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Short communication: The effect of heat treatment of bovine colostrum on the concentration of oligosaccharides in colostrum and in the intestine of neonatal male Holstein calves

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ABSTRACT

The objective of this study was to determine the effect of the heat treatment (HT, 60°C for 60 min) on the concentration of bovine colostrum oligosaccharides (bCO) in pooled bovine colostrum and the intestine of neonatal male Holstein calves after feeding. First-milking colostrum was pooled from both primiparous and multiparous cows, and half of the pooled colostrum was heat-treated at 60°C for 60 min (HC), whereas the other half was not heat-treated and remained fresh (FC). At birth, 32 male Holstein calves were randomly assigned to 1 of 3 treatment groups: (1) control calves that did not receive colostrum for the duration of the experiment and were euthanized at 6 h (NC, n = 4) or 12 h (NC, n = 4), (2) calves fed fresh colostrum (FC) and were euthanized at 6 h (FC, n = 6) or 12 h (FC, n = 6), or (3) calves fed heat-treated colostrum (HC) and euthanized at 6 h (HC, n = 6) or 12 h (HC, n = 6). All calves were fed 2 L of colostrum within 1 h after birth. At dissection, digesta of the distal jejunum, ileum, and colon was collected and analyzed by liquid chromatography-mass spectrometry to determine the concentration of bCO within each intestinal region. The heat-treated colostrum displayed numerically higher concentrations of total bCO (3,511.6 μg/g) when compared with fresh colostrum (1,329.9 μg/g), with 3′sialyllactose being the most abundant bCO in both fresh and HT colostrum. In contrast, calves fed HT colostrum displayed a lower amount of total bCO in the distal jejunum (221.91 ± 105.3 vs. 611.26 ± 265.1 μg/g), ileum (64.97 ± 48.39 vs. 344.04 ± 216.87 μg/g), and colon (25.60 ± 13.1 vs. 267.04 ± 125.81 μg/g) at 6 h of life when compared with calves fed fresh colostrum. No differences were observed in regard to the concentrations of total bCO in the intestine of FC and HC calves at 12 h of life. It is speculated that lower concentrations of bCO in the gastrointestinal tract of HC calves at 6 h of life could be due to the early establishment of beneficial bacteria, such as *Bifidobacterium*, in HC calves and their subsequent metabolism of bCO as a carbon source. These findings suggest that the heat treatment of colostrum increases the amount of free bCO, which may serve as prebiotics available to microbiota within the intestine of the neonatal calf.
Key words: heat treatment, colostrum, neonatal calf, oligosaccharide

Short Communication

The neonatal dairy calf is at high risk of morbidity and mortality (NAHMS, 2011), which causes concern not only from an economic standpoint, but also with regard to welfare. The timely feeding of high-quality, adequate volumes of uncontaminated colostrum is a key factor in determining the survival of the neonatal dairy calf (Weaver et al., 2000). However, although the consequences of poor colostrum management are well known, many farms do not assess the quality of colostrum, which may lead to feeding colostrum with a low concentration of IgG or contaminated colostrum, and do not feed the first colostrum meal in a timely manner (Vasseur et al., 2010). Unfortunately, this type of colostrum management likely plays a pivotal role in decreased calf health and welfare, which contributes to high rates of morbidity reported in neonatal calves. More specifically, neonatal calves have an alarmingly high prevalence of enteric infections, with neonatal calf diarrhea being the most common ailment resulting in illness and death (Meganck et al., 2014) and 25.3% of pre-weaned calves being affected by digestive problems (NAHMS, 2011). Therefore, knowledge regarding how to decrease the prevalence of digestive disorders in pre-weaning calves is necessary to ensure a profitable dairy industry.

In an effort to improve neonatal calf gut health, interest has been increasing in supplementing bovine colostrum or colostrum replacers with gut active carbo-

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hydrates derived from yeast (mannan-oligosaccharides, **MOS**) and bacteria (*Bifidobacterium* galacto-oligosaccharides; Brady et al., 2015). However, the majority of studies using large sample sizes have found a negative or no effect on calf performance and passive transfer of immunity when MOS or *Bifidobacterium* galacto-oligosaccharides are supplemented (Villettaz Robichaud et al., 2014; Brady et al., 2015). During early life, the gastrointestinal tract (**GIT**) of the calf is evolutionarily tailored to respond to compounds secreted by the dam into colostrum and milk, and the structure of an oligosaccharide is a major determinant of biological function (Short et al., 2016). For instance, MOS are particularly effective at adhering to *Escherichia coli* when present in an α1–3 and α1–6 configuration (Firon et al., 1987), while sialylated oligosaccharides (**OS**) are most effective as α2–6 isomers (Martin et al., 2002). Therefore, these differences in structure and configuration may provide reasoning as to why the supplementation of MOS may not have a beneficial effect on the calf GIT during early life, as it may better respond to bovine colostrum oligosaccharides (**bCO**) structures during this period. Martin-Sosa et al. (2003) determined 5 primary OS compounds present in bovine colostrum and milk, with significantly higher amounts of specific OS present in colostrum compared with mature milk. More than 70% of the identified OS in bovine colostrum and milk are sialylated (Tao et al., 2008), with 3′sialyllactose being the most abundant isoform in colostrum, followed by 6′sialyllactosamine (Martin-Sosa et al., 2003). Using in vitro experiments, it has been demonstrated that oligosaccharides are able to resist enzymatic hydrolysis throughout the upper GIT and it was previously thought that the majority of OS reach the colon intact for fermentation by commensal microbiota (Engfer et al., 2000). However, a recent study using a rat model showed that the intestinal bacteria might metabolize human milk-derived OS as early as the jejunum and that smaller molecular weight OS may actually never reach the colon (Jantscher-Krenn et al., 2013).

Currently, knowledge is lacking regarding methods to increase the availability of oligosaccharides in bovine colostrum for supplementation in dairy calves, as well as the characterization of bCO concentrations in the neonatal calf intestine. Therefore, the objectives of the present study were to (1) determine the effect of the heat treatment of colostrum on the concentration of bCO, and (2) to determine the concentrations of bCO in the distal jejunum, ileum, and colon of neonatal calves fed heat-treated (**HT**) colostrum compared with calves fed fresh colostrum. It was hypothesized that HT colostrum would have higher concentrations of free bCO when compared with fresh colostrum, and as a consequence, calves fed HT colostrum would have higher concentrations of bCO within the intestine compared with calves fed fresh colostrum.

The experimental procedures were conducted at the Dairy Research and Technology Centre, University of Alberta in accordance with the Canadian Council of Animal Care (CCAC, 1993), and all protocols were approved by the University of Alberta Animal Care and Use Committee for Livestock (AUP00001012). Colostrum (first milking) containing ≥50 mg/mL of IgG was collected from 16 primiparous and multiparous cows and immediately frozen at −20°C after collection. Once the required volume was collected, colostrum was thawed and pooled. Half of the pooled colostrum (24 L) was heat-treated for 60 min at 60°C using a pasteurizer (DT 10G, Dairy Tech Inc., Greeley, CO). Both fresh and HT colostrum were frozen at −20°C until needed. At birth, male Holstein calves were randomly assigned to 1 of 3 treatment groups: (1) control calves that did not receive colostrum for the duration of the experiment and euthanized at 6 h (**NC**, n = 4) or 12 h (NC, n = 4), (2) calves fed fresh colostrum and euthanized at 6 h (**FC**, n = 6) or 12 h (FC, n = 6), or (3) calves fed pasteurized colostrum and euthanized at 6 h (**HC**, n = 6) or 12 h (HC, n = 6). Prior to the study, because no previous research has been conducted with regard to the concentrations of oligosaccharides in the intestine of neonatal calves, studies regarding the proportion of *Bifidobacterium* in the small intestine of neonatal calves were used as a variable to determine the amount of biological replicates required to have sufficient power for the experiment. It was determined that a minimum of 4 biological replicates was required to detect a 20% difference at a power of 80%. The average birth BW of FC calves euthanized at 6 and 12 h were 40.9 ± 3.4 and 39.1 ± 1.5 kg, respectively, and for HC calves euthanized at 6 and 12 h were 47.7 ± 3.3 and 41.4 ± 2.1 kg, respectively. Using a water bath, colostrum was thawed to 38°C and 2 L was fed to each calf using an esophageal tube feeder within an hour after birth. Immediately before euthanasia, FC and HC calves euthanized at 6 h achieved serum IgG concentrations of 9.7 ± 0.74 and 9.7 ± 0.70 mg/mL, respectively, whereas FC and HC calves euthanized at 12 h achieved 15.8 ± 1.37 and 12.9 ± 1.37 mg/mL, respectively (Kent-Dennis, 2014). Calves were euthanized by penetrative captive bolt followed by exsanguination. The digesta samples were collected following the procedures previously reported by Malmuthuge et al. (2015). Briefly, closed intestinal segments (10 cm) of the distal jejunum, ileum, and colon were collected with the distal jejunum defined as 30 cm proximal to the collateral branch of the mesenteric artery, the ileum defined as 30 cm proximal to the ileo-cecal junction, and the colon defined as 30 cm distal to the colon-cecal junction.

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