



J. Dairy Sci. 98:1–10
<http://dx.doi.org/10.3168/jds.2015-9607>
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Heat-treated colostrum feeding promotes beneficial bacteria colonization in the small intestine of neonatal calves

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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-

Received March 21, 2015.

Accepted July 13, 2015.

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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

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