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Symposium review: Dairy-derived oligosaccharides—Their influence on host–microbe interactions in the gastrointestinal tract of infants*

Erinn M. Quinn,[1,2] Lokesh Joshi,[2] and Rita M. Hickey[1]†
[1]Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork P61 C996, Ireland
[2]Advanced Glycoscience Research Cluster, National Centre for Biomedical Engineering Science, National University of Ireland Galway, Galway H91 TK33, Ireland

ABSTRACT

Oligosaccharides are the third most abundant component in human milk. It is widely accepted that they play several important protective, physiological, and biological roles, including selective growth stimulation of beneficial gut microbiota, inhibition of pathogen adhesion, and immune modulation. However, until recently, very few commercial products on the market have capitalized on these functions. This is mainly because the quantities of human milk oligosaccharides required for clinical trials have been unavailable. Recently, clinical studies have tested the potential beneficial effects of feeding infants formula containing 2′-fucosyllactose, which is the most abundant oligosaccharide in human milk. These studies have opened this field for further well-designed studies, which are required to fully understand the role of human milk oligosaccharides. However, one of the most striking features of human milk is its diversity of oligosaccharides, with over 200 identified to date. It may be that a mixture of oligosaccharides is even more beneficial to infants than a single structure. For this reason, the milk of domestic animals has become a focal point in recent years as an alternative source of complex oligosaccharides with associated biological activity. This review will focus specifically on free oligosaccharides found in bovine and caprine milk and the biological roles associated with such structures. These dairy streams are ideal sources of oligosaccharides, given their wide availability and use in so many regularly consumed dairy products. The aim of this review was to provide an overview of research into the functional role of bovine and caprine milk oligosaccharides in host–microbial interactions in the gut and provide current knowledge related to the isolation of oligosaccharides as ingredients for incorporation in functional or medical foods.

Key words: animal milk, oligosaccharides, gut health, host–microbe interactions

INTRODUCTION

In recent times, awareness has been growing that breast milk beneficially supports the infant immune system (Cacho and Lawrence, 2017) and modulates microbiota composition during the first months of life. Among the various bioactive components in breast milk, human milk oligosaccharides (**HMO**) are suggested to play a leading role (Ayechu-Muruzabal et al., 2018; Xiao et al., 2019). Human milk oligosaccharides are made of linear or branched monosaccharides, such as galactose, glucose, *N*-acetylglucosamine, fucose, and sialic acid, varying in size from 3 to 22 monosaccharide units (German et al., 2008; Bode, 2012). In contrast to the milk of other mammals, human breast milk contains a very high amount and a structurally diverse set of oligosaccharides that even exceeds the protein content of breast milk (Zivkovic et al., 2011; Bode, 2012; Triantis et al., 2018). Indeed, HMO constitute the third largest solid component of human milk after lactose and lipids (Kunz et al., 2000; Thurl et al., 2010). Table 1 outlines the overall concentrations of oligosaccharides found in human and dairy animal milks and highlights the major oligosaccharide structures common between these milks.

Surprisingly, HMO offer no direct nutritional value to infants and involve only minor absorption across the intestinal wall, with approximately 0.1 and 4% detectable in plasma and urine, respectively (Goehring et al., 2014; Dotz et al., 2015; Underwood et al., 2015). They are now recognized to have various additional benefits for the developing infant. They are favored substrates for several species of gut bacteria and act as prebiotics, promoting the growth of a beneficial microflora

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†Corresponding author: rita.hickey@teagasc.ie

Table 1. Milk oligosaccharides found in human milk and dairy milks[1]

Oligosaccharide	Human, g/L	Bovine, g/L	Caprine, g/L
Total in colostrum milk	20–23	1.0	0.4–1.35
Total in mature milk	12–15	0.03–0.06	0.25–0.3
2′-Fucosyllactose (2′-FL)	1.88–4.9	Trace	Trace
3′-Fucosyllactose (3′-FL)	0.25–0.86	Trace	Trace
Lacto-*N*-tetraose (LNT)	0.5–1.5	Trace	Trace
Lacto-*N*-neotetraose (LNnT)	0.04–0.2	Trace	—
Lacto-*N*-fucopentaose I (LNFPI)	1.2–1.7	—	—
Lacto-*N*-fucopentaose II (LNFPII)	0.3–1.0	—	—
Lacto-*N*-fucopentaose III (LNFPIII)	0.01–0.2	—	Trace
3′-Sialyllactose (3′-SL)	0.1–0.3	0.035–0.119	0.03–0.05
6′-Sialyllactose (6′-SL)	0.3–0.5	0.014–0.088	0.05–0.07
Sialyllacto-*N*-tetraose (a) (LSTa)	0.03–0.2	Trace	—
Sialyllacto-*N*-tetraose (b) (LSTb)	0.01–0.16	—	—
Sialyllacto-*N*-tetraose (c) (LSTc)	0.1–0.6	Trace	—
6′-Sialyllactosamine (6′ SLN)	—	0.009–0.176	Trace
Disialyllactose (DSL)	—	0.002–0.07	0.001–0.005
Disialyllactose-*N*-tetraose (DSLNT)	0.2–0.6	Trace	—
α-3′-Galactosyllactose (α3′-GL)	—	Trace	0.03–0.05
β-3′-Galactosyllactose (β3′-GL)	Trace	Trace	0.03
β-4′-Galactosyllactose (4′-GL)	Trace	—	—
β-6′-Galactosyllactose (6′-GL)	0.002	Trace	Trace
α-3′-*N*-acetylgalactosaminyllactose (α-3′-GalNAcL)	—	0.003–0.065	Trace
Lacto-*N*-difucohexaose I (LNDFH-I)	0.58	—	—
Lacto-*N*-neohexaose (LNnH)	Trace	—	—
Lacto-*N*-hexaose (LNH)	0.13	—	0.001–0.005
6′-*N*-Acetyl-glucosaminyl-lactose (NAL)	—	Trace	0.02–0.04

[1]The ranges shown reflect differences due to variations in the analytical methods used in the different studies, and they reflect changes in abundance over lactation (i.e., from colostrum to mature milk). Compiled data are from Gopal and Gill (2000); Kunz et al. (2000); Wang et al. (2001); Nakamura et al. (2003); Nakamura and Urashima (2004); Uemura et al. (2009); Fong et al. (2011); Oliveira et al. (2012); Aldredge et al. (2013); Meyrand et al. (2013); Albrecht et al. (2014); Oliveira et al. (2015); Austin et al. (2016); Sprenger et al., (2017); Ma et al. (2018); Samuel et al., (2019); Sousa et al. (2019b); Tonon et al. (2019).

and shaping the gut microbiome, in turn beneficially influencing immune responses (Doherty et al., 2018). Fermentation products, such as short-chain fatty acids generated from gut bacteria breaking down HMO, are immunoregulating and serve as nutrients for beneficial gut commensals and epithelial cells (Trompette et al., 2014). Human milk oligosaccharides also directly modulate host epithelial responses, resulting in reduced binding of pathogens to the gut epithelium. In addition, HMO act as decoy receptors, inhibiting the binding of enteric pathogens to prevent infection and subsequent illness (Smilowitz et al., 2014). Furthermore, HMO provide a selective advantage for colonization by favorable bacteria, in turn inhibiting the colonization of pathogenic species. Human milk oligosaccharides and their metabolic products, such as sialic acid, also play a role in brain development, neural transmission, and synaptogenesis (Bienenstock et al., 2013; Jacobi et al., 2016).

However, the protective effects ascribed to HMO are for the most part unavailable to formula-fed infants, with the exception of 2′-fucosyllactose and lacto-*N*-neotetraose, which have been added to some formulas recently (European Union, 2017; Puccio et al., 2017). Despite this advancement, the complexity of HMO makes it almost impossible for their associated functions to be duplicated in formulas. Infant milk formulas are based on bovine milk and to a lesser extent caprine milk, which contain lower concentrations of oligosaccharides (~0.03 and 0.30 g/L, respectively; Kunz et al., 2000; Martinez-Ferez et al., 2006a). However, at least 10 bovine milk oligosaccharides (**BMO**) and 9 caprine milk oligosaccharides (**CMO**) share the same structure as certain HMO (outlined in Table 1), which could imply common functionalities (Barile et al., 2009; Silanikove et al., 2010; Mariño et al., 2011; Robinson, 2019). Therefore, value may lie in extracting and concentrating oligosaccharides from domestic animal milks with a view to adding them as an active ingredient to infant formulas. This review highlights recent studies from our group and others that demonstrate novel bioactivities associated with BMO and CMO, with a particular focus on their influence on host–microbial interactions in the gut. The review also provides an overview of current developments in industrial-scale processes for milk oligosaccharide production from dairy streams.

MILK OLIGOSACCHARIDE STRUCTURE

Milk oligosaccharides have a lactose core and are enzymatically elongated by β1–3 and β1–6 linkages to units of lacto-*N*-biose and *N*-acetylactosamine, respectively. Milk oligosaccharides are further elongated by the addition of fucose and sialic acid at the terminal positions (Figueroa-Lozano and de Vos, 2019). A large number (100 to 200) of different HMO can be found in individual milk samples (Totten et al., 2012; Urashima et al., 2018). Concentrations in mature human milk ranges from 12 to 15 g/L, but relative proportions and amounts vary depending on the lactating stage, Lewis blood group, secretor status, feed practices, and environmental factors (Bode, 2012; Azad et al., 2018; Zúñiga et al., 2018; Seppo et al., 2019). Among these elements, the individual maternal genetic disposition has a large effect on the HMO profile of human milk, particularly the individual expression pattern of Lewis and secretor gene alleles that encode different fucosyltransferases.

The significant difference between human and animal milk oligosaccharide pools is that human milk contains high levels of fucosylated oligosaccharides, accounting for approximately 70% of oligosaccharides in human milk, with high levels of 2′-fucosyllactose detected (e.g., 4.65 g/L; Chaturvedi et al., 2001; Asakuma et al., 2008; Marriage et al., 2015). Another difference between the milks is the predominance of type 1 oligosaccharides in human milk (Urashima et al., 2012, 2013, 2016). In contrast, animal milk counterparts contain higher levels of sialylated oligosaccharides containing *N*-acetylneuraminic acid or *N*-glycolylneuraminic acid (Urashima et al., 2001, 2013). Added to this, only about 40 BMO and CMO structures have been identified to date (Tao et al., 2008; Albrecht et al., 2014). However, despite these differences, structurally identical oligosaccharides are found in human and animal milk oligosaccharide pools (Table 1). Indeed, many of these individual structures have been identified as having specific biological activities, so it is likely these structures may offer the same physiological benefits as oligosaccharides derived from human milk (Vicaretti et al., 2018).

THE ROLE OF MILK OLIGOSACCHARIDES IN HOST–MICROBE INTERACTIONS

The diverse functions of milk oligosaccharides on the gut microbiome are as follows: they act as prebiotics and stimulate the growth of beneficial microbes; they promote the colonization of beneficial microbes; they exert direct and indirect defense mechanisms against pathogens and protect infants from infections; and they act as signaling molecules and interact directly with host cells (Figure 1). For insights into how milk oligosaccharides affect immune system development, which is outside the scope of this article, we refer the reader to some excellent recent reviews (Plaza-Díaz et al., 2018; Triantis et al., 2018).

Role of Milk Oligosaccharides as Prebiotics

The human gut lacks glycoside hydrolases and intestinal membrane transporters, which can degrade milk oligosaccharides; therefore, HMO are not digested in the upper part of the gastrointestinal tract of infants (Engfer et al., 2000; German et al., 2008). As a result, the majority of HMO reach the colon, where they act as a substrate for specific bacteria, influencing the composition of the gastrointestinal microbiota (Garrido et al., 2011). Human milk oligosaccharides are specifically known to influence populations of beneficial bacteria, such as *Bifidobacterium* (Garrido et al., 2011; Akkerman et al., 2019), a dominant species in the intestine of breastfed infants. These bacteria have the ability to use HMO with dedicated enzymes (glycoside hydrolases), transporters, and other molecules that contribute to degradation (Goh and Klaenhammer, 2015). Genomic analysis of a prototypical infant-derived bifidobacteria, *Bifidobacterium longum* ssp. *infantis*, which grows well on HMO, revealed a single cluster of genes coding for enzymes dedicated to the degradation of HMO, suggesting co-evolution of this strain with human milk (Ward et al., 2006; Sela and Mills, 2010). Analysis of other infant strains of *Bifidobacterium longum* ssp. *longum, Bifidobacterium bifidum,* and *Bifidobacterium breve,* which also grow on HMO (LoCascio et al., 2009; Asakuma et al., 2011; Ruiz-Moyano et al., 2013), were shown to also possess specific milk glycan transporters and glycosyl hydrolases linked to milk glycan usage (Garrido et al., 2013; Ruiz-Moyano et al., 2013; Matsuki et al., 2016; Sakanaka et al., 2019; Zabel et al., 2019). The role of HMO as prebiotics is well characterized and we refer the reader to many excellent reviews on this topic (Marcobal and Sonnenburg, 2012; Moossavi et al., 2018; Reverri et al., 2018; Zúñiga et al., 2018).

The use of BMO as prebiotics is less well investigated; only a limited number of in vitro studies have been documented. A recent example is a study by Jakobsen et al. (2019), in which proton nuclear magnetic resonance metabolomics and molecular biology methods were combined to quantify bacteria and compare the effect of BMO and synthetic galacto-oligosaccharides on mono- and co-cultures of 8 major bacteria related to a healthy infant microbiome. The results revealed that BMO treatments supported the growth of *B. longum* ssp. *longum* and *Parabacteroides distasonis*, while at the same time inhibiting the growth of *Clostridium*

perfringens and *Escherichia coli*. Perdijk et al. (2019) investigated the effect of sialyllactose (isolated from bovine milk) on microbiota composition and short-chain fatty acid production using in vitro fecal batch cultures. Sialyllactose resulted in a distinct modulation of microbiota composition, promoting the outgrowth of *Bacteroides* and bifidobacteria, which resulted in distinct changes in short-chain fatty acid production profiles.

Caprine milk oligosaccharides have also been shown to have prebiotic characteristics in vitro (Oliveira et al., 2012). Increased numbers of *Bifidobacterium* spp. in the presence of CMO have been demonstrated using in vitro fermentation models (Thum et al., 2015; Barnett et al., 2018). A recent study by Leong et al. (2019) examined the presence of naturally occurring oligosaccharides in commercial caprine milk–based stage 1 and stage 2 infant formulas and their prebiotic properties. Fourteen quantifiable oligosaccharides in caprine milk–based infant formula were detectable by LC/MS. These oligosaccharides significantly enhanced the growth of bifidobacteria and lactobacilli in vitro.

The ability of BMO to modulate the gut microbiota in vivo has been the subject of several recent studies. Meli et al. (2014) revealed positive trends in stool bacterial counts in infants fed BMO-supplemented formulas. The BMO in this case were generated from whey permeate. In a randomized controlled double-blind clinical trial, Simeoni et al. (2016) tested the effect of feeding a formula supplemented with a mixture

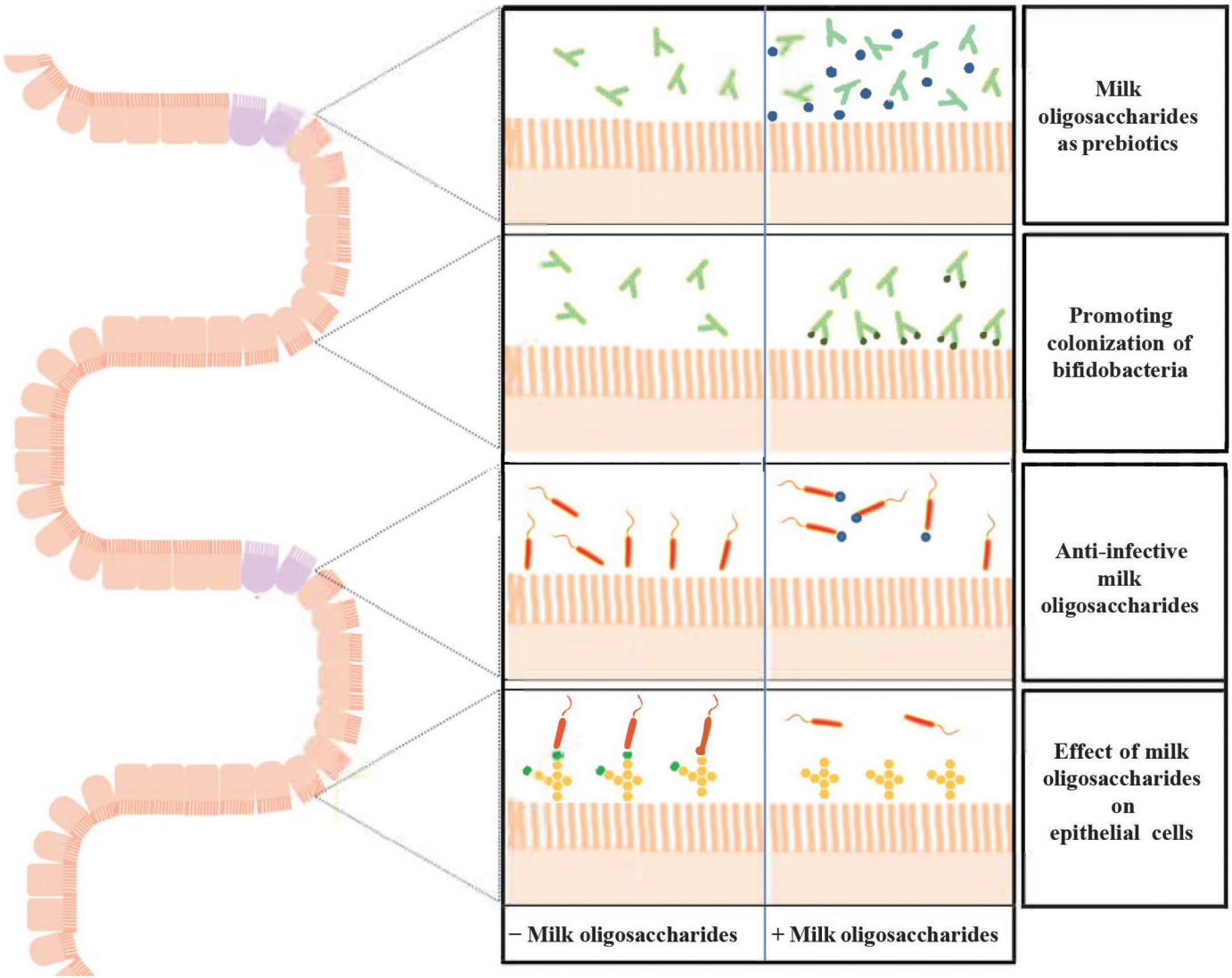

Figure 1. Schematic overview of the suggested mechanisms of action of milk oligosaccharides in the human intestine. Here, oligosaccharides may serve as prebiotics, promote the colonization of bifidobacteria, act as decoys for pathogens, and modify epithelial glycan receptor expression.

of BMO generated from whey permeate, containing galacto-oligosaccharides and 3′- and 6′-sialyllactose, and the probiotic *Bifidobacterium animalis* ssp. *lactis* strain CNCM I-3446. The same formula without addition of the prebiotic–probiotic blend was used in the control group. Breastfed infants served as the reference group. The test formula with the prebiotic–probiotic blend was well tolerated, and a strong bifidogenic effect was observed. The control group, but not the test group, differed from the breastfed reference group, showing a higher fecal pH and a significantly higher diversity in the fecal microbiota. In the test group, the probiotic *B. animalis* ssp. *lactis* increased by 100-fold in feces and was detected in all supplemented infants. The BMO stimulated a marked shift to a *Bifidobacterium*-dominated fecal microbiota via increases in endogenous bifidobacteria (e.g., *B. longum, B. breve*, *B. bifidum*, *Bifidobacterium pseudocatenulatum*; Simeoni et al., 2016).

In another study, germ-free mice and newborn piglets were colonized with a consortium of cultured bacterial strains isolated from the fecal microbiota of a severely stunted Malawian infant and fed a representative Malawian diet with or without the addition of sialylated BMO (Charbonneau et al., 2016). The results demonstrated that this BMO preparation produced a microbiota-dependent promotion of growth and metabolic changes indicative of improved nutrient use in both host species. A study by Boudry et al. (2017) investigated whether a combination of dietary BMO and *B. longum* ssp. *infantis* could reverse the gut microbial dysbiosis and altered gut permeability induced by ingestion of the Western diet. Male C57BL/6 mice were fed a Western diet (40% fat/kcal) or normal chow (14% fat/kcal) for 7 wk. During the final 2 wk of the study, the diet of a subgroup of mice fed the Western diet was supplemented with BMO (7% wt/wt). Supplementation with BMO normalized the cecal and colonic microbiota, and the authors observed an increased abundance of *Lactobacillus* compared with the mice fed the Western diet alone and the control (chow-fed) mice. In addition, restoration of *Allobaculum* and *Ruminococcus* levels in the BMO group was observed, which was comparable to that of the control group. A similar study by Hamilton et al. (2017) investigated C57BL/6 mice that were fed a control diet, a high-fat diet (40% fat/kcal), or a high-fat diet and 6%/kg BMO for 1, 3, or 6 wk. Gut microbiota and intestinal permeability were assessed in the ileum, cecum, and colon. The BMO completely abolished the increase in paracellular and transcellular permeability in the small and large intestine induced by the high-fat diet, and increased the abundance of *Bifidobacterium* and *Lactobacillus* in the ileum.

Using preterm piglets, Obelitz-Ryom et al. (2018) investigated the effects of bovine milk supplements enriched with oligosaccharides to improve gut development and colonization. Supplements with BMO were well tolerated, but did not improve gut maturation or clinical outcomes in artificially reared preterm piglets. The authors concluded that immaturity at birth, coupled with artificial rearing, may render the neonate unresponsive to the gut-protective effects of milk oligosaccharides. A study by Jena et al. (2018) examined the effects of BMO and *B. longum* ssp. *infantis* in preventing nonalcoholic steatohepatitis in bile acid receptor FXR (farnesoid × receptor) knockout mice fed a Western diet. The authors found that BMO increased the abundance of butyrate-generating bacteria, which has a beneficial effect in nonalcoholic steatohepatitis treatment. In a recent study, Cowardin et al. (2019) colonized germ-free mice with cultured bacterial strains from a 6-mo-old stunted infant and fed the mice a diet supplemented with bovine sialylated milk oligosaccharides. Although this study was focused on bone biology, the diet was associated with BMO-dependent and microbiota-dependent increases in cecal levels of succinate, increased numbers of small intestinal tuft cells, and evidence for the activation of a succinate-induced tuft cell signaling pathway linked to T helper (Th)2 immune responses. In contrast, similar studies on CMO are limited. Thum et al. (2016) found that consumption of CMO by mice during gestation and lactation improved the development of their pups, and the relative abundance of bifidobacteria and butyric acid in the colon at weaning. Overall, these studies highlight the potential of domestic animal milks as a source of oligosaccharides that can aid in shaping the enrichment of a protective intestinal microbiota.

Effect of Milk Oligosaccharides on the Colonization of Bifidobacteria

Bifidobacteria are associated with several health-related benefits for the host, including inhibiting the growth of pathogenic organisms, modulating mucosal barrier function, and promoting appropriate immunological and inflammatory responses (Gareau et al., 2010; Buffie and Pamer, 2013). To exert a beneficial effect, a sufficient population of bifidobacteria must colonize the host and initially adhere to intestinal cells (Westermann et al., 2016). Our group has recently found that oligosaccharides from domestic animal milks may contribute not only to the selective growth of bifidobacteria but also to their specific adhesive ability. Kavanaugh et al. (2013) showed that treatment of *B. longum* ssp. *infantis* ATCC 15697 with a mixture of 3′- and 6′-sialyllactose found in human and domestic animal milks substantially increased bacterial adhesion (up to 9.8-fold) to human HT-29 intestinal cells

in vitro. Moreover, transcriptomic analysis revealed that the increased adherence phenotype of the strain resulting from exposure to the oligosaccharide is likely multifaceted, involving transcription factors, chaperone proteins, adhesion-related proteins, and a glycoside hydrolase (Kavanaugh et al., 2013). More recently, our group found that CMO also resulted in increased adhesion (8.3-fold) of *B. longum* ssp. *infantis* to HT-29 intestinal cells when used at physiological concentrations (Quinn et al., 2018).

Bovine milk components may also modulate intestinal epithelial cell surface glycans, thereby reducing or increasing the prevalence of attachment sites for bacteria (Angeloni et al., 2005). In a recent study by our group, HT-29 intestinal cells were exposed to a bovine colostrum fraction rich in free oligosaccharides (Morrin et al., 2019a). The adherence of several commensal bacteria (comprising mainly bifidobacteria) to the intestinal cells was significantly enhanced (up to 52-fold) for all strains tested, which spanned species that are found across the human lifespan. Importantly, the changes to the HT-29 cell surface did not support enhanced adhesion of the enteric pathogens tested. The gene expression profile of the HT-29 cells following treatment with the colostrum fraction was evaluated by microarray analysis (Morrin et al., 2019a). Many so-called glyco-genes (glycosyltransferases and genes involved in the complex biosynthetic pathways of glycans) were found to be differentially regulated, suggesting modulation of the enzymatic addition of sugars to glycoconjugate proteins. Changes in the glycosylation pattern of the intestinal cells upon exposure to the colostrum fraction were also demonstrated through the use of lectin arrays (Morrin et al., 2019b). These studies highlight the potential of bovine milk components as functional ingredients that could increase the attachment of health-promoting bacteria in the gut, which may be important to individuals with lower counts of such bacteria.

Protective Role of Milk Oligosaccharides Against Pathogens and Infection

Milk oligosaccharides are considered to be soluble receptor analogs of epithelial cell surface carbohydrates, because they are generated from similar enzymes (Kunz et al., 2000; Smilowitz et al., 2014). These structures display structural homology to host cell receptors and thus function as receptor decoys that pathogens can bind to instead of the host. Oligosaccharides can also inhibit pathogens by competitive binding with the host cell surface receptor (Morrow et al., 2005). An expansive literature exists describing the action of HMO against a variety of pathogens (Hickey, 2012; Li et al., 2014; Manthey et al., 2014; Laucirica et al., 2017), but information on the role of oligosaccharides from domestic animal milks in preventing infection is more limited. Examples exist of 3′ and 6′- sialyllactose being used as anti-infectives. Mysore et al. (1999) investigated the effect of 3′-sialyllactose on colonization of *Helicobacter pylori* in rhesus monkeys. Of the 6 monkeys given the milk oligosaccharide, 2 were cured permanently, 1 was transiently cleared, and 3 animals remained persistently colonized, suggesting that these oligosaccharides can cure or decrease *H. pylori* colonization in some cases. Martín et al. (2002) investigated whether BMO had the potential to protect against 7 enterotoxigenic *E. coli* strains isolated from diarrheic calves. Inhibition of hemagglutination in the presence of oligosaccharides was used as an indicator of interaction between the oligosaccharides and bacterial adhesins. Mid-lactation milk oligosaccharides, in particular, proved to be the most efficient at inhibiting hemagglutination.

Coppa et al. (2006) included a *Salmonella fyris* strain in their study on inhibition of pathogen adhesion to Caco-2 cells. The study showed that 6′-sialyllactose had an anti-adhesive effect on *S. fyris*. In a study by our group, we examined oligosaccharides isolated and purified from the colostrum of Holstein-Friesians for anti-infective activity against a highly invasive strain of *Campylobacter jejuni* (Lane et al., 2012). During our initial studies, we structurally defined 37 bovine colostrum oligosaccharides (**BCO**) in our sample by hydrophilic interaction liquid chromatography (**HILIC**)–HPLC coupled with exoglycosidase digests and offline mass spectroscopy (Mariño et al., 2011). We then examined the effect of the BCO on *C. jejuni* adhesion to, invasion of, and translocation of intestinal HT-29 cells. We found that the BCO dramatically reduced the cellular invasion and translocation of *C. jejuni*, in a concentration-dependent manner. Periodate treatment of the BCO before inhibition studies resulted in a loss of anti-infective activity of the oligosaccharides, suggesting a direct oligosaccharide–bacterial interaction. We found that 5 mg/mL BCO, which is similar to physiological concentrations of HMO in breastmilk, dramatically decreased the level of internalized bacteria by up to 50% compared with the control (Lane et al., 2012). Maldonado-Gomez et al. (2015) also demonstrated that BCO could prevent the adhesion of enteropathogenic *E. coli*, *Cronobacter sakazakii* and *Salmonella enterica* serovar Typhimurium to HEp-2 cell monolayers cultured in vitro (Maldonado-Gomez et al., 2015). Recently, neutral and acidic oligosaccharides isolated from caprine milk and bovine colostrum were compared for their potency to inhibit the adhesion of *S. enterica* IID604 to Caco-2 cells using HMO as a positive control (Urakami et al., 2018). Both the CMO and BCO inhibited the ad-

hesion of *S. enterica* to Caco-2 cells at concentrations ranging from 2.5 to 5.0 mg/mL. The ability of CMO to prevent the adhesion of *E. coli* NCTC 10418 and a *Salmonella* Typhimurium isolate to Caco-2 cells was also recently demonstrated (Leong et al., 2019). These studies build a strong case for exploring the possibility of using oligosaccharides sourced from dairy streams as potential anti-infectives.

Effect of Milk Oligosaccharides on Intestinal Epithelial Cells

Intestinal health and barrier function are considered to be the first line of defense in the gastrointestinal tract (Holscher et al., 2014). In this respect, HMO can modify host–microbe interactions by affecting epithelial cell turnover (Kuntz et al., 2008) and intestinal glycocalyx formation (Angeloni et al., 2005; Straubinger et al., 2010; Kong et al., 2019). The HMO are able to directly affect the intestinal cell response by reducing the cell growth and by inducing differentiation and apoptosis (Kuntz et al., 2008). They have also been reported to increase intestinal cell maturation (Holscher et al., 2017). Considering the consequences of pathogen invasion and the advantages of commensal colonization, it favors the gastrointestinal environment, and ultimately the host, to possess a means by which to rapidly adapt to possible threats while maintaining a hospitable environment for beneficial organisms. Some evidence shows that 3′-sialylactose, one of the main oligosaccharides of both human and domestic milks, may modulate intestinal epithelial cell surface glycans, thereby reducing or increasing the prevalence of attachment sites for bacteria (Angeloni et al., 2005). Exposure of 3′-sialylactose to Caco-2 cells altered the glycan profile of the cell surface. The expression of the sialyltransferases ST3Gal1, 2, and 4 were reduced, but ST3Gal3, 5, and 6 were unaffected by 3′-sialylactose. The subsequent reduction in α-2,3– and α-2,6–linked sialic acid coincided with reduced adhesion of enteropathogenic *E. coli* to the cells (Angeloni et al., 2005). These results suggest that 3′-sialyllactose modulates the intestinal cell surface glycome via differential regulation of genes associated with glycosylation in much the same way as described by our group (Morrin et al., 2019a,b). Interestingly, recent studies examining the effect of a CMO on barrier function of epithelial cell co-cultures found that the CMO at the maximum concentration tested (4.0 mg/mL) enhanced transepithelial electrical resistance, mucin gene expression, and mucin protein abundance in epithelial co-cultures, all of which are essential components of intestinal barrier function (Barnett et al., 2016, 2018). Collectively, these findings suggest that milk components can directly modulate the intestinal cell surface and potentially alter the glycosylation state of the cells, which in turn may facilitate the adherence of distinct communities of bacteria.

INDUSTRIAL-SCALE STRATEGIES TO PRODUCE DAIRY OLIGOSACCHARIDES

Considering the wide availability of dairy side streams from which oligosaccharides can be isolated, BMO and CMO show promise as future therapeutics that could be used to provide HMO-associated health benefits to infants and adults on a large scale. Whey, the liquid part of milk that separates from the curd during cheese production, is a particularly attractive source of oligosaccharides. This stream is rich in lactose and protein, but has a high biochemical and chemical oxygen demand, making it costly to dispose of within environmental regulations (Peters, 2005). Martinez-Ferez et al. (2006b) were among the first to describe the use of membrane technology for the isolation of oligosaccharides from animal milk, in this case pasteurized skimmed caprine milk. A 2-stage tangential filtration process was used, and at the end of the process, 80% of the oligosaccharides had been obtained in the final retentate. Oliveira et al. (2012) also used ultrafiltration to remove proteins and fat globules from caprine whey, and then further processed the ultrafiltered permeate using a 1-kDa "tight" ultrafiltration membrane. The final retentate was fractionated by preparative scale molecular size exclusion chromatography to yield 28 oligosaccharide-rich fractions.

Ultrafiltered whey permeate is disposed of at a cost to the whey processor or used to produce food-grade lactose by crystallization (Mehra et al., 2014). Milk oligosaccharides pass through the ultrafiltration membranes, ending up in the whey permeate (Barile et al., 2009; Mehra et al., 2014). The liquid that is separated from lactose crystals is known as mother liquor, and is usually disposed of in sewage plants or sold as animal feed. A study by Mehra et al. (2014) resulted in the concentration of BMO from mother liquor using membrane filtration technology. A combination of HPLC and accurate MS allowed the identification of optimal processing conditions, which resulted in the production of kilogram amounts of BMO-enriched powders. Among the BMO identified in the powder, 18 had high molecular weights and corresponded in size to the most abundant oligosaccharides present in human milk. Interestingly, 6 oligosaccharides contained fucose, which is rarely detected in bovine milk (Mehra et al., 2014).

More commonly, a combination of lactose hydrolysis and membrane filtration is used to isolate oligosaccharides from milk to increase purity. Recently, de Moura Bell et al. (2018) developed a novel pilot-scale approach

for the recovery of highly pure oligosaccharides, from colostral bovine whey permeate. Because the concentration of BMO in colostrum is much higher than in mature milk (Nakamura et al., 2003; Fong et al., 2011), it represents an available source from which to separate BMO on a large scale. The method described by de Moura Bell et al. (2018) relies on the integration of optimized processing conditions that favor maximum lactose hydrolysis and monosaccharide fermentation before concentrating the oligosaccharides by selective membrane filtration. Upon complete lactose hydrolysis and fermentation of the monosaccharides by yeast, nanofiltration of fermented whey permeate from colostrum enabled the recovery of 95% of the oligosaccharides at high purity (de Moura Bell et al., 2018). This processing strategy has also been applied to the recovery of CMO at a pilot scale, with a 75% recovery of oligosaccharides (Aquino et al., 2017), and recently further optimized in terms of enzymatic hydrolysis (Thum et al., 2019). Overall, processing conditions using temperatures $\leq$40°C and an enzyme concentration of $\leq$0.25% resulted in a higher preservation or formation of caprine whey oligosaccharides. Martín-Ortiz et al. (2019) were also successful in the selective removal of lactose, and the resulting glucose and galactose, from pooled caprine colostrum using a biotechnological procedure based on the combined use of β-galactosidase from *Kluyveromyces lactis* (optimal conditions: 0.68 U/mL enzyme, 37°C, 15 min, and pH 7) and yeast from *Saccharomyces cerevisiae* (optimal conditions: 37°C, 24 h).

Another recent study investigated the characterization and concentration of oligosaccharides naturally present in caprine cheese whey obtained from 2 types of caprine milk (Sousa et al., 2019a). The caprine cheese whey was processed by a 2-step cross-flow filtration process and a HILIC–UPLC coupled to high-definition mass spectrometry. A quadrupole time-of-flight (HILIC UPLC-HDMS-Q-TOF) method was used to identify and measure oligosaccharides in the samples. A final product with recovery of 63 to 96% of oligosaccharides was obtained compared with the original whey. Although membrane filtration is the most commonly investigated technique for producing dairy-derived oligosaccharides on a large scale, our group has had some recent success using scalable chromatography approaches to produce bovine oligosaccharides from whey streams (European Patent Application No. EP18214230.7), an area we continue to explore.

CONCLUSIONS

Because HMO are supplied through breastfeeding, their valuable effects are largely lost to formula-fed infants. Substitution of infant formula with dairy-derived oligosaccharides to impart HMO functions is a potential solution, in addition to the benefits already observed by supplementation of formulas with 2′-fucosyllactose. Oligosaccharides generated from bovine or caprine milk may have many applications in foods (e.g., in infant milk formula, infant foods, follow-on formula, beverages, and fermented milks). It may be that a mixture of oligosaccharides such as those generated from dairy streams may be even more beneficial to infants than individual oligosaccharides. A mixture could better represent the great variety of chemical structures found in human milk and as such allow more than one function. More studies are required to investigate the different in vivo actions of such ingredients on intestinal modulation and their technological implications when added to formula. Future clinical research should be directed at addressing the following questions: which specific milk oligosaccharides in the BMO and CMO pools confer beneficial activity, in what quantity are they required, for how long should they be administered, and can production on a large scale be cost-effective?

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ORCIDS

Erinn M. Quinn https://orcid.org/0000-0003-4118-8954
Lokesh Joshi https://orcid.org/0000-0002-3612-0747
Rita M. Hickey https://orcid.org/0000-0002-2128-2575