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

Amanda J. Fischer, Nilusha Malmuthuge, Le Luo Guan, and Michael A. Steele¹

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

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 $\mu\text{g/g}$) when compared with fresh colostrum (1,329.9 $\mu\text{g/g}$), 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 $\mu\text{g/g}$), ileum (64.97 ± 48.39 vs. 344.04 ± 216.87 $\mu\text{g/g}$), and colon (25.60 ± 13.1 vs. 267.04 ± 125.81 $\mu\text{g/g}$) 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

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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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¹Corresponding author: mastelee@ualberta.ca

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 α 1–3 and α 1–6 configuration (Firon et al., 1987), while sialylated oligosaccharides (**OS**) are most effective as α 2–6 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.

After collection, digesta samples were snap-frozen in liquid nitrogen and transferred to -80°C until further analysis.

After thawing digesta samples for 5 min on ice, approximately 0.10 g of each sample was obtained and placed in a 2-mL microcentrifuge tube. This was followed by the addition of 150 μL of HPLC-grade water and defatting by centrifugation at $6,000 \times g$ for 15 min at room temperature, and the supernatant was removed and placed in a new tube. After repeating the above step twice, 1 mL of 2:1 chloroform:methanol was added to the supernatant and centrifugation was performed at $300 \times g$ for 60 min at room temperature to remove any proteins and impurities. The lower phase was then re-extracted using 500 μL of 50% methanol, and the resulting supernatant was cooled at 4°C for 30 min. The sample was then centrifuged at $11,000 \times g$ for 15 min at room temperature to remove any residual contaminants and diluted 5-fold using 95% acetonitrile (**AcN**). Colostrum was processed using the same procedures, except that it was only defatted by centrifugation once and diluted 5-fold before chloroform:methanol extraction. All samples were stored at 4°C until liquid chromatography (LC)-MS analysis. The recovery rate of the OS extraction method was assessed by spiking a known amount of internal standard (β 1-3-gal-*N*-acetyl-galactosaminyl- β 1-4-gal β 1-4-Glc; **GalNAc**) before OS extraction and was measured by LC-MS and estimated to be 97%.

Oligosaccharide standards, including disialyllactose (**DSL**), 3'-sialyllactose (**3'-SL**), 6'-sialyllactose (**6'-SL**), 3'-sialyllactosamine (**3'-SLN**), and 6'-sialyllactosamine (**6'-SLN**), and GalNAc (internal standard), were purchased from Dextra Laboratories Ltd. (Reading, UK) and diluted using 95% AcN to give a 9-point calibration curve. An LC system with a binary pump and autosampler (Agilent Technologies, Palo Alto, CA) coupled to a 4000 QTRAP mass spectrometer (AB SCIEX, Concord, ON, Canada) was used to determine the concentration of bCO in colostrum and intestinal digesta.

Liquid chromatography separation was performed using an Ascentis Express hydrophilic interaction liquid chromatography column (10 cm $\times$ 2.1 mm, 2.7 μm in particle size; Sigma, St. Louis, MO), with the mobile phase being composed of (A) water with 50 mM ammonium acetate, and (B) AcN. The total run time was 35 min (including re-equilibration) with a constant flow rate of 200 $\mu\text{L}/\text{min}$. The gradient used was as follows: 0 to 18 min, 5 to 30% A; 18 to 20 min, 30% A; 20.1 min increased to 50% A; 20 to 24 min held at 50% A, and then decreased back to 5% A at 24.1 min for column re-equilibrium over 11 min before the next injection. The auto-sampler temperature was set to 15°C with 5 μL of injection volume. A turbo spray ion source (electrospray ionization) was used under negative ion mode and multiple reaction-monitoring (MRM) scan mode was developed for the quantification of the analytes of interest. Nitrogen was used as curtain gas, nebulizing gas, and drying gas. The instrument was operated using the following settings: curtain gas, gas 1, and gas 2 at 20, 50, and 50 arbitrary units, respectively, and ionspray voltage at -4.5 kV. The ion source temperature was 400°C and quadrupoles Q1 and Q3 were operating at unit mass resolution. The multiple reaction-monitoring transitions and optimized mass spectrometer parameters for each analyte and their reference internal standard (GalNAc) are summarized in Table 1. The data were processed using Analyst 1.6 software (AB SCIEX, Concord, ON, Canada).

To determine the effect of colostrum treatment on the concentration of bCO within intestinal region, all data were analyzed using the MIXED procedure of the Statistical Analysis system (ver. 9.4, SAS Institute Inc., Cary, NC). Data were analyzed using the animal as a random effect, and the treatment (HC, FC), sample time (6 h, 12 h), and sample type (distal jejunum, ileum, colon) and their interactions as fixed effects, and BW was included as a covariate. For the effect of treatment on the concentration of each bCO (3'-SL, 6'-SL,

Table 1. Multiple reaction-monitoring (MRM) transitions and optimized parameters¹ for each compound

Compound(s) ²	MRM transitions (amu)	DP (eV)	EP (eV)	CE (eV)	CXP (eV)
3'-SLN/6'-SLN	673 $\rightarrow$ 290	-105	-10	-44	-13
3'-SLN/6'-SLN	673 $\rightarrow$ 572	50	4	-38	-13
3'-SL/6'-SL	632 $\rightarrow$ 290	40	4	35	3
3'-SL/6'-SL	632 $\rightarrow$ 572	45	4.5	40	3
DSL	932 $\rightarrow$ 632	50	4	40	3
DSL	932 $\rightarrow$ 581	45	4.5	40	3
Glucose	706 $\rightarrow$ 628	50	6	25	6
Glucose	706 $\rightarrow$ 201	50	6	32	6

¹DP, EP, CE, and CXP are declustering potential, entrance potential, collision energy, and collision cell exit potential.

²3'-SL = 3'-sialyllactose; 6'-SL = 6'-sialyllactose; 3'-SLN = 3'-sialyllactosamine; 6'-SLN = 6'-sialyllactosamine; DSL = disialyllactose.

3'-SLN, 6'-SLN, and DSL) and total bCO, data were analyzed among treatment group by sampling time and sample type and the interaction (treatment $\times$ type, treatment $\times$ time, time $\times$ type, and treatment $\times$ type $\times$ time). All values reported are least squares means with significance declared at $P \leq 0.05$ and tendencies at $0.05 < P < 0.10$.

Using LC-MS, 4 main types of bCO were detected in bovine colostrum, regardless of treatment (Figure 1a). Our study revealed that 3'-SL was the dominant bCO in both HT (2,390.0 $\mu\text{g/g}$) and fresh (840.0 $\mu\text{g/g}$) colostrum samples (Figure 1b,c), which is consistent with previous reports (Martin-Sosa et al., 2003; Nakamura et al., 2003; Fong et al., 2011). However, the value of 3'-SL in FC in our study differed from previous stud-

ies that had reported values of 354 and 1,245 $\mu\text{g/g}$ of 3'-SL in fresh colostrum (Martin-Sosa et al., 2003; Fong et al., 2011). The differences observed between the present study and previous reports may be attributed to differences in the sampling time after parturition, genetics, and sample size of the various experiments. Specifically, Fong et al. (2011) collected second-milking colostrum from a single Friesian cow, and Martin-Sosa et al. (2003) obtained samples from 6 multiparous Spanish-Brown cows on d 2 of lactation. In contrast to these 2 experiments, the present study collected pooled fresh, first-milking colostrum from both primiparous and multiparous Holstein cattle. Further research using large sample sizes and frequent sampling throughout the various lactation stages of both primiparous and

Figure 1. (a) The effect of heat treatment at 60°C for 60 min on the abundance of bovine colostrum oligosaccharides (bCO) between a single pooled bovine fresh colostrum sample and a single heat-treated colostrum sample (numerical differences only). Bars represent the concentration ($\mu\text{g/g}$) of a single pooled colostrum sample. (b) The proportion of bovine colostrum oligosaccharides in fresh colostrum as a percentage of total oligosaccharides. (c) The proportion of bovine colostrum oligosaccharides in heat-treated colostrum as a percentage of total oligosaccharides. HC = heat-treated colostrum; FC = fresh colostrum; 3'-SL = 3'-sialyllactose; 6'-SL = 6'-sialyllactose; 6'-SLN = 6'-sialyllactosamine; DSL = disialyllactose.

multiparous cows is needed to determine the factors that may greatly influence the concentrations of bCO in colostrum.

Although statistical analysis was not performed for pooled colostrum samples, heat treatment numerically increased 3'-SL by 2.8 times, 6'-SLN by 2.3 times, 6'-SL by 1.5 times, and DSL by 3.6 times when compared with FC, whereas 3'-SLN was low (1.1–3.8 $\mu\text{g/g}$) and not different between the 2 types of colostrum samples (Figure 1a). In contrast to human milk, bovine milk contains fewer free oligosaccharides, as they are generally found attached to lipid or protein as glycoconjugate structures (Kobata, 1977; Neeser et al., 1991). The concentration of free oligosaccharides in bovine milk can be increased through heat treatment (Neeser et al., 1991), likely from their cleavage from these structures. Therefore, it is suggested that the cleavage of bCO from colostrum lipids or proteins may provide reasoning for the increase in the concentration of free bCO in HT colostrum when compared with fresh colostrum.

In contrast to our hypothesis, although higher concentrations of bCO were detected in HT colostrum, calves fed HT colostrum had a lower concentration of bCO within intestinal regions at 6 h when compared with that of calves fed fresh colostrum. As expected, no bCO were detected in the intestine of calves not fed colostrum at either 6 or 12 h of life. More specifically, at 6 h of life FC calves tended to have a higher concentration of DSL ($P = 0.05$) and a significantly higher concentration of 6'-SLN ($P = 0.02$), 6'-SL ($P = 0.03$), and 3'-SL ($P = 0.01$) in the distal jejunum when compared with HC calves (Figure 2). In the ileum, FC calves tended to have a higher concentration of 6'-SLN ($P = 0.09$) and 6'-SL ($P = 0.06$), and a significantly higher concentration of 3'-SL ($P = 0.03$) and DSL ($P = 0.04$) compared with HC calves. Additionally, the colon of FC calves tended to have a higher concentration of 6'-SLN ($P = 0.09$), 6'-SL ($P = 0.06$), and DSL ($P = 0.06$), and a significantly higher concentration of 3'-SL ($P = 0.02$) compared with HC calves. Yet, at 12 h of life no differences were detected in bCO within the distal jejunum or ileum among treatments; however, FC calves had a higher concentration of DSL ($P = 0.04$) in the colon when compared with HC calves. Species belonging to the genus *Bifidobacteria* have been shown to exhibit robust sialidase activity, as well as produce large amounts of acidic fermentation products (e.g., lactate and SCFA) when grown on 3'-SL and 6'-SL (Yu et al., 2013). A previous study that focused on the differences of *Bifidobacteria* in the small intestine of the same calves from the present study reported that calves fed HT colostrum displayed a higher prevalence of small intestinal *Bifidobacterium* at 6 h of life compared with calves fed fresh colostrum,

whereas no differences were detected at 12 h among treatment groups in the prevalence of small intestinal *Bifidobacterium* (Malmuthuge et al., 2015). Therefore, we speculate that the low concentration of bCO in the intestine of HC fed calves at 6 h of life may be due to their utilization as a substrate for the growth of *Bifidobacteria*. Moreover, we speculate that no differences in the *Bifidobacterium* prevalence and the concentrations of bCO at 12 h of life indicates that the high concentration of bCO in FC calves at 6 h of life may have been metabolized by *Bifidobacterium* to achieve a similar prevalence this bacterial genus to that of HC calves at 12 h of life. To support our speculation, further correlation analysis was performed to explore the relationship between bCO and *Bifidobacterium* in the intestine of FC and HC calves. A negative correlation ($r = -0.47$, $P = 0.04$) between *Bifidobacterium* associated with the distal jejunum mucosa and bCO was observed, suggesting that the decreased concentrations of bCO may be due to their metabolism by *Bifidobacterium* in the small intestine of HC calves. However, additional bacterial genera in the intestine may also metabolize bCO, such as *Bacteroides*, and certain species belonging to this genus have been shown to cleave and catabolize sialic acid (Marcobal et al., 2011). Future studies involving more in-depth analysis of the gut microbiota composition and population changes between HC and FC would help us determine the exact causative factor for the observed lower concentrations of bCO in the gut of HC calves. In addition, recent studies have reported biological effects of sialylated OS, including their ability to inhibit pathogenic *E. coli* K99 (Martin et al., 2002) and their capability to enhance the absorption of IgG (Gill et al., 1999). Therefore, the high content of sialylated OS [e.g., 3'-SL (2,390.0 $\mu\text{g/g}$)] observed in HT colostrum in the present study suggests it may have a potential beneficial effect as a prebiotic to enhance the population of beneficial microorganisms. Future studies are needed to understand the role of bCO in the intestine of neonatal calves and their interactions with the immune system and intestinal microbiota.

To our knowledge, the present study is the first to characterize the concentration of bCO from fresh and HT colostrum throughout the intestine of neonatal dairy calves, as well as to investigate the effect of heat treatment on the concentration of bCO in colostrum. In conclusion, the heat treatment of colostrum increased the concentration of free bCO in colostrum when compared with fresh colostrum. Heat treatment at 60°C for 60 min may release sialylated bCO from colostrum proteins and lipids, thus increasing the amount of free bCO. It was also determined that FC calves displayed a higher concentration of bCO at 6 h of life in the intestine compared with HC calves, which may sug-

Figure 2. The concentration ($\mu\text{g/g}$) of bovine colostrum oligosaccharides in the intestine of calves fed fresh and heat-treated colostrum. Bars represent mean $\pm$ SEM, with white bars representing calves fed fresh colostrum and gray bars representing calves fed heat-treated colostrum. 3'-SL = 3'-sialyllactose; 6'-SL = 6'-sialyllactose; 6'-SLN = 6'-sialyllactosamine; DSL = disialyllactose. * $P < 0.05$, † $0.05 < P < 0.10$.

gest a variation in their metabolism due to the lower prevalence of *Bifidobacterium*. Our study suggests that heat treatment may have a potential prebiotic benefit in regard to providing more substrate to beneficial microorganisms in the gut of the neonatal calf, such as *Bifidobacterium*.

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REFERENCES

- Brady, M. P., S. M. Godden, and D. M. Haines. 2015. Supplementing fresh bovine colostrum with gut-active carbohydrates reduces passive transfer of immunoglobulin G in Holstein dairy calves. *J. Dairy Sci.* 98:6415–6422.
- CCAC. 1993. Guide to the care and use of experimental animals. 2nd ed. Vol. 1. Canadian Council on Animal Care, Ottawa, ON, Canada.
- Engfer, M. B., B. Stahl, B. Finke, G. Sawatzki, and H. Daniel. 2000. Human milk oligosaccharides are resistant to enzymatic hydrolysis

- in the upper gastrointestinal tract. *Am. J. Clin. Nutr.* 71:1589–1596.
- Firon, N., S. Ashkenazi, D. Mirelman, I. Ofek, and N. Sharon. 1987. Aromatic alpha-glycosides of mannose are powerful inhibitors of the adherence of type 1 fimbriated *Escherichia coli* to yeast and intestinal epithelial cells. *Infect. Immun.* 55:472–476.
- Fong, B., K. Ma, and P. McJarrow. 2011. Quantification of bovine milk oligosaccharides using liquid chromatography–selected reaction monitoring–mass spectrometry. *J. Agric. Food Chem.* 59:9788–9795.
- Gill, R. K., S. Mahmood, and J. P. Nagpaul. 1999. Functional role of sialic acid in IgG binding to microvillus membranes in neonatal rat intestine. *Biol. Neonate* 76:55–64.
- Jantscher-Krenn, E., C. Marx, and L. Bode. 2013. Human milk oligosaccharides are differentially metabolized in neonatal rats. *Br. J. Nutr.* 110:640–650.
- Kent-Dennis, C. 2014. Effects of heat-treatment of colostrum on the development of calves in the neonatal and pre-weaned periods. MS Thesis. University of Alberta, Edmonton, Alberta, Canada.
- Kobata, A. 1977. Milk glycoproteins and oligosaccharides. Page 423 in *The Glycoconjugates*. Vol. I. M. I. Horowitz and W. Pigman, ed. Academic Press, New York, NY.
- Malmuthuge, N., Y. Chen, G. Liang, L. A. Goonewardene, and L. L. Guan. 2015. Heat-treated colostrum feeding promotes beneficial bacteria colonization in the small intestine of neonatal calves. *J. Dairy Sci.* 98:8044–8053.
- Marcobal, A., M. Barboza, E. D. Sonnenburg, N. Pudlo, E. C. Martens, P. Desai, C. B. Lebrilla, B. C. Weimer, D. A. Mills, J. B. German, and J. L. Sonnenburg. 2011. *Bacteroides* in the infant gut consume milk oligosaccharides via mucus-utilization pathways. *Cell Host Microbe* 10:507–514.
- Martin, M. J., S. Martin-Sosa, and P. Hueso. 2002. Binding of milk oligosaccharides by several enterotoxigenic *Escherichia coli* strains isolated from calves. *Glycoconj. J.* 19:5–11.
- Martin-Sosa, S., M. J. Martin, L. A. Garcia-Pardo, and P. Hueso. 2003. Sialyloligosaccharides in human and bovine milk and in infant formulas: Variations with the progression of lactation. *J. Dairy Sci.* 86:52–59.
- Meganck, V., G. Hoflack, and G. Opsomer. 2014. Advances in prevention and therapy of neonatal dairy calf diarrhea: A systematical review with emphasis on colostrum management and fluid therapy. *Acta Vet. Scand.* 56:75.
- Nakamura, T., H. Kawase, K. Kimura, Y. Watanabe, and M. Ohtani. 2003. Concentrations of sialyloligosaccharides in bovine colostrum and milk during the prepartum and early lactation. *J. Dairy Sci.* 86:1315–1320.
- National Animal Health Monitoring System. 2011. Dairy Heifer Raiser, 2011. USDA-Anim. Plant Health Insp. Serv. Vet. Serv., Ft. Collins, CO.
- Neeser, J. R., M. Golliard, and S. D. Vedovo. 1991. Quantitative determination of complex carbohydrates in bovine milk and in milk-based infant formulas. *J. Dairy Sci.* 74:2860–2871.
- Short, D. M., D. A. Moore, and W. M. Sisco. 2016. A randomized clinical trial evaluating the effects of oligosaccharides on transfer of passive immunity in neonatal dairy calves. *J. Vet. Intern. Med.* 30:1381–1389.
- Tao, N., E. J. DePeters, S. Freeman, J. B. German, R. Grimm, and C. B. Lebrilla. 2008. Bovine milk glycome. *J. Dairy Sci.* 91:3768–3778.
- Vasseur, E., F. Borderas, R. I. Cue, D. Lefebvre, D. Pellerin, J. Rushen, K. M. Wade, and A. M. de Passille. 2010. A survey of dairy calf management practices in Canada that affect animal welfare. *J. Dairy Sci.* 93:1307–1315.
- Villetaz Robichaud, M., S. M. Godden, D. M. Haines, D. B. Haley, D. L. Pearl, J. Rushen, and S. LeBlanc. 2014. Addition of gut active carbohydrates to colostrum replacer does not improve passive transfer of immunoglobulin G in Holstein dairy calves. *J. Dairy Sci.* 97:5700–5708.
- Weaver, D. M., J. W. Tyler, D. C. VanMetrem, D. E. Hostetler, and G. M. Barrington. 2000. Passive transfer of colostral immunoglobulins in calves. *J. Vet. Intern. Med.* 14:569–577.
- Yu, Z.-T., C. Chen, and D. S. Newburg. 2013. Utilization of major fucosylated and sialylated human milk oligosaccharides by isolated human gut microbes. *Glycobiology* 23:1281–1292.