ABSTRACT

Colostrum is essential for good neonate health; however, it is not known whether different calves absorb the nutrients from colostrum equally well. In this study, the absorption of protein, IgG, and γ-glutamyl transferase was compared in newborn dairy bull calves for 1 wk after feeding colostrum from different sources. Thirty-five Holstein-Friesian bull calves were randomly allocated into 3 groups and fed colostrum within 4 h after birth. Group A calves (n = 12) were bottle fed colostrum from their own dam for 3 d. Colostrum from these group A cows was also used as foster cow colostrum for the group B calves (n = 12), such that each group A and B calf pair received identical colostrum from each milking of the respective group A dam (10% of birth weight per day). The group C calves (n = 11) were fed 1 bottle (2 L) of pooled colostrum and transition milk (referred to as pooled colostrum), as was the standard practice on the dairy farm. The pooled colostrum was collected from the other dairy cows on the farm 0 to 4 d postpartum and stored at 4°C for less than 12 h. Blood was sampled from calves before the first feeding and at 1, 2, 3, and 7 d after birth. Levels of total solids, total protein, and IgG were higher in the dam colostrum than in the pooled colostrum. At birth, there were no differences between the calf groups for any measurements, and all calves had very low IgG levels. After receiving colostrum, the glucose, plasma γ-glutamyl transferase, serum total protein, and IgG concentrations increased significantly in all calves. There were no differences in any blood measurements at any time point between the pairs of group A and group B calves that received colostrum from the same cow except for the IgG concentration 2 d after birth. However, the group A calves had a higher total serum protein level and IgG concentration than the group C calves for all the time points after the first feeding. The group B calves had a higher IgG concentration than the group C calves on d 1, 2, and 7 after birth. Compared with groups A and B, there was no difference in the proportion of calves in group C that failed to have passive immunity transferred adequately based on the IgG threshold (<10 g/L). Thus, the calves receiving identical colostrum from the same cow had the same levels of IgG, and even the pooled colostrum provided sufficient transfer of IgG as the calves were fed within 4 h after birth.

Key words: bovine, immunoglobulin G, passive transfer, failure of passive immunity transfer, passive immunity

INTRODUCTION

Management practices to ensure that calves receive adequate colostrum in the first day of life are critically important for calf health and future production (Quigley et al., 2001; Faber et al., 2005; Trotz-Williams et al., 2008; Furman-Fratczak et al., 2011). The placenta of ruminants is synepitheliochorial, which prevents the transfer of immunoglobulins from dam to calf during pregnancy; therefore, calves have little or no passive immunity when they are born (Barrington and Parish, 2001; Castro et al., 2011). Newborn calves receive passive immunity after birth by the absorption of immune-related cells and proteins, primarily IgG, from colostrum through the gut. The gut remains open for absorption only for approximately the first 24 h after birth (reviewed by Godden et al., 2019). To resist pathogens during the first weeks of life, newborn calves must receive a sufficient quantity of good-quality colostrum as soon as possible after birth. It is recommended that dairy calves be fed within 1 to 2 h of birth and receive colostrum with greater than 50 g/L IgG at a volume equivalent to 10 to 12% of their birth weight (Godden et al., 2019).
If calves receive too little colostrum before gut closure or if the colostrum quality is poor (without sufficient immunoglobulins), then failure of passive immunity transfer (FPIT) may occur. Failure of passive immunity transfer is associated with increased calf morbidity and mortality (Barrington and Parish, 2001; Moran, 2002; Furman-Fratczak et al., 2011; Vandeputte et al., 2011; Lombard et al., 2020), and the suggested threshold to define FPIT in calves is less than 10 g/L IgG in the blood 24 to 48 h after birth (Barrington and Parish, 2001; Godden et al., 2019; Oliveira et al., 2019). In addition to serum IgG, the activity of γ-glutamyl transferase (GGT) in neonatal calf blood can be used as an indicator of colostrum ingestion (Braun et al., 1982; Weaver et al., 2000). Serum GGT activity greater than 200 IU/L at 1 d after birth or greater than 75 IU/L at 7 d after birth is considered to be indicative of successful passive immunity transfer, whereas a GGT level below 50 IU/L within the first 2 wk after birth is indicative of FPIT (Parish et al., 1997). As IgG and GGT concentrations are not measured routinely on farms, FPIT can be assessed in the field using refractometry to determine the total serum protein levels in calves. The refractometry threshold for FPIT is generally agreed to be less than 52 g/L total serum protein in neonate calves 1 d after birth (McGuirk and Collins, 2004; Cuttance et al., 2017; Godden et al., 2019), although 55 g/L may be a better threshold for ruling out FPIT (Buczinski et al., 2018).

Studies have shown that calves that receive sufficient colostrum shortly after birth and avoid FPIT have improved passive immunity and health status as well as weaning weights (Furman-Fratczak et al., 2011; Priestley et al., 2013; Lago et al., 2018), which allows first insemination earlier (Furman-Fratczak et al., 2011). Nevertheless, colostrum and transition milk are often pooled from cows 0 to 4 d postpartum to feed the newborn calves in New Zealand and Australia (Denholm et al., 2017; Cuttance et al., 2018; Phipps et al., 2018; Abuelo et al., 2019). The newborn calves are frequently fed pooled colostrum and transition milk on the first day after birth and are then fed fresh mature milk thereafter (Cuttance et al., 2018; Abuelo et al., 2019). Pooling colostrum from multiple cows postpartum has benefits for commercial dairy farms, as pooling increases the volume of colostrum available for all calves, saves labor, and simplifies colostrum management. However, the quality of colostrum, in terms of IgG concentration, decreases rapidly after the first 24 h postpartum (Morin et al., 2010; Conneely et al., 2014). Therefore, if the colostrum and transition milk are combined from cows 0 to 4 d postpartum, this pooled colostrum will be of lower quality with less IgG and protein than colostrum collected within the first day of calving (Godden et al., 2019). Poor-quality pooled colostrum with less than 50 g/L IgG has been shown to result in an increased risk of FPIT (Beam et al., 2009), whereas pooled colostrum of good quality with greater than 50 g/L IgG should provide adequate transfer of passive immunity to the calves (King et al., 2020).

The objectives of this study were to (1) establish whether different neonate dairy bull calves that are bottle fed colostrum (5% of birth weight every 12 h) from the same cow for 3 d will absorb equal amounts of the available components, including IgG; (2) examine whether the level of passive immunity transfer is comparable if calves are fed colostrum from their own dam or a foster cow; and (3) determine whether the level of passive immunity transfer is comparable if calves from the same cohort are fed a single bottle (2 L) of pooled colostrum and transition milk within 4 h of birth instead of being fed individual cow colostrum and transition milk for 3 d. It was hypothesized that calves that receive colostrum from individual cows would absorb similar amounts of the colostrum components and would have an adequate level of passive immunity. In contrast, calves that receive a minimal volume of pooled colostrum would have a lower level of passive immunity and a higher level of morbidity under the same environmental conditions.

MATERIALS AND METHODS

Experimental Design and Animals

All animal experimental work was approved by The University of Adelaide Animal Ethics Committee (approval number S-2017-060). A commercial farm with 1,800 cows located in Mount Gambier, South Australia, provided the 35 newborn Holstein-Friesian bull calves used in this study, which were singletons born in February 2018 from multiparous dams. The study power calculation with α of 80% and β of 0.05 estimated that 10.5 calves would be required per group to detect 5% variation in IgG. As a dropout rate of 10% was expected, 12 calves were chosen per group. The farm provided the facilities with 24-h access so that the samples could be processed immediately upon collection. Calving ease was recorded (Supplemental Table S1; https://adelaide.figshare.com/articles/journal_contribution/Do_et_al_2021_Colostrum_source_and_passive_immunity_transfer_in_dairy_bull_calves_Journal_of_Dairy_Science_paper_Supplementary_Files/14370269, Bottema et al., 2021) and calves were separated from their dam within 30 min of calving after being licked cleaned but before suckling. The calves were
tagged, measured for weight and girth, and scored for vigor and health within 4 h of birth (Supplemental File S1; Supplemental Figure S1; https://adelaide.figshare.com/articles/journal_contribution/Do_et_al_2021_Colostrum_source_and_passive_immunity_transfer_in_dairy_bull_calves_Journal_of_Dairy_Science_paper_Supplementary_Files/14370269, Bottema et al., 2021). The calves were randomly allocated to 3 groups using block randomization, with 6 blocks of 3 calf groups (groups A, B, and C). The calf serum GGT levels measured 1 d after birth indicated that one of the group C calves had suckled; therefore, this calf was removed from the study. All matched pairs of calves (e.g., A1 and B1) were born within 12 h of each other. The 12 dams of calves assigned to group A were milked within 2 h postpartum and twice daily thereafter for 3 d. The colostrum volume from each cow was recorded. The 12 group A calves were fed colostrum from their own dam, and the 12 matched group B calves were fed the identical colostrum from each milking of the respective group A dam (Supplemental File S1). Group A and B calves were bottle fed 5% of their birth weight of colostrum twice a day for 3 d, as recommended for maximum IgG absorption (Jaster, 2005). The first feeding was within 4 h after birth, and the second feeding was within 12 h after birth. After 3 d, the group A and B calves were fed bulk tank milk twice a day for the next 4 d (2 L/calf per feed). The feeding program used for the group C calves replicated the practice on the commercial dairy farm and represented a cohort-matched contrast for the calves fed the individual cow colostrum. The purpose of group C was to provide a benchmark for the expected morbidity under the local conditions on the farm. Within 4 h of birth, the 11 group C calves were fed a single bottle of mixed colostrum and transition milk (2 L), which was collected and pooled from the dairy transition cows between 0 and 4 d postpartum (referred to as “pooled colostrum” herein). Thereafter, the group C calves were fed bulk tank milk twice a day for the next 4 d (2 L/calf per feed) for 7 d. All colostrum and milk was stored at 4°C until fed and was never stored more than 12 h.

**Colostrum Samples**

The group A cows were milked within 2 h of calving using a portable milking machine for the first milking, and then were milked in the dairy using a rotary milking parlor for the second to seventh milkings. Based on the definition of colostrum from the review of McGrath et al. (2016), the mammary secretions collected from these early milkings are referred to as “dam colostrum” herein. In addition to the bulk tank milk, the dairy provided the pooled colostrum and transition milk from multiple cows 0 to 4 d postpartum. These samples are referred to as “pooled colostrum” to distinguish from the bulk tank milk and the individual dam colostrum samples. The dam colostrum, pooled colostrum, and bulk tank milk were collected from the dairy twice a day and stored at 4°C for less than 12 h before feeding. Samples of dam colostrum, pooled colostrum, and bulk tank milk were collected into sterile 50-mL tubes immediately after each milking, frozen at −20°C, and then transported to The University of Adelaide, Roseworthy Campus, for storage at −80°C until analyzed.

**Calf Blood Samples**

Calf blood samples were collected within 4 h of calving before feeding (d 0) and on d 1, 2, 3, and 7 after birth. Blood was drawn from the jugular vein into 6-mL BD Vacutainers without anticoagulant and 6-mL BD Vacutainers with heparin. The blood samples in tubes without anticoagulant were allowed to clot for 45 to 60 min at 4°C and centrifuged (2,000 × g for 15 min at room temperature), and the serum was collected. Heparinized blood was centrifuged at 2,000 × g for 15 min at room temperature immediately after collection, and plasma was collected. Serum and plasma samples were stored at −20°C and then transported to The University of Adelaide, Roseworthy Campus, for storage at −80°C until analyzed.

**Colostrum and Serum Refractometer Measurements**

The concentration of the total soluble solids (Brix value) was measured in fresh colostrum and bulk tank milk immediately after milking using a digital optical refractometer (DBR-1, Starr Instruments). The refractometer measured proteins, carbohydrates, and other soluble molecules within a Brix value range of 0 to 50%. A different digital refractometer (Atago) with measurement range for protein from 0 to 12 g/100 mL was used to determine total soluble protein concentration by refractometer (TP-R) in the calf serum at d 0, 1, 2, 3, and 7 immediately after separating the serum by centrifugation. Both refractometers were calibrated using ultrapure water (Milli-Q).

**Colostrum Fourier-Transform Midrange Infrared Analyzer Measurements**

Total protein, lactose, and fat percentages in the thawed–frozen individual dam colostrum and pooled colostrum samples were determined using a Fourier-
transform midrange infrared spectrometer (Foss Analytics) at the National Herd Development Co-operative (Kyabram, VIC, Australia).

**Bradford Assay for Total Protein**

Total protein in colostrum, pooled colostrum, bulk tank milk, and calf serum was assayed in a 96 well-plate using a Quick Start Bradford protein assay kit following the manufacturer’s instructions and bovine serum albumin for the standard curves (Quick Start, Bio-Rad Laboratories Inc.). Calf serum, the dam colostrum samples at d 1, 2, and 3, pooled colostrum, and bulk tank milk were diluted with ultrapure water at a ratio of 1:100, and colostrum at d 0 was diluted at a ratio of 1:200 or 1:300. The diluted samples (5 µL) were mixed with Coomassie Brilliant Blue G-250 dye (250 µL) and color change was measured using a Benchmark Plus microplate spectrophotometer (Bio-Rad Laboratories Inc.) at 595 nm. The total protein by Bradford assay (TP-B) in the samples was calculated based on the standard curve.

**ELISA for IgG Concentration**

The IgG concentration in colostrum, pooled colostrum, bulk tank milk, and calf serum was assayed in 96-well plates (Coat Nunc F96 Maxisorp plates, Thermo Fisher Scientific) using 2 bovine IgG-specific antibodies (Life Technologies): goat anti-bovine IgG antibody unconjugated, affinity purified (Novex cat. no. A18753), and goat anti-bovine IgG antibody conjugated with horseradish peroxidase, affinity purified (Invitrogen cat. no. 18751). Calf serum samples were diluted in 0.05% Tween 20-PBS solution at a ratio of 1:10³ for d 0 and a ratio of 1:10⁶ for all other time points. The colostrum samples from d 0 and 1 and the pooled colostrum samples were diluted at a ratio of 1:10⁶, and the colostrum samples from d 2 and 3 and the bulk tank milk samples were diluted at a ratio of 1:10⁵. Bovine γ-globulin (Bio-Rad Laboratories Inc.) was used as a standard with serial dilutions (0, 6.3, 12.5, 25, 37.5, 50, 75, and 100 ng/mL). The 3,3′,5,5′-tetramethylbenzidine substrate (Ultra TMB-ELISA, Thermo Fisher Scientific) was added and the plates were incubated at room temperature in the dark for 15 min before the reactions were stopped with the addition of 0.1 M H₂SO₄. Antibody binding was measured as the enzymatic color change at 450 nm using a Benchmark Plus microplate spectrophotometer (Bio-Rad Laboratories Inc.). The IgG concentrations in samples were calculated based on the standard curve. The intra-assay variation was 7.2%, and the interassay variation was 9.4%.

**Calf Blood Glucose Concentration**

Fresh whole blood was collected into a Vacutainer without anticoagulant. A drop was immediately absorbed onto an Accu-Check Performa test strip, and the glucose concentration was determined using a glucose meter (Accu-Check Performa, Roche Diabetes Care).

**Calf Plasma GGT Measurements**

Calf plasma was analyzed for GGT levels using a kinetic color test with a Beckman Coulter analyzer (Coulter Manufacturing Co.) at the Veterinary Diagnostic Laboratory, The University of Adelaide, Australia. Because the data for GGT were not normally distributed, these data were log-transformed and reported as geometric means with confidence intervals.

**Statistical Analyses**

The preliminary statistical analyses were conducted in R (version 3.6.3; https://cran.r-project.org/bin/windows/base/old/3.6.3/). Descriptive statistics for the colostrum components (Brix value, TP-B, IgG, and protein, lactose, and fat percentages) and calf blood measurements (glucose, GGT, TP-R, TP-B, and IgG concentration) were calculated using the R “psych” package, and normality was determined using Shapiro-Wilk test in the R “nortest” package. T-tests were performed to compare differences between dam colostrum and pooled colostrum components when the data were normally distributed, whereas Kruskal-Wallis tests were used when the data were not normally distributed. The differences between the groups at birth were tested by 1-way ANOVA when the data were normally distributed and verified using Tukey’s honestly significant difference test, whereas Kruskal-Wallis tests were used when the data were not normally distributed.

The effect of time was estimated using mixed models in SAS (PROC MIXED, SAS version 9.4, SAS Institute Inc.), which accounted for the repeated effect of individual dams on the means of the colostrum-dependent variables (Brix value, TP-B, IgG, and protein, lactose, and fat percentages) across the time points (d 0, 1, 2, and 3). The effect of time was also estimated using mixed models (PROC MIXED), which accounted for the repeated effect of individual calves on the means of the blood-dependent variables (glucose, GGT, TP-R, TP-B, and IgG concentration) across the time points (d 0, 1, 2, 3, and 7). Last, the effects of group, time point, and the interaction between the groups and time points were estimated by mixed models in SAS (PROC MIXED), which accounted for the repeated effect of individual calves on the means of dependent variables.
(glucose, GGT, TP-B, TP-R, and IgG levels) for the 3 calf groups (A, B, and C) across time points (d 0, 1, 2, 3, and 7). Blood measurements taken on d 1 from the calves in groups A and B were compared for each calf pair by PROC GLM in SAS using a Bonferroni adjustment for nonparametric variables and Tukey adjustment for parametric variables. Colostrum components at d 0 and calf blood measurements at d 1 were compared using PROC CORR in SAS by estimation of the Pearson correlation coefficients.

The classification scheme of Lombard et al. (2020) was used to categorize the calf serum IgG levels at 48 h as excellent, good, fair, and poor, where the serum IgG levels were ≥25.0, 18.0 to 24.9, 10.0 to 17.9, and <10 g/L, respectively. The proportion of calves from the groups within each category and the proportion of calves in the groups that failed to reach the thresholds for adequate passive immunity transfer were compared pairwise using a Fisher’s exact test (2 × 2 table) with a confidence level of 95%. Failure of passive immunity transfer was indicated by values below the thresholds of 52 g/L total protein at d 1 and 2, 10 g/L IgG at d 1 and 2, 200 U/L GGT on d 1, and 75 U/L GGT on d 7.

RESULTS AND DISCUSSION

To investigate the level of passive immunity transferred after feeding neonate calves colostrum from different sources and to establish whether calves that receive identical colostrum from the same milking of a cow will absorb similar amounts of the available nutrients, 3 groups of neonate calves were fed dam, foster cow, or pooled colostrum. Components in the colostrum were analyzed with respect to their absorption into the blood of the calves during the first 7 d after birth.

**Colostrum and Bulk Tank Milk Measurements**

The levels of the total solids (Brix value), lactose percent, protein percent, total protein (TP-B), and IgG were found to be different between the dam and pooled colostrum ($P < 0.05$; Table 1). The only exception was the concentration of lipid, as there was no difference in the fat percentage when measured by Fourier-transform midrange infrared analyzer between the dam and pooled colostrum.

**Colostrum Total Soluble Solids and IgG.** The total soluble solids of the dam colostrum were significantly higher than the pooled colostrum (mean ± SE Brix value: 25.4 ± 1.4% vs. 19.4 ± 2.1%, respectively; $P < 0.05$; Table 1). The Brix value for the dam colostrum observed in the present study ranged between 18.5 and 34% (95% CI = 22.4–28.4%), and pooled colostrum had a range of 12.3 to 25.9% (95% CI = 14.0–24.8%).

Colostrum is considered good quality if the concentration of IgG is greater than 50 g/L, and many studies have suggested that Brix values between 18 and 23% are equivalent to 50 g/L IgG (Bielmann et al., 2010; Quigley et al., 2013; Bartier et al., 2015; Morrill et al., 2015). In a meta-analysis by Buczinski and Vandeweerden (2016), the authors classified good-quality colostrum as having a Brix value greater than 22% and poor-quality colostrum as having a Brix value less than 18%, where these percentages ensure greater than 50 g/L IgG or less than 50 g/L IgG, respectively. On this basis, although the pooled colostrum generally had less total solids than the dam colostrum, it was not always considered poor quality.

The average IgG concentration in dam colostrum was 184.4 g/L (95% CI: 139.5–229.2 g/L). This was significantly higher than the pooled colostrum ($P < 0.05$), which had an average of 90.5 g/L (95% CI: 66.0–115.1 g/L). The minimum IgG concentration of the dam colostrum was 83.7 g/L, whereas the minimum of the pooled colostrum was 69.7 g/L.

The dam colostrum in this study had higher IgG levels than some studies (Marnila and Korhonen, 2011; Quigley et al., 2013; Dunn et al., 2017), most likely because it was collected within 2 h of calving and the cows were multiparous. The IgG concentrations

---

**Table 1.** Descriptive statistics and comparison of the components in the dam colostrum (n = 12) and pooled colostrum (n = 6) at first feeding on d 0

<table>
<thead>
<tr>
<th>Component</th>
<th>Dam colostrum</th>
<th>Pooled colostrum</th>
<th>$P$-value $^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total soluble solids (Brix %)</td>
<td>25.4 ± 1.4</td>
<td>19.4 ± 2.1</td>
<td>0.04</td>
</tr>
<tr>
<td>Total protein (g/L)</td>
<td>170.8 ± 16.0</td>
<td>88.7 ± 11.6</td>
<td>0.001</td>
</tr>
<tr>
<td>IgG (g/L)</td>
<td>184.4 ± 20.4</td>
<td>90.5 ± 9.6</td>
<td>0.008</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>16.6 ± 0.9</td>
<td>12.4 ± 1.3</td>
<td>0.02</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>4.6 ± 0.4</td>
<td>5.4 ± 0.9</td>
<td>0.46</td>
</tr>
<tr>
<td>Lactose (%)</td>
<td>1.9 ± 0.2</td>
<td>2.4 ± 0.1</td>
<td>0.02</td>
</tr>
</tbody>
</table>

$^1$Total protein was measured by Bradford assay; protein, fat, and lactose percentages were measured by Fourier-transform midrange infrared analysis; and IgG was measured by ELISA.

$^2P < 0.05$ was considered significantly different.
in pooled colostrum in the present study were in the ranges reported elsewhere (Lago et al., 2018: 22.7–96.9 g/L, mean of 63.6 g/L; King et al., 2020: 90.5–104.4 g/L, mean of 100.7 g/L).

Overall, the dam colostrum was good quality, whereas the pooled colostrum was clearly of lower quality. This was expected because total protein and IgG concentrations in colostrum are known to steadily decrease postpartum and are significantly higher in colostrum than in the mature milk produced by dams (McGrath et al., 2016).

**Colostrum Lactose and Fat.** The lactose and fat provided by colostrum and milk are essential energy sources for the neonate (Hammon et al., 2013). The dam colostrum collected within 2 h postpartum contained 1.9% lactose and 4.6% fat on average, whereas the pooled colostrum contained 2.4% and 5.4%, respectively. Both the lactose and fat concentrations were in the range reported in previous studies (Kehoe et al., 2007; Godden, 2008; Dunn et al., 2017). The lactose in dam colostrum was significantly lower than in pooled colostrum ($P < 0.05$), as expected, because lactose is known to be low in colostrum and increases over time as milk production increases (McGrath et al., 2016; Dunn et al., 2017). In contrast, fat concentration has been reported to decrease over time, declining from 7.0% to 6.0% within 24 h (Dunn et al., 2017) and from 6.7% to 3.9% between the first and third milkings (Godden, 2008). However, in the present study, the fat concentration in dam colostrum was similar to that in the pooled colostrum.

**Dam Colostrum Components Day 0 to 3 Postpartum.** Dam colostrum volume increased slowly between successive time points ($P < 0.05$ for colostrum volume between d 0 and 2 postpartum), whereas the concentration of colostrum components, as measured by Brix value, TP-B, and IgG, decreased ($P < 0.05$ between d 0 and all other time points), which is consistent with the observations of others (Yang et al., 2015). The mean Brix value measured in colostrum was 25.4% at the first milking postpartum (d 0) and then decreased considerably to 12.2, 10.6, and 10.5% by d 1, 2, and 3, respectively (Figure 1). Total protein, measured by Bradford assay, declined from 170.8 g/L at d 0 to 51.2 g/L on d 1 and 34.4 g/L for d 2 and 3. The mean IgG concentration in the dam colostrum was 184.4 g/L on d 0 and then rapidly declined to 29.4, 4.9, and 2.7 g/L on d 1, 2, and 3, respectively. Only the colostrum collected within 24 h of parturition had Brix value and IgG concentration above the thresholds of 22% and 50 g of IgG/L, respectively, recommended for the first calf feeding (Quigley et al., 2013; Bartier et al., 2015). Therefore, when pooling colostrum for the first feeding, it is advisable to use only colostrum from cows within 1 d postpartum.

**Bulk Tank Milk Measurements.** Twenty-five bulk tank milk samples, which were fed to the group C calves for 7 d and the group A and B calves from d 4 to 7 postpartum, were also analyzed. The average Brix value, TP-B, and IgG concentration of the bulk tank milk were 8.9 ± 0.2%, 28.6 ± 2.0 g/L, and 0.3 ± 0.1 g/L, respectively, which were all lower than the colostrum sample levels ($P < 0.05$).

**Calf Birth Measurements**

Within 4 h after birth and before feeding, no significant differences were observed in any of the physical or blood measurements for the 3 groups of calves (Table 2), and no health problems or calving difficulties were observed. The average BW at birth of the 35 calves was 38.3 ± 0.9 kg. At birth, the mean glucose, TP-R, and TP-B of 35 calves was 3.8 mmol/L, 40.4 g/L, and 52.5 g/L, respectively. The mean IgG concentration in calf serum was 0.3 g/L, indicating that the calves had not suckled from their dams.

Another good indication of whether a calf has sucked colostrum is the presence of GGT in the neonate blood (Braun et al., 1982; Parish et al., 1997). γ-Glutamyl transferase is found in very low concentrations in the blood of newborn calves until it is rapidly absorbed from the colostrum before gut closure (Weaver et al., 2000). The GGT level was used to exclude calves from this study that sucked from their dams before the initial sampling. The mean GGT level in the plasma of the newborn calves included in this study at d 0 was 11.7 U/L, with a range of 6.2 to 26.2 U/L, which is similar to the 10 to 31 U/L values reported before suckling by Braun et al. (1982).

**Calf Measurements After Receiving Colostrum from Different Sources**

Between 2 and 4 h after birth, the calves were fed colostrum from their own dam (group A calves), foster colostrum from one of the group A dams (group B calves), or pooled colostrum (group C calves). Each group A calf was paired with a group B calf that was born within 12 h (e.g., A1 and B1). For d 3, these pairs of calves received identical colostrum from each milking of the respective group A dam. In contrast, the group C calves received 1 bottle (2 L) of a pool of colostrum and transition milk from the other dairy cows 0 to 4 d postpartum and bulk tank milk thereafter.

Twenty-four hours after consumption of colostrum, the glucose, TP-B, TP-R, IgG, and GGT concentrations
Figure 1. Dam colostrum data from d 0 to 3 postpartum (LSM ± SE, n = 12). Colostrum was milked from the dams of the group A calves within 2 h postpartum and then every 12 h. The colostrum was measured and sampled, and the data were analyzed for 4 time points (2, 24, 48, and 72 h postpartum). Colostrum volume (a), total solids measured by Brix refractometer (b), total protein measured by Bradford assay (c), and IgG concentration measured by ELISA (d). Different letters (a, b) indicate significant differences between means (P < 0.05).

Table 2. Descriptive statistics and comparison of birth measurements among calf groups

<table>
<thead>
<tr>
<th>Measure</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>All</th>
<th>Minimum–maximum</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth BW (kg)</td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
<td>SE</td>
</tr>
<tr>
<td></td>
<td>38.0</td>
<td>1.5</td>
<td>36.8</td>
<td>1.8</td>
<td>40.1</td>
<td>1.3</td>
</tr>
<tr>
<td>Girth (cm)</td>
<td>43.7</td>
<td>1.3</td>
<td>44.9</td>
<td>1.7</td>
<td>45.7</td>
<td>1.4</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>3.8</td>
<td>0.4</td>
<td>4.1</td>
<td>0.3</td>
<td>3.6</td>
<td>0.4</td>
</tr>
<tr>
<td>GGT (U/L)</td>
<td>10.8</td>
<td>1.0</td>
<td>11.0</td>
<td>0.8</td>
<td>13.3</td>
<td>1.8</td>
</tr>
<tr>
<td>TP-R (g/L)</td>
<td>40.5</td>
<td>1.0</td>
<td>39.6</td>
<td>1.1</td>
<td>41.1</td>
<td>0.9</td>
</tr>
<tr>
<td>TP-B (g/L)</td>
<td>55.0</td>
<td>3.5</td>
<td>52.1</td>
<td>2.7</td>
<td>50.1</td>
<td>1.7</td>
</tr>
<tr>
<td>IgG (g/L)</td>
<td>0.3</td>
<td>0.2</td>
<td>0.3</td>
<td>0.1</td>
<td>0.2</td>
<td>0.1</td>
</tr>
</tbody>
</table>

1Group A = calves (n = 12) fed own dam’s colostrum; group B = calves (n = 12) fed foster cow colostrum; group C = calves (n = 11) fed 1 bottle of pooled colostrum. All: n = 35 calves.

2GGT = γ-glutamyl transferase activity; TP-R = total serum protein measured by refractometer; TP-B = total serum protein measured by Bradford assay.

3P < 0.05 was considered significantly different.

4Data were nonparametric.
Glucose Concentration. There was no difference in glucose concentration between the 3 calf groups, although there was a difference between the time points ($P < 0.05$; Figure 2a). The blood glucose concentration increased from a mean for all 3 groups of $3.8 \pm 0.2$ mmol/L before feeding to a mean of $6.9 \pm 0.2$ mmol/L on d 1 after receiving the dam colostrum or pooled colostrum ($P < 0.05$). This level was maintained from d 2 ($6.6 \pm 0.1$ mmol/L) and d 3 ($6.4 \pm 0.1$ mmol/L) to d 7 for all groups ($6.0 \pm 0.1$ mmol/L). These glucose concentrations were in the same range as reported in other studies (Schissler et al., 2002).

GGT Activity. The GGT activity in the calf plasma of all the groups increased 100-fold from d 0 before feeding to d 1 following colostrum feeding ($P < 0.05$; mean: $1,271.4 \pm 94.8$ U/L; range: $89.5-4,034.5$ U/L). These GGT levels are similar to those reported by Braun et al. (1982), who found a range of 10 to 31 U of GGT/L at birth (mean: $18.2$ U/L), which increased to 370 to 5,000 U/L by d 1. The GGT levels in the group A and B calves were higher than those in the group C calves on d 1 ($P < 0.05$; Figure 2b). However, the GGT level in all 3 groups declined by d 7 (mean: $200.8 \pm 94.8$ U/L), and there were no differences between the groups.

Serum Protein Measured by Refractometer. Serum protein refractometry is a tool that can be used on-farm to estimate serum total protein (TP-R) and detect possible FPIT in neonatal calves (Vandeputte et al., 2011; Deelen et al., 2014; Hernandez et al., 2016). The TP-R differed depending on the calf group and time point ($P < 0.05$; Figure 2c). There were large differences in TP-R between d 0 and all other time points (d 1, 2, 3, and 7). At birth, TP-R concentrations in group A, B, and C calves were similar. After receiving colostrum, TP-R increased dramatically by d 1 to $60.9 \pm 1.9$, $59.3 \pm 1.9$, and $52.4 \pm 1.9$ g/L for group A, B, and C calves, respectively, with a significant difference between the group A and B calves versus the group C calves ($P < 0.05$). The TP-R levels in the 3 calf groups remained at these levels for d 2 and 3, and then decreased slightly by d 7. Differences between the group A and C calves were observed for all time points, but there were no significant differences between the group A and B calves at any time point (Figure 2c). The total serum protein levels, as measured by refractometry in the present study, were similar to those in previous studies, where calf serum TP-R at 24 h postpartum was $52$ g/L following feeding $2$ L of pooled colostrum or $59$ g/L following feeding $4$ L (Williams et al., 2014).

Bradford Serum Total Protein. There was a difference in total protein in the calf blood, as measured by the Bradford assay (TP-B), across time points ($P < 0.05$), but no difference was observed between the groups at any of the time points (Figure 2d). The pattern in TP-B levels was similar to the TP-R, with a large increase by d 1 and declining by d 7. The protein concentration estimated by refractometry was lower than the total protein measured by Bradford assay ($P < 0.05$). The Bradford assay measures both soluble and nonsoluble proteins in the blood, whereas the refractometer measures the refractive index of the serum soluble components. Therefore, the refractometer underestimates total protein concentration. This has been observed in other studies where refractometer values were lower by 20 to 25 g/L compared with the Bradford assay or Biuret method (Braun et al., 2001).

Serum IgG. The group A and B calves received dam colostrum with very high IgG concentrations ($184.4$ g/L IgG for the first feed) and then dam colostrum twice daily for 3 d, whereas the calves in group C received only 1 bottle of lower quality pooled colostrum ($94.4$ g/L IgG for the first feed) and then bulk tank milk thereafter ($0.3$ g/L IgG). There were significant differences in the calf serum IgG concentrations between d 0 and all other time points for all calf groups ($P < 0.05$; Figure 2e). After 24 h, the IgG concentration in serum of group A and B calves was similar ($27.9 \pm 2.9$ and $26.4 \pm 2.9$ g/L, respectively) and higher than that in the group C calves ($15.5 \pm 3.0$ g/L), although the difference was significant only for the group A and C calves ($P = 0.004$). The IgG concentration in the calf serum on d 2 was different among all groups (group A, B, and C mean: $35.0 \pm 2.9$, $25.7 \pm 2.9$, and $15.5 \pm 3.0$ g/L, respectively; $P < 0.05$). A difference in the IgG levels between the group A calves (fed their own dam’s colostrum) and the group B calves (fed foster cow colostrum) was observed only at d 2 ($P = 0.03$). The mean IgG concentrations were higher in the group A and B calves than in the group C calves (fed only 1 bottle of pooled colostrum) at all of the time points ($P < 0.05$), except d 3, where there was no difference between the group B and C calves.

It is not clear whether the difference between the IgG levels in the group A and B calves and the levels in the group C calves was the result of the colostrum quality, the amount of colostrum provided, or both. A limitation of the experimental design is that the data from
Figure 2. Blood measurements of the 3 calf groups from d 0 to 7 after birth (LSM ± SE). Blood was sampled before receiving colostrum (d 0) and after feeding colostrum and bulk tank milk (d 1, 2, 3, and 7 after birth). Group A = calves fed own dam’s colostrum for 3 d and then bulk tank milk (black); group B = calves fed foster cow colostrum for 3 d and then bulk tank milk (dark gray); group C = calves fed 1 bottle of pooled colostrum and then bulk tank milk for 7 d (light gray). Blood glucose levels (a), plasma γ-glutamyl transferase (GGT) activity (b), serum total protein by refractometer (TP-R; c), serum total protein by Bradford assay (TP-B; d), and serum IgG concentrations (e). Different letters (a–c) indicate significant differences between means at each time point ($P < 0.05$).
the group C calves could not be compared directly with the data of the other groups in terms of the absorption of the colostrum components because the volume of colostrum fed to these calves differed from the group A and B calves. It was important to have a benchmark that reflected the conditions and the expected morbidity on the farm; however, the results from the group C calves were confounded and prevented additional conclusions regarding quality versus quantity.

The serum IgG concentrations in all calf groups were in the same range as previous studies with similar-quality colostrum (Jones et al., 2004; Poulson et al., 2010; Priestley et al., 2013; Lago et al., 2018; King et al., 2020). The classification scheme of Lombard et al. (2020) was used to categorize the calf serum IgG levels at 48 h as excellent, good, fair, and poor (Supplemental Table S3; https://adelaide.figshare.com/articles/journal_contribution/Do_et_al_2021_Colostrum_source_and_passive_immunity_transfer_in_dairy_bull_calves_Journal_of_Dairy_Science_paper_Supplementary_Files/14370269, Bottema et al., 2021). The majority of group A and B calves had excellent levels of IgG (75 and 66.7%, respectively). The majority of the group C calves, which received 1 bottle of pooled colostrum, had only fair levels of IgG (63.6%). Thus, the proportions within the categories did not differ between the group A and B calves, but they were different for the group C calves (P < 0.01). One group B calf and 1 group C calf were classified as poor, with serum IgG levels below 10 g/L.

**Pairwise Comparisons Between Calves Receiving the Same Cow Colostrum**

In the pairwise comparison of group A and B calves receiving colostrum from the same cow, no differences were observed in the levels of plasma GGT, glucose, serum total protein, or serum IgG at d 1 between the calves receiving identical colostrum, whether the colostrum was from their own dam or a foster cow (e.g., calf A1 vs. calf B1; Table 3). The lack of any significant differences suggests that calves given the same colostrum will absorb similar levels of nutrients.

Interestingly, although each pair of A and B calves received the same colostrum and the mean IgG concentrations for these groups were not statistically different, the group A calves generally had higher average IgG concentrations after d 0 compared with the group B calves (27.9 and 26.4 g/L at d 1, respectively). The dissimilarity in IgG concentrations between group A and B calves was even greater by d 2 (35.0 and 25.7 g/L, respectively; P = 0.03). The IgG levels in the group B calves were consistently between the levels of the group A and group C calves for all time points. The correlation between calf serum IgG concentration at d 1 and d 0 colostrum IgG was also lower for the group A calves (r = 0.49) than for the group B calves (r = 0.76).

This suggests that factors other than IgG itself may be involved in its absorption in the calf. The speed of ingestion could play a role, as there is evidence that bioactive factors in colostrum improve gastrointestinal development and nutrient absorption (Blum, 2006; Hammon et al., 2020). The effect of cross-fostering on the absorption of IgG and other components may warrant further investigation.

**Calf Health**

Calf health was carefully monitored for 1 wk after birth, and diarrhea, fever, and respiratory infections were noted (Table 4). No health problems were detected in the group A calves, and only 2 calves in group B and 2 calves in group C were ill in the first 7 d. Consequently, the numbers were insufficient to assess the effects of colostrum source on calf health. A constraint in this study was the small number of calves in

<table>
<thead>
<tr>
<th>Component 2</th>
<th>Group A</th>
<th>Group B</th>
<th>P-value 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SEM</td>
<td>95% CI</td>
</tr>
<tr>
<td>Parametric</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>6.8</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>TP-R (g/L)</td>
<td>60.9</td>
<td>5.4</td>
<td></td>
</tr>
<tr>
<td>TP-B (g/L)</td>
<td>78.6</td>
<td>9.9</td>
<td></td>
</tr>
<tr>
<td>IgG (g/L)</td>
<td>27.9</td>
<td>8.1</td>
<td></td>
</tr>
<tr>
<td>Nonparametric</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GGT (U/L)</td>
<td>1,511.0</td>
<td>873.2-2,148.8</td>
<td></td>
</tr>
</tbody>
</table>

1Group A = calves fed own dam’s colostrum; group B = calves fed foster cow colostrum. Paired group A and B calves received identical colostrum from the same milking of the dam of the group A calf.
2TP-R = total serum protein measured by refractometer; TP-B = total serum protein measured by Bradford assay; GGT = γ-glutamyl transferase activity. Nonparametric data are presented with 95% CI for the estimate rather than SEM.
3P < 0.05 was considered significantly different.
each group. In particular, an accurate assessment of morbidity was not possible because there were very few cases of illness.

**FPIT**

Failure of the transfer of passive immunity is defined by thresholds that are indicative of the amount of IgG that has been absorbed from the colostrum into the blood of the newborn calf. All calves in group A, which were fed their own dam’s colostrum, had total protein (TP-R), GGT, and IgG levels above the thresholds that indicated successful transfer of passive immunity. However, several calves in groups B and C had TP-R levels below the suggested threshold for d 1 (4 and 5 calves, respectively; Table 4). Compared with group A, group C had a significantly higher proportion of calves that failed to have adequate passive transfer of immunity based on the serum protein refractometry (TP-R), GGT, and IgG levels above the thresholds that indicated successful transfer of passive immunity. However, several calves in groups B and C had TP-R levels below the suggested threshold for d 1 (4 and 5 calves, respectively; Table 4). Compared with group A, group C had a significantly higher proportion of calves that failed to have adequate passive transfer of immunity based on the serum protein refractometry (TP-R), GGT, and IgG levels above the thresholds that indicated successful transfer of passive immunity. Nevertheless, on d 1, only 1 calf from group B (fed foster cow colostrum) and 1 calf from group C (fed only 1 bottle of the lower quality pooled colostrum) had IgG concentrations and GGT levels below the suggested passive immunity transfer thresholds (<10 g/L and <200 U/L, respectively; Table 4).

Few studies have focused on feeding pooled colostrum or foster cow colostrum to calves. In general, feeding colostrum and transition milk pooled from multiple cows has been found to increase the risk of FPIT because the IgG concentration is diluted by the milk from those cows that calved 3 to 4 d earlier (Pithua et al., 2013). However, calves are less at risk of FPIT if the pooled colostrum is collected within the first 2 d postpartum and has an adequate IgG concentration (Williams et al., 2014; King et al., 2020).

The results of our study indicate that calves fed pooled colostrum collected 0 to 4 d postpartum had adequate passive immunity transfer as defined by IgG and GGT thresholds, presumably because the pooled colostrum contained relatively high IgG concentrations (90.5 ± 9.6 g/L) and was fed within 4 h of birth. Given that additional factors besides IgG are transferred from the colostrum to the neonate, including other immunity-related proteins, microRNA, and cells (Gelsinger and Heinrichs, 2017; Godden et al., 2019), it would be of interest to examine other components that may be vital.

**CONCLUSIONS**

Calves receiving colostrum collected soon after birth from their own dam (group A) had significantly higher TP-R and IgG concentrations on d 1 compared with calves fed lower quality pooled colostrum (group C). The group C calves, which received only 1 bottle of pooled colostrum, had fair levels of IgG, with only 1 calf with poor IgG levels (below 10 g/L) and no demonstrable increased morbidity. Thus, the dairy farm practice of providing 1 bottle of pooled colostrum for the first feeding was generally adequate for the transfer of passive immunity in this study. However, it should be noted that the group C calves were fed within 4 h after birth and the quality of the pooled colostrum was good.

No significant concentration differences for any calf blood components (including glucose, GGT, total protein, and IgG) were observed between the calves that received their own dam’s colostrum (group A) and the paired calves that received identical colostrum (group B). Moreover, there were no differences in the transfer of passive immunity based on IgG levels. This suggests that the calves receiving the same colostrum will ab-

<table>
<thead>
<tr>
<th>FPIT threshold for measurement</th>
<th>Time point</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP-R &lt;52 g/L</td>
<td>Day 1</td>
<td>0</td>
<td>4</td>
<td>5</td>
<td>0</td>
<td>33.3ab</td>
<td>45.5a</td>
</tr>
<tr>
<td></td>
<td>Day 2</td>
<td>1</td>
<td>4</td>
<td>3</td>
<td>8.3</td>
<td>33.3</td>
<td>27.3</td>
</tr>
<tr>
<td>TP-B &lt;52 g/L</td>
<td>Day 1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>8.3</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>Day 2</td>
<td>0</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>8.3</td>
<td>91</td>
</tr>
<tr>
<td>IgG &lt;10 g/L</td>
<td>Day 1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>8.3</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>Day 2</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>8.3</td>
<td>91</td>
</tr>
<tr>
<td>GGT &lt;200 U/L</td>
<td>Day 1</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>8.3</td>
<td>91</td>
</tr>
<tr>
<td>GGT &lt;75 U/L</td>
<td>Day 7</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>16.7</td>
<td>18.2</td>
</tr>
<tr>
<td>Illness within 7 d after birth</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Frequency and proportion of calves classified as having failure of passive immunity transfer (FPIT) by calf group.

a,bMeans within a row of FPIT proportion with different superscripts differ (P < 0.05), comparing pairwise using 2 × 2 tables with Fisher’s exact test.

1Group A = calves (n = 12) fed own dam’s colostrum; group B = calves (n = 12) fed foster cow colostrum; group C = calves (n = 11) fed 1 bottle of pooled colostrum.

2TP-R = total serum protein measured by refractometer; TP-B = total serum protein measured by Bradford assay; GGT = γ-glutamyl transferase activity.
sorb the colostrum components equally. Intriguingly, though, the results hint that the calves that receive their own dam’s colostrum may absorb more IgG than those that receive foster cow colostrum; this should be explored in larger studies.

ACKNOWLEDGMENTS

This work was funded by the Davies Livestock Research Centre, The University of Adelaide, Australia. Cattle were kindly provided by a commercial farm located in Mount Gambier, South Australia. Do Thi Hue was funded by a scholarship jointly provided by the Vietnam Government (Ministry of Agriculture and Rural Development and Vietnam International Education Development) and The University of Adelaide. The authors thank the many staff and students from the School of Animal and Veterinary Science, The University of Adelaide, for their help with sample collection, laboratory work, and data analysis, particularly Michelle Hebart, Cassandra Marr, Darren Miller, Sarah Weaver, Taylah White, and Simone Wijnen (Fontys University of Applied Sciences). The authors had no conflicts of interest.

REFERENCES


ORCIDs

Hue et al.: COLOSTRUM SOURCE AND PASSIVE IMMUNITY

Do T. Hue: https://orcid.org/0000-0002-7321-4945
Rebel Skirving: https://orcid.org/0000-0001-8313-0091
Tong Chen: https://orcid.org/0000-0002-7681-9632
John L. Williams: https://orcid.org/0000-0001-5188-7957
Cynthia D. K. Bottema: https://orcid.org/0000-0001-6245-0099
Kiro Petrovski: https://orcid.org/0000-0003-4016-2576