J. Dairy Sci. 100:3883–3892
https://doi.org/10.3168/jds.2016-12388

Comparative composition, diversity, and abundance of oligosaccharides in early lactation milk from commercial dairy and beef cows

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ABSTRACT

Prebiotics are nondigestible dietary ingredients, usually oligosaccharides (OS), that provide a health benefit to the host by directly modulating the gut microbiota. Although there is some information describing OS content in dairy-source milk, no information is available to describe the OS content of beef-source milk. Given the different trait emphasis between dairy and beef for milk production and calf survivability, it is plausible that OS composition, diversity, and abundance differ between production types. The goal of this study was to compare OS in milk from commercial dairy and beef cows in early lactation. Early-lactation multiparous cows (5–12 d in milk) from 5 commercial Holstein dairy herds and 5 Angus or Angus hybrid beef herds were sampled once. Milk was obtained from each enrolled cow and frozen on the farm. Subsequently, each milk sample was assessed for total solids, pH, and OS content and relative abundance. Oligosaccharide diversity and abundance within and between samples was transformed through principal component analysis to reduce data complexity. Factors from principal component analysis were used to create similarity clusters, which were subsequently used in a multivariate logistic regression. In total, 30 OS were identified in early-lactation cow milk, including 21 distinct OS and 9 isomers with unique retention times. The majority of OS detected in the milk samples were present in all individual samples regardless of production type. Two clusters described distribution patterns of OS for the study sample; when median OS abundance was compared between the 2 clusters, we found that overall OS relative abundance was consistently greater in the cluster dominated by beef cows. For several of the structures, including those with known prebiotic effect, the difference in abundance was 2- to 4-fold greater in the beef-dominated cluster. Assuming that beef OS content in milk is the gold standard for cattle, it is likely that preweaning dairy calves are deprived of dietary-source OS. Although supplementing rations with OS is an approach to rectify this deficiency, understanding the health and productivity effects of improving OS abundance being fed to preweaning calves is a necessary next step before recommending supplementation. These studies should account for the observation that OS products are variable for both OS diversity and structural complexity, and some products may not be suitable as prebiotics.
Key words: oligosaccharides, dairy, beef, milk, prebiotics

INTRODUCTION

Diarrhea is a significant cause of morbidity and mortality in dairy calves (USDA, 2010, 2012), and there is considerable interest in approaches to reduce this disease. One idea is to affect the intestinal microbiota via the use of prebiotics to support and improve gut health (Barile and Rastall, 2013). Prebiotics are nondigestible dietary ingredients, usually oligosaccharides (**OS**), that provide a health benefit to the host by modulating the gut microbiota (Gibson et al., 2010; Barile and Rastall, 2013; Rastall and Gibson, 2015).

Studies of human infant intestinal microbiota have reported that infants exclusively fed breast milk develop a different bacterial profile from that of infants receiving formula milk (Harmsen et al., 2000; Jost et al., 2012; Azad et al., 2013). The difference is the relative dominance of anaerobic bacteria, with *Bifidobacterium* spp. being dominant in breast-fed infants and *Bifidobacterium* spp. sharing dominance with *Bacteroides* spp. in formula-fed infants (Harmsen et al., 2000). The dominance of putative health-beneficial bacteria such as *Bifidobacterium* spp. in the infant microbiome is driven by their ability to metabolize a variety of OS structures found in mammalian milk (Jost et al., 2012; Ruiz-Moyano et al., 2013). Humans lack enzymes to

Received December 1, 2016.
Accepted January 26, 2017.
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digest OS; consequently, these molecules pass to the hindgut where they promote growth of *Bifidobacterium* spp. that metabolize OS into short-chain fatty acids that are utilized by the host. Oligosaccharides in human milk are produced in the mammary gland, where 5 types of monosaccharides—glucose and galactose (hexose, **Hex**), *N*-acetylglucosamine (**GlcNAc**), fucose (**Fuc**), and sialic acid (*N*-acetylneuraminic acid, **NeuAc**)—are added to a lactose core by action of specific glycosyltransferases. The 5 monosaccharides that comprise OS are attached in various ways through at least 12 possible linkages, resulting in many possible structural combinations (Smilowitz et al., 2014).

Significant analytical efforts have generated a human milk OS library with over 200 entries and 100 fully elucidated structures (Wu et al., 2011, 2010). In contrast, information about OS in bovine colostrum is still developing, although over 40 OS structures have been described (Tao et al., 2008; Barile et al., 2010; Mariño et al., 2011). Recent studies have identified 13 OS in bovine milk that overlap with OS structures found in human milk, including several fucosylated OS (Aldredge et al., 2013; Albrecht et al., 2014). The structural complexity of OS is a key factor determining their selective prebiotic activity. In particular, the monosaccharide sialic acid is crucial to the ability of OS to enrich beneficial bacteria while being less than ideal substrates for undesirable and pathogenic bacteria (Sela et al., 2011; Lane et al., 2012; Pacheco et al., 2015). Based on the high structural homology of several bovine acidic and neutral OS with human milk OS molecules, we predict that a similar activity will be demonstrated in bovine milk. All of the work describing OS in bovine milk is focused on dairy cattle and relatively few animals are included in these studies (Tao et al., 2008; Barile et al., 2010). Bovine milk has a lower abundance of OS compared with bovine colostrum and several structures remain to be elucidated (Tao et al., 2008). Oligosaccharides in animal milk also contain *N*-acetylgalactosamine (GalNAc) besides GlcNAc; therefore, the monosaccharide is referred to as *N*-acetylhexosamine (HexNAc), which is comprehensive of both the galactose and glucose modified form. Additionally, animal milk contain a second form of sialic acid, known as *N*-gylcolylneuraminic acid (NeuGc). Similar to what is observed for human milk (Niñonuevo et al., 2008), OS abundance and structure are heterogeneous between dairy animals and breeds (Tao et al., 2009) and change over the course of lactation (Barile et al., 2010; Sundekilde et al., 2012). No information is available about the OS content of beef cow milk. Given the different trait emphasis between dairy and beef for milk production and calf survivability, it is plausible that OS composition, diversity, and abundance differ between production types. The goal of this study was to compare OS in milk from commercial dairy and beef cows in early lactation. Our hypothesis was that early-lactation beef cows will have a more abundant and diverse OS population compared with dairy cows in early lactation.

MATERIALS AND METHODS

Herd Selection

Five commercial Holstein dairy herds and 5 Angus or Angus hybrid beef herds were recruited as a convenience sample. The herds were all from Washington State and enrolled in the study between January and April 2014.

Animal Enrollment

From each enrolled herd, 5 to 8 multiparous cows between 5 and 12 d postcalving were identified and sampled with the help of on-farm personnel. Cows with overt clinical evidence of disease, history of recent antibiotic treatments, or reported with dystocia were excluded from the study. All experimental procedures involving cows were approved by the Washington State University, Office of Research, Institutional Animal Care and Use Committee (04497-002).

Demographic Data and Biological Sample Collection

The identification of each enrolled cow was collected along with demographic data including age or parity, production type (beef or dairy), body condition, and calving information. Body condition scoring for dairy cows was based on a score between 1 and 5 (Ferguson et al., 1994), and that for beef cows was based on a score between 1 and 9 (http://beef.unl.edu/learning/condition1b.shtml). For most beef herds, age was estimated by herd owner. Information describing herd-level feeds was collected for each farm.

From each cow, a 10- to 20-mL composite milk sample was aseptically collected. Before the sample was collected, the cow's teat ends were cleaned and disinfected. Then, after discarding any milk in the teat canals and 1 to 2 mL of cisternal milk, approximately 4 mL of milk was collected into a sterile screw-cap tube from each quarter and mixed to create a single composite sample per cow. From this sample, a 4-mL aliquot was immediately transferred to another tube. The larger volume sample was directly placed in a container with dry ice for transport to the laboratory. The smaller sample was used for an evaluation of pH and total solids. Once milk