanor and signs of clinical illness (e.g., diarrhea, pneumonia, or omphalophlebitis). Any calf that became ill received the appropriate care and veterinary treatment as required, and any treatments administered were recorded. Clinical illness was determined as any case in which a calf received treatment for disease.

All calves were managed similarly postweaning, continuing to receive approximately 1 kg (DM) of supplementary concentrate until they were approximately 6 mo of age.

Sample Collection and Analysis

Colostrum Collection and Management. All colostrum was collected individually from each cow via a portable milking machine immediately postpartum (DeLaval milking solutions, DeLaval, Tumba, Sweden). To ensure colostrum quality was adequate to provide

sufficient immunity to calves (i.e., >50 g/L), a hydrometer (Volac colostrometer, Royston, UK) was used to estimate IgG concentration. Only colostrum that was measured as very good quality or in the green marker region of the hydrometer was used; when colostrum was measured below this (i.e., at approximately 50 g/L or less), it was discarded. This process eliminated colostrum quality before treatment as a confounding factor. To determine volume of colostrum available to separate into treatments by each cow, total volume of colostrum produced by each cow was measured using a weigh scale before separating into treatments. When insufficient colostrum remained from a single cow, it was discarded.

Collection of Calf Serum. Blood samples were taken from the jugular vein of each calf (0 h) using a 21-gauge needle into plain 8-mL serum tubes (BD Vacutainer, Langanbach Services Ltd., Bray, Co. Wicklow, Ireland). A further blood sample was taken from each calf at 24 h of age. All blood samples were refrigerated at 4°C for 24 h to allow clotting before separating the serum from the clot by centrifugation ($3,500 \times g$ for 30 min at 4°C). Serum samples were frozen at -20°C for later determination of IgG concentration, and the clot was discarded.

IgG Analysis of Serum, Colostrum, and Transition Milk. The IgG concentration of serum, colostrum, and transition milk was determined using the radial immunodiffusion (RID) method. Samples were diluted using a 0.8% saline solution and mixed thoroughly; 0 h serum samples were tested neat (i.e., not diluted), 24 h serum samples were diluted to a ratio of 1:2, colostrum samples were diluted to 1:3, and the dilution of transition milk samples ranged from neat to 1:3 dilution. A total of 5 μ L of diluted sample was added to each well of a Bovine IgG RID test plate (Triple J Farms, Bellingham, WA). Each RID plate was incubated for 24 h and the diameter of each ring was measured using digital Vernier calipers. All samples were tested in duplicate. The concentration of IgG was calculated from a standard reference curve containing known concentrations of IgG (196, 1,402, and 2,748 mg/dL), obtained from the internal test standards. Any sample with an IgG concentration outside the range of the standard reference curve (range of $R^2 = 0.9709$ – 0.9999) was retested after further dilution according to the test recommendations. Interassay coefficients of variation (CV) were calculated for 0 and 24 h serum (CV = 0.04 and 0.09, respectively), colostrum (CV = 0.07), and transition milk (CV = 0.07) samples.

TBC Analysis. Colostrum aliquots were thoroughly mixed and diluted using serial dilution at different rates, according to colostrum treatment (i.e., the expected amount of bacteria in the colostrum). Maximum

recovery diluents of 9 mL containing 1 g of peptone/L and 8.5 g of NaCl/L of water were used. To determine the most appropriate dilution rate for each aliquot, superfluous samples were initially diluted at the same rate (10^4). If the plates were too concentrated to read, further dilutions were made. The lowest dilution rate was 10^3 for PST colostrum aliquots, while the highest dilution rate was 10^7 for ST22 colostrum aliquots. Aerobic count plates (3M Petrifilm Aerobic Count Plates; 3M, St. Paul, MN; <http://www.solutions.3m.com>) with 1 mL of diluted aliquot were incubated at 32°C for 2 d. Each aliquot was tested in duplicate, and an average of the 2 results was obtained. A 3M Petrifilm Plate Reader (3M) was used to verify TBC (cfu/mL).

pH Analysis. Sample pH was measured using an OHM Delta 2105.2 pH/mV meter (Delta OHM S.r.L, Caselle di Selvazzano, Italy). Each sample was tested in duplicate to an accuracy of 3 decimal places, and the average of the 2 readings was calculated. Probe calibration was carried out before each test period, and the probe was cleaned on a weekly basis according to the manufacturer's guidelines.

Determination of Apparent Efficiency of Absorption. Apparent efficiency of absorption (AEA) for IgG was determined as previously described (Quigley et al., 1998, 2002) using the following formula:

$$\text{AEA} = [\text{serum IgG (g/L)} \\ \times \text{plasma volume (L)/IgG intake (g)}] \times 100.$$

The plasma volume was also previously described by Quigley et al. (1998) calculated using the following formula:

$$\text{Plasma volume} = 0.089 \times [\text{BW at birth (kg)}].$$

Statistical Analysis

In all statistical analyses, $P < 0.05$ indicates significance and $P < 0.10$ and ≥ 0.05 indicates a tendency toward significance (i.e., a trend toward significant).

IgG Concentration, TBC, pH, AEA, and Average Daily Weight Gain. Serum, colostrum, and transition milk IgG concentrations; pH of colostrum and transition milk; AEA; and average daily weight gain were all normally distributed. Total bacterial count of colostrum and transition milk was right skewed, so a $\log_{10}$ transformation was performed. Mixed models in PROC MIXED (SAS version 9.3; SAS Institute Inc., Cary, NC) were used to determine if colostrum, transition milk, and serum IgG concentrations; TBC and pH of colostrum and transition milk; and calf AEA and average daily weight gain differed between colos-

tral treatments. For each model, colostrum treatment of the calf was the independent variable. Experimental block was included as a random effect (i.e., calf date of birth, breed, and weight). Cow parity and breed and colostrum volume were included as covariates in the model. In addition, depending on the dependent variable being investigated in each model, colostrum IgG concentration, transition milk IgG concentration, TBC of colostrum and transition milk, pH of colostrum and transition milk, serum IgG concentration at 0 h, number of transition milk feeds, and time of birth were considered as covariates in the model. For example, if the dependent variable being investigated was average daily weight gain, then IgG concentration, TBC, and pH of colostrum and transition milk, serum IgG concentration, AEA, number of transition milk feeds, and time of birth were considered as covariates in the model. If the dependent variable was colostrum TBC, colostrum IgG concentration, pH, and time of birth were considered as covariates in the model. Backward elimination ($P > 0.05$) was used until only significant ($P < 0.05$) variables remained in the mixed model. When the dependent variable being investigated was TBC, then pH as a covariate was included as an additional independent variable in the final model; when the dependent variable being investigated was pH, then TBC was included as a covariate in the final model. No other dependent variables were associated with any covariates considered.

The statistical model used for analysis was

$$Y_{abcdefghi} = \beta_0 + T + \beta_1 + \beta_2 + \beta_3 + \beta_4 + \beta_5 + \beta_6 \\ + \beta_7 + \beta_8 + \beta_9 + \varepsilon_{abcdefghi}$$

where $Y_{abcdefghi}$ = dependent variables (colostrum, transition milk, and serum IgG concentrations; TBC and pH of colostrum and transition milk; and calf AEA, and average daily weight gain), T = colostrum treatment, β_0 = the intercept, β_{1-9} = parameter estimates for the independent variables considered, and $\varepsilon_{abcdefghi}$ = residual error.

Because subsequent transition milk feedings took place at fixed times during the day (0800 and 1500 h), and because of the natural spread in the actual times of birth of each calf, variation existed in the number of opportunities each calf had to receive subsequent transition milk feedings before cessation of IgG absorption at 24 h of age, depending on their time of birth. Calves in the same treatment group may have received a different number of subsequent transition milk feedings before 24 h of age. Thus, the time of birth of the calf was also considered as a covariate when investigat-

ing the dependent variables serum IgG concentration, AEA, and average daily weight gain. Time of birth of the calf was categorized as (1) calf born between 0500 and 0800 h, with next transition milk feeding at 1500 h; (2) calf born between 0800 and 1200 h, with next transition milk feeding at 1500 h; (3) calf born between 1200 and 1500 h, with next transition milk feeding at 0800 h; or (4) calf born between 1500 and 0500 h, with next transition milk feeding at 0800 h.

Categorization of TBC. In a separate series of analyses, colostrum samples were categorized according to TBC (i.e., in the analyses TBC substituted treatment). Three TBC categories were formed according to amount of bacteria (TBC) the colostrum contained, regardless of colostrum treatment applied: (1) samples with $<100,000$ cfu/mL TBC (recommended minimum TBC; Godden, 2008), (2) samples with $>100,000$ and $\leq 1,000,000$ cfu/mL, and (3) samples with $>1,000,000$ cfu/mL. A mixed model in PROC MIXED (2011; SAS Institute Inc., Cary, NC) was used to establish if these TBC categories were associated with calf serum IgG concentration at 24 h, pH, colostrum IgG concentration, and serum IgG concentration at 0 h; AEA and time of birth were considered as continuous independent variables in the model.

Disease. Calf diseases recorded during the experimental period were diarrhea, pneumonia, and omphalophlebitis (navel ill). A case of diarrhea was defined as a calf repeatedly passing loose or watery feces, with or without blood. A case of pneumonia was defined as a calf with increased respiratory rate, fever, liberal bilateral nasal discharge, and repeated coughing. A case of omphalophlebitis was defined as a calf displaying a swollen and painful umbilical vein, with or without pus. The number of times a calf was classified as clinically ill was recorded as never, once, twice, or 3 times. To be classified as clinically ill a second or third time, a calf had to have had no signs of illness between illnesses. A new case of disease was recorded once the calf exhibited no signs of previous clinical illness for 6 d. No calf was sick more than 3 times. The risk of clinical illness occurring within the experimental period was modeled using binomial logistic regression with PROC GENMOD (SAS Institute Inc.). The link function used was log. Colostrum treatment was included in the model, and the number of days each calf remained in the study was included as a covariate in the model. Calf was included as a repeated measure. Significance was declared at $P < 0.05$. Other covariates that were considered and subsequently removed due to lack of significance included colostrum, transition milk, and 24-h serum IgG concentrations; TBC and pH of colostrum and transition milk; and calf AEA and time of birth. Relative risk and 95%

confidence intervals were calculated. Relative risk was calculated from the exponent of the model regression coefficients.

RESULTS

Colostrum from 49 cows was used to feed calves; 23 cows fed 1 calf each, and the remaining 26 cows fed 2 calves each. The mean birth date of all calves was February 22, 2014 (SD = 13.2 d), and the mean birth BW was 34.5 kg (SD = 5.67 kg).

IgG Concentration, TBC, and pH of Colostrum

The overall mean mass of IgG fed to all calves in the first 24 h of life was 280 g (SD = 88.1 g). The mean colostral IgG concentration fed to all calves in the present study was 94.0 g/L (range 61.6–176.6 g/L). No difference in colostral IgG concentration existed among the different experimental groups ($P = 0.96$). The mean IgG concentration of PST was 91.0 g/L (SE = 8.86), FR had a mean IgG concentration of 98.0 g/L (SE = 7.31), ST4 had 96.7 g/L IgG (SE = 8.32), ST13 had 93.7 g/L IgG (SE = 7.73), and the mean IgG concentration of ST22 was 91.0 g/L (SE = 7.70).

Total bacterial count differed ($P < 0.01$) by colostrum treatment. Numerically, pasteurized colostrum had the lowest TBC, whereas ST22 had the highest TBC (Figure 1). The mean pH of all colostrum fed to calves in the present study was 6.126 (SE = 0.1371). The pH of colostrum differed ($P < 0.01$) between experimental treatment groups; colostrum in ST22 had the lowest pH (Figure 2). The pH values of the different treatments were 6.512 (SE = 0.1368), 6.254 (SE = 0.1368), 6.474 (SE = 0.1469), 5.995 (SE = 0.1368), and 5.392 (SE = 0.1285) for PST, FR, ST4, ST13, and ST22, respectively. Colostral TBC was negatively correlated (Pearson correlation) with pH ($r = -0.87$), indicating that a greater TBC was associated ($P < 0.01$) with a lower pH. No pasteurized colostrum had a TBC result $>100,000$ cfu/mL, 4 fresh and 8 ST4 samples had a TBC $>100,000$ cfu/mL, whereas all samples in both ST13 and ST22 had $>100,000$ cfu/mL. Colostrum in the TBC category of $<100,000$ cfu/mL (mean pH = 6.472; SE = 0.1273) had a greater ($P < 0.01$) pH than colostrum with a TBC $>1,000,000$ cfu/mL, whereas colostrum with a TBC of between 100,000 and 1,000,000 cfu/mL (pH = 6.123; SE = 0.2153) tended to have a greater ($P = 0.07$) pH than colostrum with $>1,000,000$ cfu/mL TBC (pH = 5.669; SE = 0.1228). No considered covariate (colostral IgG concentration, pH, or time of birth) was associated with these variables, and hence all were excluded in final analysis.

Figure 1. Total bacterial count ($\log_{10}$ TBC cfu/mL) of colostrum stored in varying conditions to induce different bacteria levels. Values above bars indicate count in colony-forming units per milliliter. Error bars represent 1 standard error each side of the least squares means. Means differ significantly between treatments with different letters ($P < 0.05$). PST = pasteurized colostrum ($n = 15$); FR = colostrum fed when freshly collected ($n = 15$); ST4 = colostrum stored at 4°C for 2 d ($n = 15$); ST13 = colostrum stored at 13°C for 2 d ($n = 15$); ST22 = colostrum stored at 22°C for 2 d ($n = 15$).

IgG Concentration, TBC, and pH of Transition Milk

The mean transition milk IgG concentration fed to all calves in the present study was 30.9 g/L (range 6.6–76.9 g/L). No difference in transition milk IgG concentration existed among the different experimental groups ($P = 0.68$). The mean IgG concentration of PST was 30.8 g/L (SE = 4.43), FR had a mean IgG concentration of 31.5 g/L (SE = 3.61), ST4 had 30.5 g/L

Figure 2. pH of colostrum stored in varying conditions to induce different bacteria levels. Error bars represent 1 standard error each side of the least squares means. Means differ significantly between treatments with different letters ($P < 0.05$). PST = pasteurized colostrum ($n = 15$); FR = colostrum fed when freshly collected ($n = 15$); ST4 = colostrum stored at 4°C for 2 d ($n = 15$); ST13 = colostrum stored at 13°C for 2 d ($n = 15$); ST22 = colostrum stored at 22°C for 2 d ($n = 15$).

IgG (SE = 4.37), ST13 had 31.7 g/L IgG (SE = 3.93), and the mean IgG concentration of ST22 was 31.0 g/L (SE = 3.78).

Total bacterial count in transition milk did not differ between treatments ($P = 0.51$). The mean transition milk TBC of PST was 142,000 cfu/mL (SE = 14,043), FR had a mean transition milk TBC of 131,500 cfu/mL (SE = 13,161), ST4 had 125,000 cfu/mL (SE = 13,137), ST13 had 134,000 cfu/mL (SE = 13,293), and the mean transition milk TBC of ST22 was 131,000 cfu/mL (SE = 13,578). The mean pH of all transition milk fed to calves in the present study was 6.015 (SE = 0.1260). The pH of transition milk did not differ between experimental treatments ($P = 0.41$). The pH values of the different treatments were 6.114 (SE = 0.1268), 6.145 (SE = 0.1268), 6.174 (SE = 0.1269), 6.095 (SE = 0.1268), and 6.092 (SE = 0.1285) for PST, FR, ST4, ST13, and ST22, respectively. None of the considered covariates (transition milk IgG concentration, and pH) were associated with these variables and hence were excluded in final analysis.

Serum IgG Concentration

The mean IgG concentration of serum collected from calves at 0 h (i.e., before colostrum feeding) was 0.20 g/L (SE = 0.30) and was not different between treatments ($P = 0.59$). Colostral treatment had a significant effect ($P < 0.01$) on 24-h calf serum IgG concentrations (Figure 3). At 24 h of age, PST calves had 34.2 g/L

IgG (SE = 3.29), FR calves had 41.4 g/L IgG (SE = 2.65), ST4 calves had 46.4 g/L IgG (SE = 2.96), ST13 calves had 36.0 g/L IgG (SE = 2.76), and ST22 calves had 24.0 g/L IgG (SE = 2.82; Figure 3). No calf had failure of passive transfer; however, the lowest serum IgG concentration was 10 g/L IgG, and this concentration was only in ST22.

When all colostrum samples were considered and categorized according to TBC value rather than colostral treatment, an association was detected between TBC and serum IgG of calves sampled at 24 h. Calves ($n = 36$) fed colostrum $< 100,000$ cfu/mL TBC (mean serum IgG = 38.4 g/L; SE = 2.20) and calves ($n = 20$) fed colostrum between 100,000 and 1,000,000 cfu/mL TBC (mean serum IgG = 43.0 g/L; SE = 6.59) did not have different serum IgG at 24 h ($P = 0.30$). Calves in both of these categories had greater ($P < 0.05$) IgG at 24 h than calves ($n = 19$) fed colostrum with $> 1,000,000$ cfu/mL TBC (mean serum IgG = 31.2; SE = 2.11). Serum IgG and colostrum TBC value were negatively correlated ($r = -0.60$). For every 1-unit increase in logTBC, serum IgG concentration decreased by 3.57 units (SE = 2.756) on average.

Apparent Efficiency of Absorption

The difference in AEA of calves among treatments tended toward significance ($P = 0.05$). Mean AEA in PST calves was 36.1% (SE = 3.78), and in FR calves, the mean AEA was 45.6% (SE = 3.96). Calves fed ST4 had a mean AEA of 48.2% (SE = 3.74), whereas calves fed ST13 and ST22 had an AEA of 37.9% (SE = 3.15) and 26.2% (SE = 3.56), respectively. No difference in AEA existed between PST, FR, ST4, and ST13 ($P > 0.05$), but ST22 had a lesser AEA than FR ($P = 0.04$) and ST4 ($P < 0.01$).

Average Daily Weight Gain

The mean colostral treatment birth weights were PST 33.6 kg (SD = 7.07), FR 34.4 kg (SD = 4.46), ST4 34.1 kg (SD = 5.42), ST13 34.0 kg (SD = 5.79), and ST22 36.5 kg (SD = 4.87). The mean treatment weights at 6 mo were PST 109.9 kg (SD = 6.69), FR 119.1 kg (SD = 9.91), ST4 117.7 kg (SD = 9.74), ST13 119.4 kg (SD = 9.49), and ST22 115.2 kg (SD = 12.17) ($P = 0.18$). The mean average daily weight gain of all calves in the present study from birth to weaning (February 4 to June 19; i.e., including birth weights of the first calves to the weaning weights of the last calves in the study) was 0.62 kg/d (range 0.48 to 0.79 kg/d; Table 1). No difference existed between experimental groups in weight at 6 mo ($P = 0.12$), average daily weight gain

Figure 3. Serum IgG concentration of calves fed colostrum stored in varying conditions to induce different bacteria levels. Error bars represent 1 standard error each side of the least squares means. Means differ significantly between treatments with different letters ($P < 0.05$). PST = pasteurized colostrum ($n = 15$); FR = colostrum fed when freshly collected ($n = 15$); ST4 = colostrum stored at 4°C for 2 d ($n = 15$); ST13 = colostrum stored at 13°C for 2 d ($n = 15$); ST22 = colostrum stored at 22°C for 2 d ($n = 15$).

from birth to weaning ($P = 0.18$; Table 1), or from birth to 6 mo ($P = 0.16$; Table 1).

Disease

A total of 60 disease episodes (i.e., treatments for clinical illness) were recorded during the experimental period, which involved a total of 40 calves experiencing some form of illness. In the 75 calves (15 per treatment group) monitored during the study period, 32 cases of diarrhea (4 calves in PST, SE = 1.692; 3 calves in FR, SE = 2.569; 5 calves in ST4, SE = 3.451; 5 calves in ST13, SE = 2.710; and 6 calves in ST22, SE = 2.708), 16 cases of pneumonia (2 calves in PST, SE = 2.067; 1 calf in FR, SE = 2.883; 1 calf in ST4, SE = 3.134; 2 calves in ST13, SE = 2.326; and 4 calves in ST22, SE = 3.434), and 12 cases of omphalophlebitis (navel ill; 2 calves in PST, SE = 3.904; 2 calves in FR, SE = 4.595; 0 calves in ST4, SE = 4.447; 2 calves in ST13, SE = 4.047; and 1 calf in ST22, SE = 5.039) were observed. Colostral treatment did not affect the likelihood of a calf receiving treatment for a disease ($P = 0.86$). No calf died during the study period.

DISCUSSION

Colostrum Quality

In the present study, colostrum contained IgG concentrations (mean = 96.8 g/L) that far exceeded the 50 g/L recommended minimum threshold. Although this value is greater than the findings in some studies (Pritchett et al., 1991; Morrill et al., 2012), it is similar to others (Conneely et al., 2013, 2014). However, cow breed, cow diet, and seasonal management systems were common between the present experiment and those that previously recorded high colostrum IgG values (Conneely et al., 2013, 2014). Another factor to consider is the protocol for collecting colostrum in these studies. Colostrum was collected immediately postpartum, when IgG concentration would be at its greatest. This timing is not typical in a commercial setting; farm-

ers instead wait until the next milking before collecting the colostrum (Cummins et al., 2016a). Storage treatment did not affect colostrum IgG concentration, but the storage of colostrum at $\geq 4^{\circ}\text{C}$ resulted in greater TBC and lower pH, compared with FR colostrum, which is in agreement with previous research (Stewart et al., 2005).

Although all precautions were taken in the present study to minimize bacterial contamination during colostrum collection, TBC of FR colostrum in the present study was almost 400,000 cfu/mL (5.6 cfu/mL $\log_{10}\text{TBC}$), exceeding the current suggested maximum bacteria level of 100,000 cfu/mL (Godden, 2008). Because cleaned equipment was used for collection, the present recommendations may be unrealistic in a commercial setting. Furthermore, although some studies have reported exceptionally low bacteria counts in colostrum (Johnson et al., 2007), multiple studies correspond to the TBC identified in the fresh samples in the present study (Stewart et al., 2005; Godden et al., 2012; Morrill et al., 2012), indicating that the results in the present study are more realistic with regard to commercial farms.

The colostrum pH difference of 1.120 units between the highest (PST) and lowest (ST22) was similar to declines seen in previous research in which colostrum was stored at a mean temperature of 23°C (Stewart et al., 2005). However, in a comparison of ST4 to colostrum stored at 4°C in the previous work, the pH difference between PST and ST4 (0.0382 units) was less than the decrease reported between colostrum at collection and after refrigeration (0.08 units; Stewart et al., 2005) than in the present study (0.03 units difference between ST4 and colostrum stored at 4°C). Stewart et al. (2005) also reported a much lower pH at the time of colostrum collection than in the current study (5.59 units; Stewart et al., 2005). Although the pH in colostrum is influenced by the temperature at which colostrum is stored (Singh et al., 1997), the lack of difference in colostrum pH during storage in the present study may be due to the buffering capacity of the colostrum collected (i.e., the difference in protein content; Park, 1991). The protein

Table 1. Average daily weight gain (ADWG; SE in parentheses) from birth to weaning and from birth to 6 mo of age in calves fed different colostrum treatments

ADWG (kg/d)	Treatment ¹					P-value
	PST	FR	ST4	ST13	ST22	
Birth to weaning	0.59 (0.026)	0.61 (0.022)	0.62 (0.024)	0.63 (0.023)	0.58 (0.023)	0.182
Birth to 6 mo	0.52 (0.017)	0.53 (0.016)	0.57 (0.016)	0.56 (0.016)	0.54 (0.015)	0.164

¹PST = pasteurized (n = 15); FR = fed when freshly collected (n = 15); ST4 = stored at 4°C for 2 d (n = 15); ST13 = stored at 13°C for 2 d (n = 15); ST22 = stored at 22°C for 2 d (n = 15). Average weaning age = 86 d.

content of the colostrum was not tested in the present study, however, and it is only possible to speculate that it had a potential role.

Serum IgG Concentration

Previous research that investigated the effect of colostrum storage on IgG, TBC, and pH did not examine the effect it may have on passive transfer in calves (Jenny et al., 1977; Argüello et al., 2003; Stewart et al., 2005). Early research reported lower passive transfer in calves receiving colostrum with a pH of 4.65 (Foley et al., 1978), but a more recent study suggested that a pH as low as 5.0 did not affect the absorption of IgG in calves (Quigley et al., 2000). Neither of these studies attempted to quantify the TBC in the colostrum. The lowest mean colostrum pH in the present study was 5.24, and it is probable that TBC has the greatest role affecting passive transfer of IgG in dairy calves. This outcome also supports earlier suggestions that bacteria in the gut of the calf interfere with IgG absorption (James et al., 1981), explaining why calves that received colostrum with a greater bacterial load (i.e., ST22) had a reduced IgG absorption rate. Research analyzing IgG with the same technology (i.e., RID) also support the results in the present study, stating that IgG was greater in serum of calves that were fed heat-treated colostrum (i.e., colostrum with reduced total bacteria; Elizondo-Salazar and Heinrichs, 2009). Furthermore, when data in the present study were considered according to colostrum TBC, rather than colostrum treatment, a significant association between colostrum treatment and calf serum IgG concentration was observed. The lesser serum IgG at 24 h in calves fed colostrum with >1,000,000 cfu/mL supports the hypothesis that TBC has an important role in influencing IgG absorption by dairy calves.

Consistent with previous research (Burton et al., 1989; Morin et al., 1997; Conneely et al., 2014), the concentration of IgG in calf serum immediately after birth was negligible in the present study. At 24 h, calves fed PST had lower serum IgG concentrations than ST4, which may be due to the denaturation of IgG in colostrum reducing the absorption by the calf (Elizondo-Salazar et al., 2010). Although previous studies using the similar heat treatment (i.e., pasteurize at 60°C for 60 min) have reported no difference in serum IgG concentrations in calves fed heat-treated colostrum versus raw colostrum (Johnson et al., 2007; Godden et al., 2012), these studies tested serum IgG concentration using different methods (turbidimetric immunoassay, serum total protein). Previously, losses in colostrum IgG concentration were observed during heat treatment (60°C for 60 min; Donahue et al., 2012); however, the labora-

tory test used to identify colostrum IgG concentrations differed (turbidimetric immunoassay) from that used in the present study (i.e., RID). Thus, RID may be able to identify IgG when turbidimetric immunoassay cannot, and some other mechanism may be occurring that prevents absorption by the calf. In a previous study, heat treatment resulted in a loss in 3-dimensional functional formation of proteins (Law and Leaver, 2000). Because unfolding of the protein structure occurs in the Fab and Fc regions of the IgG molecule during heat treatment (Indyk et al., 2008), identification of IgG by laboratory test kits such as RID may be facilitated, but identification of IgG by the binding sites in the calf's intestinal wall may be obstructed. Because colostrum in the present study had extremely high concentrations of IgG, rate of passive transfer is not a particular concern, but it suggests that storing colostrum at 4°C for up to 2 d, rather than pasteurizing it, may be more reliable to maintain the viability of IgG for absorption in the calf. However, feeding unpasteurized colostrum can have its own set of negative implications. If disease such as paratuberculosis exists on the farm, feeding unpasteurized colostrum increases the risk of transferring disease to the calves (Godden et al., 2006).

Although colostrum stored at 4°C in the present study had a mean TBC >2 million cfu/mL, adequate passive transfer still occurred in all calves (serum IgG concentration >10 g/L; Furman-Fratczak et al., 2011). Because the majority of colostrum in the present study had very high concentrations of IgG, adequate passive transfer was achievable. If colostrum has a lower IgG concentration than in the present study, insufficient IgG may be available to compete with TBC, resulting in failure of passive transfer of IgG by the calf. To achieve passive transfer, a minimum total mass of approximately 150 g of IgG is required by a calf (Chigerwe et al., 2008); in the present study, the average total mass of IgG delivered at the first feed to calves between all treatments combined (280 g; SD = 88.1 g) was almost twice the minimum required to ensure adequate passive transfer. Unlike the calves in the present study, not all calves on a commercial farm receive colostrum within the first 2 h of birth (Cummins et al., 2016a), and this possibility is another consideration that must be acknowledged.

A minimum TBC cutoff of 100,000 cfu/mL in colostrum was previously proposed (Godden, 2008). In the present study, the PST colostrum treatment had a TBC <100,000 cfu/mL, while FR and stored colostrum (4°C, 13°C, and 22°C storage) had TBC >100,000 cfu/mL. Nonetheless, significantly reduced calf serum IgG concentrations were only observed in calf fed ST22 colostrum. An interaction may have occurred between colostrum TBC and IgG after consumption by the calf. The

bacteria may bind to IgG and thus alter its structure and prevent its identification by the binding sites in the intestinal wall. Alternatively, TBC may act as physical barriers that prohibit the passage of IgG through the intestinal wall to the bloodstream (James et al., 1981).

Although, calves achieved adequate passive transfer and obtaining sufficient IgG is important for protecting neonates against disease, colostrum also contains many other nutritional factors including specific and nonspecific immune factors that may influence short- and long-term health and performance of the animal (Kehoe et al., 2007). Because average daily weight gain and the incidence of disease of calves did not differ between experiments, the effect of storing colostrum or the effect that colostral TBC may have on colostral immune components may be a minor contribution. Nonetheless, acknowledging the extremely high colostrum and 24-h serum IgG concentrations is important; if colostrum had a lesser concentration of IgG or if failure of passive transfer had occurred, a greater effect on calf growth rates and the incidence of disease may have been observed. On a commercial basis, failure of passive transfer is quite common; for example, more than 50% of Irish dairy calf serum submitted regional veterinary laboratories had failure of passive transfer in 2014 (AFBI and DAFM, 2015). Furthermore, the statistical power calculation in the present study focused on serum IgG concentration; if a greater sample size had been used, greater differences in disease incidences may have been observed.

CONCLUSIONS

Storing colostrum at warmer temperatures results in greater quantities of bacteria and reduced pH, but it does not affect IgG concentration in the colostrum. Storing colostrum at 4°C for 2 d did not negatively affect the absorption of colostral IgG by the calf; however, storing it at greater temperatures led to decreased absorption of IgG from colostrum by the calf, even though all colostrum was > 50 g/L and fed promptly after birth. Bacteria levels appear to play an important role in determining the rate of IgG absorption in the calf because calves fed with extremely high levels of bacteria (>1,000,000 cfu/mL) have decreased serum IgG concentrations at 24 h. However, when calves are fed colostrum with high concentrations of IgG (>50 g/L) promptly after birth, but with bacteria levels >100,000 cfu/mL, calves achieve adequate passive transfer. If colostrum cannot be pasteurized before feeding or if it cannot be fed to calves when it is freshly collected, storage ≤4°C for 2 d sufficiently minimizes bacterial growth to ensure that adequate passive transfer can occur when the colostrum is consumed by the calf.

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