Effect of delaying colostrum feeding on passive transfer and intestinal bacterial colonization in neonatal male Holstein calves

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ABSTRACT

The objective of this study was to investigate the effect of time of first colostrum feeding on the passive transfer of IgG and on bacterial colonization in the intestine of neonatal dairy calves. Twenty-seven male Holstein calves were randomly assigned to 1 of 3 treatments at birth: calves were fed colostrum at 45 min (0 h, n = 9), 6 h (n = 9), or 12 h after birth (n = 9). Calves were fed pooled, heat-treated colostrum (62 g of IgG/L) at their respective feeding times at 7.5% of birth body weight and fed milk replacer at 2.5% of birth body weight per meal every 6 h thereafter. Blood samples were taken every 3 h using a jugular catheter and were analyzed for determination of serum IgG by radial immunodiffusion. At 51 h after birth, calves were euthanized for collection of tissue and digesta of the distal jejunum, ileum, and colon. Quantitative real-time PCR was used to estimate the prevalence of Bifidobacterium spp., Lactobacillus spp., Fecalibacterium prausnitzii, Clostridium cluster XIVa, and total Escherichia coli. Delaying colostrum feeding by 6 h (35.6 ± 1.88%) and 12 h (35.1 ± 3.15%) decreased the maximum apparent efficiency of absorption of IgG compared with feeding colostrum immediately after birth (51.8 ± 4.18%) and delayed the time to maximum serum IgG concentration (24 h vs. 15 h, respectively). Moreover, 12-h calves tended to have a lower prevalence of Bifidobacterium spp. (0.12 ± 0.017%) and Lactobacillus spp. (0.07 ± 0.019%) associated with the colon mucosa compared with 0-h calves (1.24 ± 0.648% and 0.26 ± 0.075%, respectively). In addition, 6-h (0.26 ± 0.124%) and 12-h (0.49 ± 0.233%) calves had a lower prevalence of total E. coli associated with ileum mucosa compared with 0-h calves (1.20 ± 0.458%). These findings suggest that delaying colostrum feeding within 12 h of life decreases the passive transfer of IgG and may delay the colonization of bacteria in the intestine, possibly leaving the calf vulnerable to infections during the preweaning period.

Key words: passive transfer, neonatal calf, bacterial colonization

INTRODUCTION

Preweaning dairy calves are at high risk of morbidity and mortality, with recorded rates as high as 50% and 11%, respectively (USDA, 2007). Calves are born without passive immunity because the placenta of the cow separates the maternal and fetal blood supply, thus preventing the transfer of immunoglobulins during gestation (Godden, 2008). To protect the calf, the dam produces colostrum, which contains high levels of IgG in addition to nutrients and other bioactive factors. The termination of the absorption of macromolecules, including IgG, across the gut epithelium is termed “gut closure” and is thought to occur by approximately 24 h postpartum in calves (Stott et al., 1979). The passive transfer of IgG across the small intestinal epithelium has been shown to be optimal within the first 4 h of life and rapidly declines after 12 h postpartum (Stott et al., 1979; Weaver et al., 2000). It is therefore generally accepted that calves fed colostrum immediately after birth will achieve higher maximum serum IgG concentrations than those fed colostrum more than 4 h after birth. Unfortunately, poor colostrum management continues to be a problem on dairy farms, with approximately 31% of mortality in the first 3 wk of life being due to failure of passive transfer of IgG (Wells et al., 1996). Multiple factors are responsible for insufficient circulating IgG, including feeding contaminated or low-quality colostrum and—of particular interest in the current study—the first ingestion of colostrum occurring more than 6 h after birth (Vasseur et al., 2010).

Successful passive transfer of IgG is generally perceived as an indicator of decreased risk of disease and mortality in the neonatal calf. Similarly, the early establishment of gut microbiota has been reported to be associated with reduced disease risk in calves (Oikonomou
et al., 2013). For example, enterotoxigenic *Escherichia coli* is typically associated with an increased incidence of diarrhea, whereas beneficial species, such as *Bifidobacterium* and *lactobacillus* are associated with a healthy gut microbiome and immunity (Apgar et al., 1993; Picard et al., 2005; Uhde et al., 2008). The establishment of gut microbiota in the neonate is associated with the development of the mucus in immune system and secondary lymphoid structures, and certain bacterial genera are able to produce energy substrates for the intestinal epithelia (Guarner, 2006; Sommer and Bäckhed, 2013). Current knowledge of the commensal bacteria present in the calf microbiota and their effects on the host are limited. Variation of microbial composition in the rumen has been shown to be higher in neonates or young animals than in adults (Jami et al., 2013). This knowledge may translate to the intestinal microbial composition, and if so, implies that the gut microbiome may be more easily influenced during this time. This may provide the opportunity to establish beneficial bacteria during early life to decrease the possibility of pathogenic bacterial colonization and subsequent disease (Malmuthuge et al., 2015b). Feeding colostrum within the first 12 h of life can result in a higher prevalence of small intestinal mucosa associated *Bifidobacterium* and lower total *E. coli* compared with calves not fed colostrum (Malmuthuge et al., 2015a). However, it is unknown how the extent to which the first colostrum feeding is delayed after birth affects the bacteria colonizing the small and large intestine.

The objective of the present study was to investigate the effect of time of first colostrum feeding on the passive transfer of IgG and bacterial colonization in the intestine of neonatal dairy calves. We hypothesized that delaying colostrum feeding in the first 12 h of life would progressively decrease the passive transfer of IgG as well as the prevalence of beneficial bacteria in the small intestine (distal jejunum and ileum) and colon.

**MATERIALS AND METHODS**

**Calving and Early Calf Life**

The animal experiment was conducted following the guidelines of the Canadian Council of Animal Care (CCAC, 1993) at the Dairy Research and Technology Centre of the University of Alberta. The animal use protocol was approved by The Livestock Care Committee of the University of Alberta (AUP00001595). Approximately 3 to 10 d before parturition, Holstein heifers and cows were moved to maternity pens, which were bedded with fresh shavings daily and disinfected and cleaned between calvings. After the area was cleaned using 1% iodine, an iVET birth-monitoring device (iVET, Papenburg, Germany) was inserted into the vagina. All iVET devices were thoroughly cleaned and disinfected before insertion.

Only bull calves from a singleton birth with a BW between 35 and 55 kg were included in the current study. Bulls were removed from the dam immediately after birth and thus there was no contact between the dam and calf. Bulls were weighed in a calibrated electronic scale (Digi-Star SW300, Digi-Star LLC, Fort Atkinson, WI) and transferred in the scale to individual pens bedded with shavings and fresh straw. Between calves, pens were thoroughly disinfected using Virkon (Lanxess Ag, Cologne, Germany) and lime. Calves were dried using 2 clean towels for 10 min, after which calves’ navels were dipped with 7% iodine.

**Animal Experiment and Feeding**

Male Holstein calves (n = 27) born from February to September 2016 were randomly allocated into 3 treatment groups: calves fed colostrum at 45 min (0 h, n = 9), 6 h (n = 9), or 12 h after birth (n = 9). A single batch of pooled, heat-treated colostrum containing 62 g of IgG/L was provided by the Saskatoon Colostrum Company Ltd. (Saskatoon, SK, Canada) and fed to calves at their respective feeding times at 7.5% of birth BW. Before feeding, colostrum was thawed and heated to 39°C in a water bath kept at a consistent temperature of 50°C. Once heated, colostrum was poured into two 2-L esophageal tubing bottles and transferred to the calf pen in a bucket of warm (~39°C) water, where it was tube fed to the calf in less than 5 min. Twelve hours after their colostrum feeding, calves were fed milk replacer with a 26:18 CP:fat ratio (Excel Pro-Gro Calf Milk Replacer, Grober Nutrition, Cambridge, ON, Canada) at a volume of 2.5% of birth BW per meal every 6 h until euthanasia at 51 h after birth. Milk replacer was prepared by mixing 150 g of milk replacer powder in 1 L of water in a clean bucket, poured into a clean bottle with a unique nipple, and heated in the water bath to 39°C. If calves did not consume the milk replacer meal within 30 min, the remainder of the meal was fed using an esophageal tube. If calves refused more than 50% of their milk replacer intake by nipple bottle per 24 h, they were excluded from the study.

**Blood Sampling**

At approximately 20 min of life, a 3-mL serum sample was collected from the jugular vein using a Vacutainer (Becton, Dickinson and Co., Franklin Lakes, NJ) to establish baseline values. At 2 h after birth, a 2-inch, 16-gauge catheter was inserted into the jugular vein of each calf for the duration of its life. To insert the cath-
et, the calf was gently restrained by a handler and its neck was shaven and disinfected with chlorohexidine and ethanol before the catheter was inserted. Blood samples were collected every 3 h and left at room temperature for 3 h to clot; serum was collected following centrifugation at 3,000 × g at 4°C for 20 min. The serum was transferred into three 1.5-mL microcentrifuge tubes in equal aliquots and frozen at −20°C.

**Intestinal Tissue and Digesta Sampling**

Intestinal samples were collected from all calves at 51 h of life (3 h after the final meal). Calves were euthanized with a pentobarbital sodium injection (Euthanyl, Vetoquinol, Lavaltrie, QC, Canada) at 0.125 mL/kg of BW administered through the jugular catheter. Once the calf reached a surgical plane of anesthesia, exsanguination was performed, the rectum and esophagus were ligated, and the entire gut contents were removed. Following this, 10-cm-long intestinal segments of predefined gut regions were collected. The distal jejunum segment was defined as 30 cm proximal to the collateral branch of the cranial mesenteric artery; the ileum segment was defined as 30 cm proximal to the ileo-cecal junction; and the colon segment was defined as 30 cm distal to the ileo-cecal junction (Malmuthuge et al., 2015b). Intestinal content was removed from the sample using tweezers, and placed in a 50-mL Falcon tube. Then, the tissue was washed in PBS until clean (~3–4 washes) and placed in a sterile bag. Both intestinal content and tissue samples were immediately snap-frozen in liquid nitrogen and transferred to a −80°C freezer until further use.

**Analysis of Serum IgG**

Serum samples were thawed and centrifuged at 3,000 × g for 20 min at 4°C, after which supernatant was transferred to a new tube. Serum samples were refrozen in −20°C for 24 h and then shipped overnight on ice to the Saskatoon Colostrum Company quality assurance laboratory for determination of serum IgG concentrations by radial immunodiffusion analysis (Chelack et al., 1993) with modifications as described in Chamorro et al. (2014). The same method was used for determination of IgG in the single pooled colostrum batch.

The maximum apparent efficiency of absorption (AEA, %) of IgG for each treatment group was calculated using calf birth weight, calf serum IgG concentration, and colostrum IgG mass. The formula used was previously described by Quigley et al. (2002), with the assumption of a plasma volume of 9.9% of birth weight. Parameters relative to colostrum feeding were calculated from the raw data, including time to reach maximum concentration (Tmax), maximum concentration reached (Cmax), ratio of Cmax/Tmax, change in concentration (delta change), and IgG concentrations at 12, 24, and 36 h after the colostrum meal (IgG12, IgG24, IgG36). The positive incremental area under the curve (AUC) for IgG was determined using the trapezoidal rule over the first 12 h (AUC12), 24 (AUC24), and 36 (AUC36) h after birth.

**DNA Extraction from Tissue and Digesta Samples**

The total DNA from intestinal digesta was extracted using the repeated bead beating plus column method (Yu and Morrison, 2004). Briefly, the digesta sample (~0.3 g) was washed twice with Tris-EDTA buffer. After the addition of cell lysis buffer containing 4% SDS, samples were subjected to physical disruption at 4,800 rpm for 3 min using Biospec Mini Beads Beater 8 (BioSpec, Bartlesville, OK), followed by incubation at 70°C for 15 min and centrifugation for 5 min at 16,000 × g. The bead beating, incubation, and centrifugation were repeated once and impurities were removed from the supernatant using 10 M ammonium acetate, followed by DNA precipitation using isopropanol. After precipitation, DNA was further purified using QIAamp fast DNA stool mini kit (Qiagen Inc., Germantown, MD). The quantity and purity of DNA were evaluated using NanoDrop 1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE), and DNA was stored at −20°C until further use. For processing of tissue samples, the tissue was ground in liquid nitrogen before DNA extraction. Approximately 0.1 g of the ground tissue was subjected to DNA extraction using the bead-beating method as described by Li et al. (2009). The DNA quantity and purity were evaluated as described above.

**Quantification of Bacterial Groups in the Calf Distal Jejunum, Ileum, and Colon Using Quantitative Real-Time PCR**

For intestinal digesta and tissue samples, the densities of total bacteria, Bifidobacterium, Lactobacillus, Clostridium cluster XIVa, Fecalibacterium prausnitzii, and total E. coli were estimated by measuring their respective 16S rRNA gene copy numbers using quantitative real-time PCR. Bacterial populations from digesta samples were estimated using the StepOnePlus real-time PCR system (Applied Biosystems/ThermoFisher Scientific, Waltham, MA), and bacterial populations associated with the tissue were estimated using the high throughput Viia 7 Real-Time PCR System.
effect of colostrum treatment, all data were analyzed using the MIXED procedure of SAS software (version 9.4, SAS Institute Inc., Cary, NC). For serum IgG concentrations and AEA, repeated measures were used with the model, including the fixed effects of treatment, age, and treatment by age interaction. For IgG parameters calculated relative to the meal, as well as the AEAmax, only the colostrum treatment was included as a fixed effect. For the prevalence and copy number of 16S rRNA genes per gram for the bacterial groups, data were analyzed by colostrum treatment by region of the intestine (distal jejunum, ileum, colon). For IgG and AEA, repeated measures were used with the model, including the fixed effects of treatment, age, and treatment by age interaction. Results were expressed as the mean ± SEM.

### Results

#### Effect of Delaying Colostrum Feeding on Passive Transfer of IgG

Feeding colostrum within the first hour of life (0 h) increased the passive transfer of IgG compared with feeding colostrum at 6 and 12 h of life (Figure 1; Table 2). Calves fed at 0 h had a higher AUC for the first 12 h after the colostrum meal (AUC12) compared with 6-h and 12-h calves (Table 2). Similarly, 0-h calves had higher AUC24 and AUC36 compared with 6-h and 12-h calves. No differences were observed among treatments for Tmax relative to the colostrum meal (Table 2).

#### Effect of Delaying Colostrum Feeding on Bacteria Associated with Mucosa

In general, high variation was observed between calves for the copy number of 16S rRNA genes per gram of fresh sample for both mucosa- and digesta-associated bacterial groups. Calves fed at 6 h tended to have a lower (P = 0.08) total bacteria density associated with the distal jejunum mucosa than those fed at 0 h, whereas no differences were detected between 0-h and 12-h calves (Figure 2). The prevalence of F. prausnitzii associated with the distal jejunum mucosa was higher in 12-h calves (P = 0.06) and 6-h calves (P = 0.04) than in calves fed immediately after birth (Table 2).

### Statistical Analysis

To determine the effect of colostrum treatment, all data were analyzed using the MIXED procedure of SAS software (version 9.4, SAS Institute Inc., Cary, NC). For serum IgG concentrations and AEA, repeated measures were used with the model, including the fixed effects of treatment, age, and treatment by age interaction. For IgG parameters calculated relative to the meal, as well as the AEAmax, only the colostrum treatment was included as a fixed effect. For the prevalence and copy number of 16S rRNA genes per gram for the bacterial groups, data were analyzed by colostrum treatment by bacterial target (Bifidobacterium, Lactobacillus, total E. coli, Clostridium cluster XIVa, F. prausnitzii, and total bacteria), by type (tissue, content) within each region of the intestine (distal jejunum, ileum, colon). All values reported are least squares means with significance declared at P ≤ 0.05 and tendencies at 0.05 < P < 0.10.

### Table 1. Bacterial primers (F = forward; R = reverse) used to determine the copy number of 16S rRNA genes in the calf intestine

<table>
<thead>
<tr>
<th>Bacterial group</th>
<th>Primer</th>
<th>Product size (bp)</th>
<th>Annealing temperature (°C)</th>
<th>Reference</th>
</tr>
</thead>
</table>
| Total bacteria               | F: 5′-actctcctgagggagcagc-3′  
R: 5′-gactacagggtaatacct-3′ | 467               | 62                        | Stevenson and Weimer, 2007          |
| Lactobacillus                | F: 5′-ggagccgagctggagac-3′  
R: 5′-gagccgtaactttctctcttc-3′ | 120               | 62                        | Dehoisise et al., 2008              |
| Bifidobacterium             | F: 5′-actctcctgagggagcagc-3′  
R: 5′-gactacagggtaatacct-3′ | 196               | 66                        | Cleusix et al., 2010                |
| Escherichia coli             | F: 5′-ggagccgaagctttctgac-3′  
R: 5′-agccgctctctctcactgac-3′ | 544               | 60                        | Sabat et al., 2010                  |
| Fecalibacterium prausnitzii | F: 5′-ggagccgaagctttctgac-3′  
R: 5′-agccgctctctctcactgac-3′ | 248               | 60                        | Vital et al., 2013                  |
| Clostridium cluster XIVa    | F: 5′-ggagccgaagctttctgac-3′  
R: 5′-agccgctctctctcactgac-3′ | 415               | 60                        | Rintili et al., 2004                |
3). No further differences among treatment groups were detected in the bacterial groups associated with the mucosa of the distal jejunum.

In the ileum, a 33.3% decrease ($P = 0.08$) in $F. prausnitzii$ associated with the mucosa was observed in 6-h calves compared with 0-h calves, whereas no differences were detected between 12-h and 0-h calves. Moreover, a lower prevalence of $E. coli$ was associated with the ileum mucosa when calves were fed colostrum at 6 h ($P = 0.05$) and 12 h ($P = 0.09$) compared with calves fed colostrum at 0 h (Table 3).

In regards to bacterial groups associated with the mucosa of the colon, 12-h calves tended to have a lower prevalence of $Bifidobacterium$ ($P = 0.08$) and $Lactobacillus$ ($P = 0.05$) than 0-h calves, whereas no differences were observed between 0-h and 6-h calves for

Table 2. Calf characteristics (mean ± SEM) among treatment groups and the effect of delaying colostrum feeding on IgG parameters relative to ingestion of first colostrum meal. 

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0</th>
<th>6</th>
<th>12</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth BW (kg)</td>
<td>43.5 ± 1.51</td>
<td>42.0 ± 1.74</td>
<td>42.2 ± 0.96</td>
<td>0.324</td>
</tr>
<tr>
<td>IgG intake (g)</td>
<td>202.3 ± 7.02</td>
<td>195.3 ± 8.09</td>
<td>196.1 ± 4.49</td>
<td>0.618</td>
</tr>
<tr>
<td>Baseline serum IgG (mg/mL)</td>
<td>0.4 ± 0.17</td>
<td>0.4 ± 0.10</td>
<td>0.38 ± 0.04</td>
<td>0.902</td>
</tr>
<tr>
<td>$AEA_{\text{max}}$ (%)</td>
<td>51.8 ± 4.18a</td>
<td>35.6 ± 1.88b</td>
<td>35.1 ± 3.15b</td>
<td>0.007</td>
</tr>
<tr>
<td>$AUC_{12}$</td>
<td>130.4 ± 14.20b</td>
<td>98.4 ± 5.90b</td>
<td>96.0 ± 7.60b</td>
<td>0.038</td>
</tr>
<tr>
<td>$AUC_{24}$</td>
<td>408.3 ± 35.00b</td>
<td>297.8 ± 16.00b</td>
<td>293.1 ± 22.30b</td>
<td>0.006</td>
</tr>
<tr>
<td>$AUC_{36}$</td>
<td>657.2 ± 50.60b</td>
<td>483.7 ± 26.30b</td>
<td>485.0 ± 38.40b</td>
<td>0.006</td>
</tr>
<tr>
<td>IgG$_{12}$ (mg/mL)</td>
<td>23.2 ± 2.00b</td>
<td>15.2 ± 0.80b</td>
<td>15.3 ± 1.30b</td>
<td>0.001</td>
</tr>
<tr>
<td>IgG$_{24}$ (mg/mL)</td>
<td>22.3 ± 1.40b</td>
<td>17.0 ± 1.00b</td>
<td>16.9 ± 1.40b</td>
<td>0.008</td>
</tr>
<tr>
<td>IgG$_{36}$ (mg/mL)</td>
<td>19.3 ± 1.50b</td>
<td>14.9 ± 0.90b</td>
<td>15.6 ± 1.40b</td>
<td>0.058</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>17.0 ± 0.71</td>
<td>21.0 ± 1.22</td>
<td>20.7 ± 2.03</td>
<td>0.111</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (mg/mL)</td>
<td>25.5 ± 2.00b</td>
<td>18.2 ± 1.10b</td>
<td>18.5 ± 1.40b</td>
<td>0.003</td>
</tr>
<tr>
<td>$C_{\text{max}}/T_{\text{max}}$ (mg/mL per hour)</td>
<td>1.5 ± 0.13b</td>
<td>1.2 ± 0.90b</td>
<td>1.0 ± 0.14b</td>
<td>0.003</td>
</tr>
<tr>
<td>Delta change (mg/mL)</td>
<td>25.0 ± 2.03b</td>
<td>17.8 ± 1.08b</td>
<td>18.1 ± 1.34b</td>
<td>0.004</td>
</tr>
</tbody>
</table>

$^a,b$Means within a row with different letters are significantly different at $P < 0.05$.

$^1AEA_{\text{max}} = $ maximum apparent efficiency of absorption; $AUC_{12}$, $AUC_{24}$, $AUC_{36} = $ area under the curve during the first 12, 24, and 36 h after colostrum feeding, respectively; $IgG_{12}$, $IgG_{24}$, $IgG_{36} = $ immunoglobulin G concentration at 12, 24, and 36 h after the colostrum feeding; $T_{\text{max}} =$ time to maximum concentration; $C_{\text{max}} =$ maximum concentration.
these genera (Figure 3). No differences were detected among treatment groups with regards to the prevalence of Clostridium cluster XIVa, *F. prausnitzii*, or total *E. coli* in the colon mucosa–associated microbiota.

**Effect of Delaying Colostrum Feeding on Bacteria Associated with Digesta**

Compared with mucosa-associated bacteria, fewer differences were detected in the bacterial groups associated with intestinal digesta. In the distal jejunum digesta, 12-h calves displayed a lower (*P* = 0.04) prevalence of *E. coli* than 0-h calves (Table 4). In the ileum digesta, there was a tendency (*P* = 0.09) for a higher prevalence of Clostridium cluster XIVa in 6-h calves than in 0-h calves, whereas no differences were observed between 12-h and 0-h calves. Similarly, 6-h calves tended (*P* = 0.09) to have a higher prevalence of Clostridium cluster XIVa in the colon digesta than 0-h calves. No differences were observed for total bacteria (Figure 2), *Bifidobacterium*, *Lactobacillus*, or *F. prausnitzii* prevalence in the intestinal digesta among treatment groups.

**DISCUSSION**

To our knowledge, the present study is the first to determine how a delay in colostrum feeding using current colostrum feeding recommendations, highly standardized colostrum, and frequent blood sampling affects the passive transfer of IgG in the neonatal calf. We hypothesized that delaying the first colostrum meal would progressively decrease the passive transfer of IgG in neonatal calves. In accordance with our hypothesis,
Delaying colostrum feeding in dairy calves increased the maximum concentration of serum IgG reached, as well as the apparent efficiency of absorption of IgG compared with calves fed colostrum at 6 or 12 h after birth. The increased absorption of IgG demonstrated by calves fed within the first hour of life was consistent with previous reports, which stated that IgG transfer across the enterocyte is optimal within the first 4 h after birth (Stott et al., 1979). Moreover, 0-h calves displayed a peak in IgG absorption at approximately 15 h of life (Figure 1), which is different from earlier studies that report maximal levels at 24 h (Stott et al., 1979). The current study found peak serum levels at 24 h only for 6-h- and 12-h-fed calves. However, peak IgG concentrations may have been observed at 15 h for 0-h calves in the current study because only one meal of colostrum was fed to calves, instead of 2 meals, which would provide a prolonged supply of IgG. Therefore, this is an important finding that deserves further consideration and study because many sampling protocols for passive transfer experiments have relied upon the view that peak serum IgG levels occur 24 h after birth, irrespective of the time of the first feeding. After reading the results of earlier studies, we hypothesized that colostrum was fed to calves before 6 h of life, and that this delayed the absorption of IgG into the bloodstream. The Table 3 shows the effect of delaying colostrum feeding by 0, 6, or 12 h on the prevalence (% of total bacteria) of intestinal bacterial groups associated with the intestinal mucosa in neonatal calves (mean ± SEM).

<table>
<thead>
<tr>
<th>Bacterial group</th>
<th>Distal jejunum</th>
<th>Ileum</th>
<th>Colon</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 h</td>
<td>6 h</td>
<td>12 h</td>
</tr>
<tr>
<td><em>Bifidobacterium</em></td>
<td>0.03 ± 0.003</td>
<td>0.02 ± 0.009</td>
<td>0.03 ± 0.006</td>
</tr>
<tr>
<td><em>P-value</em></td>
<td>0.85</td>
<td>0.21</td>
<td>0.54</td>
</tr>
<tr>
<td><em>Lactobacillus</em></td>
<td>0.03 ± 0.007</td>
<td>0.08 ± 0.029</td>
<td>0.24</td>
</tr>
<tr>
<td><em>P-value</em></td>
<td>0.41</td>
<td>0.31</td>
<td>0.34</td>
</tr>
<tr>
<td><em>Clostridium</em></td>
<td>0.03 ± 0.007</td>
<td>0.08 ± 0.029</td>
<td>0.24</td>
</tr>
<tr>
<td><em>Fecalibacterium</em> praunasi</td>
<td>0.04 ± 0.005a</td>
<td>0.05 ± 0.008ab</td>
<td>0.05 ± 0.005b</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>0.09 ± 0.025</td>
<td>0.11 ± 0.047</td>
<td>0.07 ± 0.011</td>
</tr>
<tr>
<td><em>P-value</em></td>
<td>0.66</td>
<td>0.30</td>
<td>0.11</td>
</tr>
</tbody>
</table>

a*,b*Means with an asterisk (*) and different superscript are significant at P < 0.05 within intestinal region and bacterial target among treatment groups.

a,bMeans with a different superscript at significant at 0.10 < P < 0.05 within intestinal region and bacterial target among treatment groups.

Table 3. Effect of delaying colostrum feeding by 0, 6, or 12 h on the prevalence (% of total bacteria) of intestinal bacterial groups associated with the intestinal mucosa in neonatal calves (mean ± SEM).
reduced ability of 6-h and 12-h calves to absorb IgG as efficiently as 0-h calves may be due to the turnover of fetal intestinal cells into mature enterocytes with a decreased ability to absorb IgG from colostrum (Smeaton and Simpson-Morgan, 1985). Early studies also suggest that a hormonal influence might be involved in closure of the small intestine, as it has been reported that when the first colostrum meal is delayed, calves experience a “cortisol shock,” which may induce changes in the absorptive capacity of the intestine (Kruse and Buus, 1972; Nightengale, 1979). However, other reports found no relationship between glucocorticoid concentrations and absorption of IgG (Stott and Reinhard, 1978; Johnston and Oxender, 1979), thus leaving this mechanism still to be elucidated.

The gut microbial community plays a key role in developing the immune system, utilizing nutrients, and influencing the overall physiology of the host (Mazmanian et al., 2005; Peterson et al., 2007). In the current study, calves fed colostrum at 12 h after birth tended to have a lower prevalence of Bifidobacterium and Lactobacillus associated with the colon mucosa than calves fed colostrum immediately after birth. Species belonging to these genera are considered beneficial bacteria, because they produce lactic acid and short-chain fatty acids, which have trophic and regulatory effects on colonoocytes (Boffa et al., 1992; Cummings, 1995). A study conducted by Malmuthuge et al. (2015a) determined that feeding colostrum to neonatal calves increased the prevalence of Bifidobacterium and total bacteria in the small intestine within the first 12 h of life compared with calves not fed colostrum. The delay in the delivery of colostral nutrients for 12 h after birth in the present study likely affected microbial dynamics during early life by shifting the establishment of certain bacterial groups to correspond with the timing of the delivery of nutrients. Preventing the immediate initiation of the growth and establishment of beneficial genera may have lasting effects on the dynamic bacterial community in the large intestine and hinder the ability of the gut to face challenges later in life, such as neonatal calf diarrhea (Oikonomou et al., 2013). However, a potential limitation of the present study was the assessment of intestinal bacterial groups at only one time point after birth. We speculate that sampling at an earlier time
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(e.g., at 24 h of life) may have provided conclusive evidence in regards to the effect of delayed colostrum feeding on colonization of beneficial intestinal bacteria in the intestine. Therefore, we believe that future research should focus on the microbial community dynamics as this delay occurs, using dissection at frequent intervals as well as control calves not fed colostrum.

In addition, although some microorganisms are beneficial to health, others may be harmful (Picard et al., 2005). For example, enterotoxigenic Escherichia coli (ETEC) K99, Cryptosporidium parvum, and rotavirus account for 5.5, 58.0, and 55.0% of diarrhea incidence in pre-weaning calves, respectively (O'Brien et al., 2005). Pathogenic E. coli, including ETEC K99, typically binds to membrane E. coli, including ETEC K99, typically binds to membrane

Table 4. Effect of delaying colostrum feeding by 0, 6, or 12 h on the prevalence (% of total bacteria) of intestinal bacterial groups associated with the digesta in neonatal calves (mean ± SEM)

<table>
<thead>
<tr>
<th>Bacterial group</th>
<th>Distal jejunum</th>
<th>Ileum</th>
<th>Colon</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 h</td>
<td>6 h</td>
<td>12 h</td>
</tr>
<tr>
<td>Bifidobacterium</td>
<td>0.05 ± 0.018</td>
<td>0.08 ± 0.030</td>
<td>0.07 ± 0.028</td>
</tr>
<tr>
<td>P-value</td>
<td>0.65</td>
<td>0.07</td>
<td>0.22</td>
</tr>
<tr>
<td>Lactobacillus</td>
<td>0.66 ± 0.254</td>
<td>0.41 ± 0.166</td>
<td>0.63 ± 0.280</td>
</tr>
<tr>
<td>P-value</td>
<td>0.74</td>
<td>0.23</td>
<td>0.27</td>
</tr>
<tr>
<td>Clostridium</td>
<td>0.01 ± 0.003</td>
<td>0.02 ± 0.019</td>
<td>0.01 ± 0.001</td>
</tr>
<tr>
<td>P-value</td>
<td>0.48</td>
<td>0.16</td>
<td>0.22</td>
</tr>
<tr>
<td>Fecalibacterium prausnitzii</td>
<td>0.05 ± 0.008</td>
<td>0.05 ± 0.019</td>
<td>0.05 ± 0.016</td>
</tr>
<tr>
<td>P-value</td>
<td>0.99</td>
<td>0.30</td>
<td>0.38</td>
</tr>
<tr>
<td>Total Escherichia coli</td>
<td>13.29 ± 3.554&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.48 ± 5.744&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.21 ± 0.243&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>P-value</td>
<td>0.11</td>
<td>0.89</td>
<td>0.54</td>
</tr>
</tbody>
</table>

<sup>a</sup><sup>b</sup>Means with an asterisk (*) and different superscript are significant at P < 0.05 within intestinal region and bacterial target among treatment groups.

<sup>a</sup><sup>b</sup>Means with a different superscript at significant at 0.10 < P < 0.05 within intestinal region and bacterial target among treatment groups.

Many of the bacterial groups associated with the mucosa and digesta of the distal jejunum, ileum, and colon did not exhibit any statistical differences among treatment groups. This may be due to the large individual variation in regards to the detected copy number of the 16S rRNA gene per gram of fresh sample. High genetic, microbial, and transcriptomic variation in the neonatal calf has been reported previously, with the exact reasoning for the variation not yet determined (Liang et al., 2014; Malmuthuge et al., 2015). The specific microbial environment of the birth canal of the dam and the site of parturition may have an effect on the phenotype, as well as the nutrition provided to the animal. Regardless of the exact causative factors for the high individual variation, the neonatal calf intestines deprived of microorganisms before birth are at this time undergoing colonization in regards to the effect of delayed colostrum feeding. Therefore, we believe that future research should focus on the microbial community dynamics as this delay occurs, using dissection at frequent intervals as well as control calves not fed colostrum.
unfamiliar, dynamic microbial changes. The newborn microbiome is only just beginning to establish itself, and thus the microbes within each individual would be expected to interact with the host in a unique way, leading to the observed high individual variation.

CONCLUSIONS

Delaying colostrum feeding by 6 or 12 h after birth decreased the passive transfer of IgG and the time to maximum serum IgG concentration compared with feeding colostrum immediately after birth. We speculate that the gut decreases in permeability when colostrum is not fed immediately to newborn calves; however, the exact mechanism by which this occurs is unknown and warrants further investigation. Delaying colostrum feeding tended to decrease the prevalence of beneficial bacteria associated with the colon mucosa, specifically *Bifidobacterium* and *Lactobacillus* spp., which play important roles in gut health. How the presence of these specific bacterial groups during the first days of life may affect future growth and productivity needs to be explored further.

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