Heat-treated colostrum feeding promotes beneficial bacteria colonization in the small intestine of neonatal calves

Nilusha Malmuthuge, Yanhong Chen, Guanxiang Liang, Laksiri A. Goonewardene, and Le Luo Guan¹

Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada T6G 2P5

ABSTRACT

The present study investigated the effect of heat-treated colostrum feeding on the bacterial colonization in calf small intestine of neonatal calves within the first 12 h of life. Newborn Holstein bull calves ($n = 32$) were assigned to 3 treatment groups and fed with either fresh colostrum (FC, $n = 12$) or heat-treated (60°C , 60 min) colostrum (HC, $n = 12$) soon after birth, whereas the control (NC, $n = 8$) group did not receive colostrum or water. Small intestinal tissues and contents were collected from proximal jejunum, distal jejunum, and ileum at 6 and 12 h after birth, following euthanasia. Quantitative real time-PCR was used to explore the colonization of total bacteria, *Lactobacillus*, *Bifidobacterium*, and *Escherichia coli*. The feeding of colostrum soon after birth increased the colonization of total bacteria in calf gut within the first 12 h compared with NC. In contrast, the prevalence of *Lactobacillus* was lower in HC and FC compared to NC. Remarkable changes in the prevalence of small intestinal tissue-attached *Bifidobacterium* were observed with the feeding of HC, but not that in small intestinal contents. The prevalence of *Bifidobacterium* was 3.2 and 5.2 fold higher in HC than FC and NC, respectively, at 6 h. Although the feeding of FC did not enhance the prevalence of tissue-attached *Bifidobacterium* at 6 h compared with NC, it displayed a gradual increase over the time that was higher than NC, but similar to that of HC at 12 h. Moreover, the colonization of *E. coli* was drastically reduced in HC calves compared with FC and NC. Thus, the present study suggests that the feeding of HC enhances the colonization of *Bifidobacterium* but lessens *E. coli* in the calf small intestine immediately postpartum compared with that of FC and NC. The increased colonization of beneficial bacteria along with the decreased colonization of potential pathogens in calf gut may also

diminish the neonatal calf diarrhea when calves are fed heat-treated colostrum soon after birth.

Key words: neonatal calf, colostrum, gut bacteria

INTRODUCTION

Colostrum management and feeding is crucial for passive immune transfer in calf management. Calves are immunodeficient at birth, as no placental transferring of immunoglobulin into the fetus occurs in cattle (Godden, 2008). Thus, calves are solely dependent on the absorption of immunoglobulin present in colostrum until the development of their own immune system (Godden, 2008). The feeding of high-quality colostrum (IgG >50 mg/mL) soon after birth plays a vital role in the passive transfer of immunity (Jaster, 2005; Chigerwe et al. 2008), which decreases calf mortality and morbidity while increasing calf weaning weight and BW gain (Priestley et al., 2013). Despite recommendations for industry that might decrease calf mortality, many producers in North America do not follow best practices regarding colostrum management (Vasseur et al., 2010; Morrill et al., 2012). The feeding of calves with contaminated (high bacterial count), low-quality colostrum (<50 mg/mL of IgG; Morrill et al., 2012) as well as low surveillance of calf birth at night and relying on dams to feed colostrum (Vasseur et al., 2010) are some of the major concerns observed in the current North American dairy industry.

Heat-treated colostrum feeding is one of the management practices introduced recently to the dairy industry that aims to decrease bacterial contaminations and increase passive immune transfer (Donahue et al., 2012; Godden et al., 2012; Teixeira et al., 2013; Gelsinger et al., 2014). Heat treatments successfully (60°C , 60 min) decrease total bacterial count, including pathogenic bacteria, while maintaining IgG concentration (Donahue et al., 2012). Additionally, the feeding of heat-treated colostrum increases serum colostrum concentration (Godden et al., 2012; Teixeira et al., 2013) as well as reduces the risk for illnesses and treatment for scours in dairy farms when compared to that of fresh colostrum (Godden et al., 2012). This suggests a decrease in dis-

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¹Corresponding author: lguan@ualberta.ca

ease transmission among the calves in dairy herds when they receive heat-treated colostrum soon after birth. Although decreased total bacteria, including pathogens in colostrum, is one of the possibilities to reduced enteric infections in heat-treated colostrum fed calves (Godden et al., 2012), the effect of heat-treated colostrum on gut colonization is not well studied. The present study hypothesized that the feeding of heat-treated colostrum influences bacterial colonization in the calf intestine and facilitates the colonization of beneficial bacteria. Herein, we investigated the effect of colostrum feeding (heat-treated and fresh) soon after birth on the colonization of total bacteria, *Lactobacillus*, *Bifidobacterium*, and *Escherichia coli* in the calf small intestine within the first 12 h of life.

MATERIALS AND METHODS

Colostrum Preparation and Animal Experiment

Prior to the animal experiment, first-milking colostrum containing ≥ 50 mg/mL of IgG was collected from cows raised at Dairy Research and Technology Center (DRTC), University of Alberta, Edmonton, Canada, and immediately laid flat on wire racks and frozen at -20°C . Once ~ 48 L of colostrum was collected, all the samples were thawed slowly for 24 h in 4°C cold room and mixed thoroughly to obtain the pool of colostrum that will be using during the entire animal experiment. Half of the colostrum (24 L) was pasteurized (60 min at 60°C) using commercial batch pasteurizer DT 10G (Dairy Tech Inc., Greeley, CO). Colostrum was held at 60°C for 60 min apart from the time (~ 30 min) taken to reach 60°C , followed by rapid cooling. The heat-treated colostrum and the remaining half of fresh colostrum were aliquoted into 1-L plastic freezer bags and stored at -20°C .

The animal experiment was conducted at DRTC, University of Alberta, following the guidelines of the Canadian Council on Animal Care (CCAC, 1993). The Livestock Care Committee of the University of Alberta approved all the protocols (AUP00001012) before beginning the experiment. Near parturition, Holstein cows predicted to have bull calves were transferred into individual maternity pens and monitored closely with video cameras. Calves were removed from the dams soon after birth, transferred into individual calf units with fresh wood shavings, and then dipped navels with 7% (vol/vol) iodine. Frozen colostrum (2 L/calf) was thawed to 37 to 38°C using a water bath and bottle-fed to calves within an hour after birth. Calves ($n = 32$) were randomly allocated into 3 treatment groups: fresh colostrum-fed calves (FC, $n = 12$), heat-treated colostrum-fed calves (HC, $n = 12$), and control calves

that did not receive either colostrum or water during the experimental period (NC, $n = 8$).

Intestinal Sample Collection from Calves

Intestinal samples from all the calves were collected at 6 (HC, $n = 6$; FC, $n = 6$; NC, $n = 4$) and 12 h (HC, $n = 6$; FC, $n = 6$; NC, $n = 4$) after birth. All calves were euthanized following captive bolt gun stunning and small intestinal tissues and contents (proximal jejunum, distal jejunum, and ileum) were collected together as closed gut sections within 30 min after euthanasia. The esophagus and rectum were first ligated to occlude the lumen and prevent environmental contamination of the intestine. Then, 10-cm long closed intestinal segments were collected in the middle of predefined gut regions. Ileum was defined as 30 cm proximal to the ileo-cecal junction; distal jejunum was defined as 30 cm proximal to the collateral branch of the cranial mesenteric artery; and proximal jejunum was defined as 100 cm distal to the pylorus sphincter. All the samples were snap-frozen and transferred into -80°C freezer until further processing.

Intestinal Sample Collection from Newborn Calves

Dams predicted to have bull calves were transferred into calving pens 3 d before the predicted due date and monitored via remote video cameras. Newborn calves ($n = 6$) were separated from dams soon after birth to make sure no interactions occurred between calves and dams. Then, the calves were transferred into the surgical room at DRTC and euthanized immediately following captive bolt gun stunning. The collection of small intestinal segments from the newborn calves was completed within 30 min after the calf birth, similar to that of 6- and 12-h calves used in colostrum feeding trial.

DNA Extraction from Tissue and Content Samples

A portion of frozen intestinal section was thawed on ice and content separated from tissue. Then, genomic DNA from tissues and contents was extracted separately using the repeated bead beating plus column method (Yu and Morrison, 2004). Briefly, content (0.5 g) and tissue (0.5 g) samples were subjected to physical disruption with a cell lysis buffer containing 4% SDS using BioSpec Mini Beads beater 8 (BioSpec, Bartlesville, OK) at 4,800 rpm for 3 min. Then, the tubes containing lysed cells were incubated at 70°C for 15 min and the supernatant was separated. The bead beating and the incubation steps were repeated once more. Any remaining impurities and SDS from the supernatant

were removed with 10 M ammonium acetate and DNA was precipitated using isopropanol. Genomic DNA was then further purified using QIAmp fast DNA stool mini kit (QIAmp fast DNA stool mini kit, Qiagen Inc., Germantown, MD). The DNA quantity was measured using NanoDrop 1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE) and stored at −20°C.

Quantification of Total Bacteria, Lactobacillus, Bifidobacterium, and E. coli in Calf Small Intestine

Quantitative real time-PCR was performed to estimate densities of total bacteria, *Lactobacillus*, *Bifidobacterium*, and *E. coli* using SYBR green chemistry (Fast SYBR Green Master Mix, Applied Biosystems Foster City, CA) with StepOnePlus real-time PCR system (Applied Biosystems) and bacterial primers (Table 1). Standard curves for total bacteria, *Lactobacillus*, *Bifidobacterium*, and *E. coli* were generated using purified 16S rRNA genes of *Butyrivibrio hungatei*, *Lactobacillus acidophilus* ATCC4356, *Bifidobacterium longum*, and *Escherichia coli* K12, respectively. The copy number of 16S rRNA genes per gram of fresh tissue or content was then calculated using the equation described by Li et al. (2009). The prevalence of *Lactobacillus*, *Bifidobacterium*, and *E. coli* was calculated by dividing the copy number of 16S rRNA gene of each genus or species by the copy number of total bacteria.

Statistical Analysis

All bacterial density data were analyzed using MIXED procedure in SAS (SAS 9.4, SAS Institute Inc., Cary, NC) with small intestinal region as the repeated measurement and animal as the experimental unit. Compound symmetry covariance structure was selected as the best fit by the Bayesian information criteria. The following statistical model was fitted to test the effect of colostrum treatment, time point, gut region, and sample type on bacterial density:

Y_{ijklm} = \mu + C_i + T_j + R_k + S_l + CT_{ij} + CR_{ik} + CS_{il} + TR_{jk} + TS_{jl} + RS_{kl} + CTR_{ijk} + CTS_{ijl} + CRS_{ikl} + TRS_{jkl} + e_{ijklm},

where Y = bacterial density (total bacteria, *Lactobacillus*, *Bifidobacterium*, and *E. coli*), μ = mean, C = colostrum treatment, T = time point, R = small intestinal region, S = sample type (tissue, content), and e = residual error.

The same model was fitted when comparing newborn calves against 6- and 12-h-old calves under each diet after removing colostrum treatment effect from the model ($Y_{ijkl} = \mu + T_i + R_j + S_k + TR_{ij} + TS_{ik} + RS_{jk} + TRS_{ijk} + e_{ijkl}$). Differences in LSM were declared at $P < 0.05$ using the PDIFF option in SAS when applicable.

RESULTS

Effect of Colostrum Feeding on the Newborn Calf Gut Bacteria Within the First 12 h of Life

The feeding of colostrum (heat-treated or fresh) accelerated bacterial colonization in calf small intestine compared with the calves that did not receive colostrum soon after birth. The density of total bacteria was significantly higher ($P < 0.01$) in HC ($9.77 \pm 3.44 \times 10^9$ 16S rRNA gene copy/g of sample; mean $\pm$ SEM) and FC ($1.37 \pm 0.73 \times 10^{10}$ 16S rRNA gene copy/g of sample) calf small intestine when compared to that of NC ($5.26 \pm 2.17 \times 10^8$ 16S rRNA gene copy/g of sample), regardless of time point and small intestinal region (Figure 1A). Besides the colostrum feeding effect, a type by time interaction effect on total bacterial density was also significant ($P < 0.01$). The content-associated total bacterial density was higher at 12 h compared with that at 6 h as well as tissue-attached bacteria at 12 h (Figure 1B). When the prevalence of beneficial bacteria in calf small intestine was explored, the prevalence of *Lactobacillus* was higher ($P < 0.01$) in NC calves

Table 1. Bacterial primers used to estimate the copy number of 16S rRNA gene in the calf small intestine

Bacterial group	Primer	Product size (bp)	Annealing temperature (°C)	Reference
Total bacteria	Forward (F): 5'-actctacgggaggcag-3' Reverse (R): 5'-gactaccagggtatctaatcc-3'	467	62	Stevenson and Weimer, 2007
Lactobacillus	F: 5'-gaggcagcagtagggaatcttc-3' R: 5'-ggccagttactacctctctcttc-3'	120	62	Delroisse et al., 2008
Bifidobacterium	F: 5'-tacaccggaatagctctgg-3' R: 5'-cgtcaagctgataggacgc-3'	115	64	Liang et al., 2014
Escherichia coli	F: 5'-ggaagaagcttgcttctttgtgac-3' R: 5'-agcccggggatttcacatctgactta-3'	544	62	Sabat et al., 2000

compared with the colostrum-fed calves (Figure 2A). In contrast, the prevalence of *Bifidobacterium* was high ($P < 0.01$) in tissue-attached community of HC ($28.6 \pm 10.3\%$) calves than that of FC ($8.9 \pm 2.5\%$) and NC ($5.5 \pm 1.5\%$) calves at 6 h (Figure 2B). Moreover, the prevalence of *Bifidobacterium* was higher ($P < 0.01$) in proximal jejunum ($19.0 \pm 8.0\%$) of the calves when compared to that of distal jejunum ($11.1 \pm 4.5\%$) and ileum ($10.4 \pm 3.7\%$) regardless of colostrum treatment, time point, and sample type. In contrast, the feeding of colostrum significantly decreased the colonization of *E. coli* in the calf small intestine within the first 12 h of life (Figure 3). The prevalence of *E. coli* in the tissue and

content-associated small intestinal communities of NC (tissue = $-0.17 \pm 0.03\%$, content = $-0.16 \pm 0.01\%$) was higher compared with that of FC (tissue = $-0.019 \pm 0.005\%$, content = $-0.069 \pm 0.061\%$) and HC (tissue = $-0.001 \pm 0.0004\%$, content = $-0.004 \pm 0.002\%$).

Effect of Colostrum Feeding on the Bacterial Colonization Process

The bacterial densities of 6- and 12-h-old calves were also compared with that of newborn calves obtained within 30 min after birth to explore the changes in initial bacterial densities within the first 12 h of life and

Figure 1. Effect of colostrum feeding on small intestinal total bacterial density of neonatal calves. (A) Total bacterial density in the calf small intestine within the first 12 h of life with differing colostrum feeding methods. (B) Total bacterial density in tissue and content-associated communities within the first 12 h postpartum (NC = no colostrum; FC = fresh colostrum; HC = heat-treated colostrum). Bars represent mean $\pm$ SEM; means with different letters (a,b) are different at $P < 0.05$; double asterisks (**) mean densities of total bacteria within content- or tissue-associated communities are different between 6 and 12 h at $P < 0.05$.

Figure 2. Effect of colostrum feeding on beneficial bacterial establishment in calf small intestine. (A) Prevalence of *Lactobacillus* in the calf small intestine content- and tissue-associated communities with differing colostrum feeding methods. (B) Prevalence of *Bifidobacterium* in the small intestinal tissue-attached community within the first 12 h of life with differing colostrum feeding methods. Different letters (a,b) mean *Bifidobacterium* prevalence is different among 3 colostrum feeding methods at 6 h after birth ($P < 0.05$), or (x,y) at 12 h after birth ($P < 0.05$); NC = no colostrum; FC = fresh colostrum; HC = heat-treated colostrum. Bars represent mean $\pm$ SEM.

how this process is influenced by the feeding of colostrum (Figure 4). The total bacterial density observed at birth ($1.21 \pm 0.56 \times 10^9$ 16S rRNA gene copy/g of sample) was significantly increased ($P < 0.01$) within the first 12 h of life when the calves were fed with heat-treated colostrum ($1.25 \pm 0.50 \times 10^{10}$ 16S rRNA gene copy/g of sample) or fresh colostrum ($2.03 \pm 1.13 \times 10^9$ 16S rRNA gene copy/g of sample) soon after birth. Although the prevalence of *Lactobacillus* was lower ($P = 0.01$) in HC calves at 6 ($0.008 \pm 0.002\%$) and 12 h ($0.006 \pm 0.003\%$) after birth compared with newborn calves ($0.046 \pm 0.024\%$), no such changes ($P = 0.12$) were observed in FC calves (6 h = $-0.018 \pm 0.010\%$;

12 h = $-0.010 \pm 0.006\%$). The prevalence of *Bifidobacterium* in tissue-attached community was significantly ($P = 0.03$) lower at 6 h in FC ($8.9 \pm 2.5\%$) calves compared with newborn ($31.2 \pm 9.0\%$); however, no such differences ($P = 0.51$) were observed in HC calves ($28.6 \pm 10.3\%$). When the colonization of *E. coli* within the first 12 h of life was compared with that of newborn calves, no differences ($P = 0.32$) were observed between newborn and FC calves. However, the HC group had lower ($P < 0.01$) colonization of *E. coli* in the small intestinal tissue-associated communities at 6 ($0.0004 \pm 0.0002\%$) and 12 h ($0.0008 \pm 0.0003\%$) after birth compared with that of newborn calves ($0.054 \pm$

Figure 3. Effect of colostrum feeding on the colonization of *Escherichia coli* in the calf small intestine. NC = no colostrum; FC = fresh colostrum; HC = heat-treated colostrum. Different letters mean *E. coli* prevalence in the small intestinal content-associated community (a,b) or in tissue-associated community (x,y) are different among the colostrum feeding methods ($P < 0.05$); double asterisks (**) mean *E. coli* prevalence within NC is different between 6 and 12 h after birth at $P < 0.05$. Bars represent mean $\pm$ SEM.

0.001%). No differences were observed when comparing total bacteria, *Lactobacillus*, and *Bifidobacterium* of NC calves to that of newborn calves. However, the prevalence of *E. coli* in the small intestinal tissue of NC increased ($P < 0.01$) at 12 h ($0.13 \pm 0.02\%$) compared with that of newborn calves.

Effect of Heat-Treated Colostrum Feeding on Small Intestinal Bacteria Within the First 12 h of Life

The feeding of HC to calves soon after birth did not significantly influence ($P = 0.22$) the total bacteria density when compared to that of FC; however, a reduction in the number of bacteria colonized in the small intestinal regions was noted (Table 2). When the prevalence of *Lactobacillus* was compared, it was 4 times higher ($P < 0.01$) in small intestinal content-associated community ($0.023 \pm 0.015\%$) of FC compared that of tissue ($0.005 \pm 0.002\%$), regardless of the time point. In general, the feeding of HC decreased *Lactobacillus* prevalence in the calf small intestine compared with FC (Table 2). The feeding of heat-treated colostrum, however, had remarkable effect on the prevalence of *Bifidobacterium* in small intestinal tissue-associated community. The prevalence of *Bifidobacterium* was 3.2 fold

higher in HC calves than FC calves at 6 h after birth ($P < 0.01$). In contrast, the feeding of fresh colostrum gradually increased the colonization of *Bifidobacterium* in the small intestine, and no effect of type of colostrum fed to the calves on *Bifidobacterium* prevalence was observed at 12 h after birth (Figure 2B). When the colonization of *E. coli* in the calf small intestine was compared between 2 colostrum-feeding methods, FC calves had higher ($P < 0.01$) density of *E. coli* but not higher prevalence than HC calves (Table 2).

DISCUSSION

Neonatal diarrhea is responsible for ~50% of the calf deaths occur in dairy industry (Uetake, 2013). Good colostrum management is one of the most important preventive measures of neonatal calf diarrhea because colostrum transfers passive immunity to the newborn calves. The bioactive compounds, such as oligosaccharides (OS), present in bovine colostrum also inhibit the adherence of pathogens to the intestinal epithelial cells and prevent infections (Maldonado-Gomez et al., 2015). The feeding of heat-treated colostrum to dairy calves is one of the industry-suggested good practices to increase passive transfer of immunity (Godden et al.,

Figure 4. Bacterial colonization within the first 12 h of life when calves fed with differing colostrum feeding methods. (A) Total bacterial density in the calf small intestine. (B) The prevalence of *Lactobacillus* in the calf small intestine. (C) The prevalence of *Bifidobacterium* in the calf small intestine. (D) The prevalence of *Escherichia coli* in the calf small intestine (data presented as mean $\pm$ SEM; means with different letters (a,b) are different at $P < 0.05$). NC = no colostrum; FC = fresh colostrum; HC = heat-treated colostrum.

2012; Teixeira et al., 2013; Gelsinger et al., 2014) and to minimize calf mortality and morbidity. Heat treatments (60°C, 60 min) decrease total bacterial count as well as eliminate pathogens, such as *Mycobacterium avium* ssp. *paratuberculosis*, present in colostrum while maintaining IgG concentration (Godden et al., 2006; Johnson et al., 2007). Although the effect of heat-treated colostrum feeding on passive immune transfer has been widely studied, very limited knowledge exists regarding its effects on gut microbial colonization. Gut microbiota plays a key role in host health and susceptibility to diseases (Round and Mazmanian, 2009), and the importance of early gut microbiota on immune system development has been well described in humans (Russell et al., 2013; Jakobsson et al., 2014).

The present study revealed that the feeding of colostrum (either fresh or heat-treated) facilitated gut microbial colonization, allowing the bacterial numbers to reach 10^{10} 16S rRNA genes/g in fresh samples within the first 12 h of life. In contrast, the calves that did not receive colostrum soon after birth had a very low number of total bacteria, which was similar to that of newborn calves, suggesting slower bacterial colonization in the absence of colostrum. The low total bacterial density in mice pups has been shown to be associated with differed mucosal and systemic immune responses compared with the pups with normal bacterial densities (Lamousé-Smith et al., 2011). We speculated that the timed feeding of colostrum is important for the establishment of stable gut microbiome that plays a

Table 2. Effect of heat-treated colostrum feeding on small intestinal bacterial densities within the first 12 h¹ (mean $\pm$ SEM)

Item	Colostrum treatment			Time point		Small intestinal region				Sample type	
	FC	HC		6 h	12 h	PJ	DJ	IL		Tissue	Content
Total bacteria ²	1.3 $\pm$ 0.3 $\times 10^{10}$	8.4 $\pm$ 1.4 $\times 10^9$		6.3 $\pm$ 0.7 $\times 10^{10}$	1.5 $\pm$ 0.4 $\times 10^{10}$	5.8 $\pm$ 1.1 $\times 10^9$	1.3 $\pm$ 0.4 $\times 10^{10}$	1.7 $\pm$ 0.5 $\times 10^{10}$		8.2 $\pm$ 2.4 $\times 10^{10}$	1.5 $\pm$ 0.8 $\times 10^{10}$
P-value	0.22			<0.01			0.14			0.03	
<i>Lactobacillus</i> ²	4.4 $\pm$ 2.6 $\times 10^5$	3.7 $\pm$ 1.6 $\times 10^5$		3.6 $\pm$ 1.6 $\times 10^5$	4.5 $\pm$ 2.6 $\times 10^5$	3.5 $\pm$ 1.9 $\times 10^5$	3.7 $\pm$ 1.8 $\times 10^5$	4.7 $\pm$ 2.6 $\times 10^5$		3.3 $\pm$ 1.3 $\times 10^5$	5.1 $\pm$ 3.2 $\times 10^5$
P-value	0.56			0.44			0.61			0.25	
<i>Bifidobacterium</i> ²	6.0 $\pm$ 2.1 $\times 10^8$	9.4 $\pm$ 3.2 $\times 10^8$		8.3 $\pm$ 2.9 $\times 10^8$	7.2 $\pm$ 2.4 $\times 10^8$	6.3 $\pm$ 2.2 $\times 10^8$	8.4 $\pm$ 3.0 $\times 10^8$	8.5 $\pm$ 2.9 $\times 10^8$		1.2 $\pm$ 0.4 $\times 10^8$	3.7 $\pm$ 1.6 $\times 10^8$
P-value	0.16			0.65			0.26			<0.01	
<i>Escherichia coli</i> ²	7.9 $\pm$ 3.4 $\times 10^5$	7.8 $\pm$ 4.3 $\times 10^4$		1.3 $\pm$ 0.5 $\times 10^5$	7.4 $\pm$ 3.3 $\times 10^5$	1.5 $\pm$ 0.6 $\times 10^5$	5.9 $\pm$ 2.7 $\times 10^5$	5.6 $\pm$ 2.4 $\times 10^5$		2.6 $\pm$ 0.9 $\times 10^5$	5.7 $\pm$ 2.8 $\times 10^5$
P-value	<0.01			<0.01			0.18			0.07	
<i>Lactobacillus</i> ³	0.01 $\pm$ 0.003	0.007 $\pm$ 0.001		0.01 $\pm$ 0.003	0.008 $\pm$ 0.002	0.02 $\pm$ 0.005	0.01 $\pm$ 0.002	0.01 $\pm$ 0.002		0.005 $\pm$ 0.002 ^a	0.016 $\pm$ 0.010 ^b
P-value	0.28			0.20			0.12			<0.01	
<i>Bifidobacterium</i> ³	14.3 $\pm$ 2.0	16.2 $\pm$ 2.0		15.0 $\pm$ 1.8	15.5 $\pm$ 2.2	19.7 $\pm$ 3.4	12.7 $\pm$ 2.2	12.6 $\pm$ 2.4		21.2 $\pm$ 8.1 ^a	7.8 $\pm$ 2.3 ^b
P-value	0.79			0.95			0.07			<0.01	
<i>E. coli</i> ³	0.022 $\pm$ 0.02	0.001 $\pm$ 0.0008		0.004 $\pm$ 0.002	0.019 $\pm$ 0.015	0.004 $\pm$ 0.002	0.03 $\pm$ 0.02	0.004 $\pm$ 0.001		0.005 $\pm$ 0.001	0.017 $\pm$ 0.014
P-value	0.17			0.28			0.37			0.35	

^{a,b}Means with different superscript within a row are significantly different at $P < 0.05$.¹FC = fresh colostrum; HC = heat-treated colostrum; PJ = proximal jejunum; DJ = distal jejunum; IL = ileum.²Copy number of 16S rRNA gene/g of fresh sample.³Prevalence of *Lactobacillus*, *Bifidobacterium* and *E. coli* as a % of total bacteria.

crucial role in the development of naïve newborn calf immune system. Therefore, besides the efficient and effective absorption of immunoglobulin, the timed feeding of colostrum also plays a vital role in calf health via prompting gut microbial colonization.

The calf small intestine was already colonized with higher levels of *Bifidobacterium* within 30 min after birth; however, only the feeding of heat-treated colostrum was capable of maintaining these higher levels throughout the first 12 h of life. *Bifidobacterium* is widely studied due to its use as a probiotic, and it can modulate host immune responses as well as immune cell phenotypes in vitro (Hart et al., 2004). Moreover, *Bifidobacterium* protects host against enteropathogenic infections via the production of acetate (Fukuda et al., 2011). The elevated *Bifidobacterium* colonization, especially in tissue-attached community immediately post-partum, may play an important role in the development of calf immune system and gut barrier. Also this may be one of the reasons for the reduction of enteric infections, when calves are fed with heat-treated colostrum, besides the improved IgG absorption (Godden et al., 2012). The feeding of heat-treated colostrum soon after birth was also associated with the reduced colonization of *E. coli* within the first 12 h of life. The prevalence of *E. coli* in the calf small intestine was 58 and 15 fold higher in NC and FC, respectively, compared with that of HC. *Escherichia coli* is one of the most common pathogens in neonatal calf diarrhea (Foster and Smith, 2009). Thus, the reduced pathogenic bacteria and increased beneficial bacteria colonization in the small intestine can be considered one of the advantages of feeding heat-treated colostrum to the calves soon after birth.

The estimation of total bacteria in colostrum using quantitative real time-PCR revealed that detectable bacteria were only present in fresh colostrum (1.8×10^3 16S rRNA gene copy/mL of colostrum), but not in the heat-treated colostrum (not detected) used in the present study. Besides, 70.6% (1.31×10^3 16S rRNA gene copy/mL of colostrum) of these detectable bacteria consisted of *E. coli*, whereas *Lactobacillus* or *Bifidobacterium* were not detected in the fresh colostrum samples used in the present study. Thus, the increased colonization of *Bifidobacterium* observed in HC calves was not due to the introduction of this bacterium via colostrum, rather this increase might have been promoted by the bioactive components. Oligosaccharides are one of the main energy sources for *Bifidobacterium*, and *B. longum* ssp. *infantis* grows easily in human milk OS due to their ability to break down sialylated OS (LoCascio et al., 2007, 2010; Sela et al., 2011). Moreover, sialylated OS promotes adhesion of *Bifidobacterium* to epithelial cells in vitro (Kavanaugh et al., 2013). Sialylated OS,

the main oligosaccharides present in bovine colostrum (ten Bruggencate et al., 2014), is mainly bound to milk proteins (Nesser et al., 1991). Moreover, the availability of sialylated OS in heat-treated bovine milk is higher than that of fresh colostrum (Nesser et al., 1991). Thus, we proposed that the increased availability of sialylated OS in heat-treated colostrum retained the higher level of *Bifidobacterium* observed in newborn small intestine when they received heat-treated colostrum soon after birth.

In conclusion, the feeding of heat-treated colostrum soon after birth enhanced the colonization of *Bifidobacterium* and reduced the colonization of *E. coli* in the calf small intestine immediately postpartum. This may be one of the reasons for the observed lower prevalence of enteric infections in calves fed heat-treated colostrum compared with calves fed fresh colostrum (Godden et al., 2012). However, the present study only explored the microbial colonization immediately postpartum (with the first 12 h); therefore, the long-term effects of heat-treated colostrum feeding on microbial colonization and succession are not clearly defined. Moreover, the effect of enhanced colonization of *Bifidobacterium* on calf performance (BW gain, resistance to enteric infections, weaning weight) needs to be studied in detail. Lastly, to our knowledge this is the first study to understand the influence of heat-treated colostrum feeding on calf gut colonization.

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