Chapter 7
Milk Oligosaccharides

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7.1 Abbreviations of Carbohydrate Structures

Oligosaccharide	Abbreviation	Structure
Lactose	Lac	Galβ1-4Glc
Galactose	Gal	Gal
Glucose	Glc	Glc
Fucose	Fuc	Fuc
N-Acetylgalactosamine	GalNAc	GalNAc
N-Acetylglucosamine	GlcNAc	GlcNAc
N-Acetylneuraminic acid	Neu5Ac	Neu5Ac
N-Glycolylneuraminic acid	Neu5Gc	Neu5Gc
Lacto-*N*-biose	LNB	Galβ1-3GlcNAc
Hexose	Hex	Hex
Hexosamine	HexNAc	HexNAc
Lacto-*N*-novopentaose 1	*novo*-LNP 1	Galβ1-3(Galβ1-4GlcNAcβ1-6)Galβ1-4Glc
Isoglobotriose	α3′-GL	Galα1-3Galβ1-4Glc
3′-Sialyllactosamine	3′-SLN	Neu5Acα2-3Galβ1-4GlcNAc
Lacto-*N*-hexaose	LNH	Galβ1-3GlcNAcβ1-3(Galβ1-4GlcNAcβ1-6) Galβ1-4Glc

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Oligosaccharide	Abbreviation	Structure
β6′-Galactosyllactose	6′-GL	Galβ1-6Galβ1-4Glc
β3′-Galactosyllactose	β3′-GL	Galβ1-3Galβ1-4Glc
N-Acetyllactosamine	LacNAc	Galβ1-4GlcNAc
N-Acetylgalactosaminyllactose	GNL	GalNAcα1-3Galβ1-4Glc
6′-Sialyllactosamine	6′-SLN	Neu5Acα2-6Galβ1-4GlcNAc
Lacto-*N*-fucopentaose I	LNFPI	Fucα1-2Galβ1-3GlcNAcβ1-3Galβ1-4Glc
Lacto-*N*-fucopentaose II	LNFPII	Galβ1-3(Fucα1-4)GlcNAcβ1-3Galβ1-4Glc
Lacto-*N*-fucopentaose III	LNFPIII	Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4Glc
Disialyllacto-*N*-tetraose	DSLNT	Neu5Acα2-3Galβ1-3(Neu5Acα2-6) GlcNAcβ1-3Galβ1-4Glc
Disialyllacto-*N*-hexaose I	DSLNH I	Neu5Acα2-3Galβ1-3GlcNAcβ1-3(Neu5Acα2-6Galβ1-4GlcNAcβ1-6)Galβ1-4Glc
Disialyllacto-*N*-hexaose II	DSLNH II	Neu5Acα2-3Galβ1-3(Neu5Acα2-6)GlcNAcβ1-3(Galβ1-4GlcNAcβ1-6)Galβ1-4Glc
Fucodisialyllacto-*N*-hexaose I	FDSLNH I	Neu5Acα2-3Galβ1-3(Neu5Acα2-6)GlcNAcβ1-3(Fucα1-2Galβ1-4GlcNAcβ1-6)Galβ1-4Glc
Fucodisialyllacto-*N*-hexaose II	FDSLNH II	Neu5Acα2-3Galβ1-3(Neu5Acα2-6)GlcNAcβ1-3[Galβ1-4(Fucα1-3)GlcNAcβ1-6]Galβ1-4Glc
Fucodisialyllacto-*N*-hexaose III	FDSLNH III	Neu5Acα2-3Galβ1-3(Fucα1-4)GlcNAcβ1-3(Neu5Acα2-6Galβ1-4GlcNAcβ1-6) Galβ1-4Glc
Disialyllacto-*N*-tetraose	DSLNT	Neu5Acα2-3Galβ1-3(Neu5Acα2-6) GlcNAcβ1-3Galβ1-4Glc
Sialyl-3′-galactosyllactose	S3′-GL	Neu5Acα2-3Galβ1-3Galβ1-4Glc
Disialyllactose	DSL	Neu5Acα2-8Neu5Acα2-3Galβ1-4Glc
Lacto-*N*-tetraose	LNT	Galβ1-3GlcNAcβ1-4Galβ1-4Glc
Lacto-*N*-neotetraose	LN*n*T	Galβ1-4GlcNAcβ1-3Galβ1-4Glc
Lacto-*N*-hexaose	LNH	Galβ1-3GlcNAcβ1-3(Galβ1-4GlcNAcβ1-6) Galβ1-4Glc
Lacto-*N*-neohexaose	LN*n*H	Galβ1-4GlcNAcβ1-3(Galβ1-4GlcNAcβ1-6) Galβ1-4Glc
2′-Fucosyllactose	2′-FL	Fucα1-2Galβ1-4Glc
3-Fucosyllactose	3-FL	Galβ1-4(Fucα1-3)Glc
3′-Sialyllactose	3′-SL	Neu5Acα2-3Galβ1-4Glc
6′-Sialyllactose	6′-SL	Neu5Acα2-6Galβ1-4Glc
Sialyllacto-*N*-tetraose a	LSTa	Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc
Sialyllacto-*N*-tetraose b	LSTb	Galβ1-3(Neu5Acα2-6)GlcNAcβ1-3Galβ1-4Glc
Sialyllacto-*N*-tetraose c	LSTc	Neu5Acα2-6Galβ1-4GlcNAcβ1-3Galβ1-4Glc
Lacto-*N*-difucohexaose I	LNDFH-I	Fucα1-2Galβ1-3(Fucα1-4) GlcNAcβ1-3Galβ1-4Glc
6′-*N*-Acetyl-glucosaminyl-lactose	NAL	GlcNAcβ1-6Galβ1-4Glc

7.2 Introduction

Milk contains from trace to ~13% carbohydrate, of which lactose (Galβ1-4Glc) usually constitutes more than 80%. The milk of most mammals also contains a variety of oligosaccharides, many of which have *N*-acetylgalactosamine, *N*-acetylglucosamine, galactose, glucose, fucose, and/or sialic acid (*N*-acetylneuraminic acid and *N*-glycolylneuraminic acid) residues attached to lactose, which is usually located at the reducing end (Urashima et al. 2013). Certain oligosaccharides in the milks of domestic animals including bovine milk contain Galβ1-4GlcNAc (*N*-acetyllactosamine) at the reducing ends. The ratio of milk oligosaccharides to free lactose in milk varies, depending on the mammalian species. For example, in mature human milk, milk oligosaccharides constitute 20% of the total carbohydrate content, whereas mature bovine and goat milk contain much lower amounts of oligosaccharides. The diversity of human milk oligosaccharides (HMOs) is large and major innovations in the field of glycomics have enabled the identification of over 200 HMO structures (Bode 2019; Urashima et al. 2018).

The biological functions of HMO have been extensively researched in recent years. According to many in vitro, in vivo, and clinical studies, the intake of HMO is associated with many benefits on the infant gastrointestinal and immune physiological systems. Other systems, such as the respiratory, central nervous, circulatory, locomotor, and urinary systems have also been found to be affected by HMO consumption. However, these protective effects ascribed to HMOs for the most part have been unavailable to formula-fed infants, with the exception of 2′-fucosyllactose (Fucα1-2Galβ1-4Glc, 2′-FL) and lacto-*N*-neotetraose (Galβ1-4GlcNAcβ1-3Galβ1-4Glc, LN*n*T), which have been added to some formulas recently (Puccio et al. 2017; European Union 2017). Despite this advancement, the complexity of HMOs makes it almost impossible for their associated functions to be duplicated in formulas. Infant milk formulas are based on bovine and goat milk, which as mentioned contain lower concentrations of oligosaccharides (~0.03 g L^{-1} and ~0.3 g L^{-1}, respectively) (Kunz et al. 2000; Martinez-Ferez et al. 2006; Meyrand et al. 2013). However, a number of bovine (BMOs) and goat milk oligosaccharides (GMOs) share the same structure as certain HMOs, which could imply common functionalities (Barile et al. 2009; Mariño et al. 2011; Robinson 2019). Moreover, Meli et al. (2014) have shown that BMO-supplemented infant formulas were well tolerated and supported normal growth of healthy term infants. 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.

In this chapter, we describe the biological significance of milk oligosaccharides, their gastrointestinal digestion and biosynthesis, their chemical structures, and methods for their structural analysis. In particular, we pay special attention to their prebiotic properties, their ability to prevent pathogen attachment to mucosal surfaces, thereby reducing infections, their involvement in improving gut barrier function, promoting immune development and tolerance, and modulating intestinal cell responses in addition, to providing the infant with a source of sialic acid, an essential nutrient in brain development and cognition. Current investigations into the production of milk oligosaccharides as functional ingredients will also be discussed.

7.3 The Chemical Structures of Milk Oligosaccharides

Oligosaccharides in milk are assembled in the mammary gland by combining the monosaccharides glucose (Glc), galactose (Gal), *N*-acetylglucosamine (GlcNAc), *N*-acetylgalactosamine (GalNAc), fucose (Fuc), and the sialic acids *N*-acetylneuraminic acid (Neu5Ac) and *N*-glycolylneuraminic acid (Neu5Gc). The oligosaccharide structures contain either lactose (Galβ1-4Glc) or *N*-acetyllactosamine (Galβ1-4GlcNAc, LacNAc) at their reducing end, with additional monosaccharide residues branching off from the non-reducing galactose. BMOs and GMOs can possess lacto-*N*-biose (Galβ1-3GlcNAc, LNB) or LacNAc units linked to the lactose core, which are defining features of the type 1 and type 2 oligosaccharide structures contained within many HMOs (Robinson 2019; Urashima et al. 2001). However, the type 1 oligosaccharides, which contain LNB,

Table 7.1 Quantities of neutral oligosaccharides found in human milk and dairy milks

Oligosaccharide (abbreviation)	Human (g/L)	Bovine (g/L)	Goat (g/L)
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 (LN*n*T)	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
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 (LN*n*H)	Trace	–	–
Lacto-*N*-hexaose (LNH)	0.13	–	0.001–0.005
6′-*N*-Acetyl-glucosaminyl-lactose (NAL)	–	Trace	0.02–0.04

2′-FL; Fucα1-2Galβ1-4Glc, 3-FL; Galβ1-4(Fucα1-3)Glc, LNT; Galβ1-3GlcNAcβ1-4Galβ1-4Glc, LN*n*T; Galβ1-4GlcNAcβ1-3Galβ1-4Glc, LNFP I; Fucα1-2Galβ1-3GlcNAcβ1-3Galβ1-4Glc, LNFP II; Galβ1-3(Fucα1-4)GlcNAcβ1-3Galβ1-4Glc, LNFP III; Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4Glc, α3′-GL (isoglobotriose); Galα1-3Galβ1-4Glc, β3′-GL; Galβ1-3Galβ1-4Glc, 4′-GL; Galβ1-4Galβ1-4Glc, 6′-GL; Galβ1-6Galβ1-4Glc, α3′-GalNAcL; GalNAcα1-3Galβ1-4Glc, LNDFH-I; Fucα1-2Galβ1-3(Fucα1-4)GlcNAcβ1-3Galβ1-4Glc, LN*n*H; Galβ1-4GlcNAcβ1-3(Galβ1-4GlcNAcβ1-3)Galβ1-4Glc, LNH; Galβ1-3GlcNAcβ1-3(Galβ1-4GlcNAcβ1-6)Galβ1-4Glc, NAL (*iso*-lacto-*N*-triose, *iso*-LNTri); GlcNAcβ1-6Galβ1-4Glc. The ranges shown reflect differences due to variations in the analytical methods used in the different studies and reflect changes in abundance over lactation, i.e., from colostrum to mature milk. Compiled data from: Kunz et al. (2000), Gopal and Gill (2000), Wang et al. (2001), Nakamura et al. (2003), Nakamura and Urashima (2004), Sumiyoshi et al. (2004), Fong et al. (2011), Oliveira et al. (2012), Meyrand et al. (2013), Aldredge et al. (2013), Albrecht et al. (2014), Oliveira et al. (2015), Austin et al. (2016), Sprenger et al. (2017), Thurl et al. (2017), Ma et al. (2018), Tonon et al. (2019), Samuel et al. (2019), Sousa et al. (2019), van Leeuwen et al. (2020)

predominate over the type 2 oligosaccharides, which contain LacNAc, in human milk, while the type 1 oligosaccharides are rare in the milk of domesticated dairy animals (Urashima et al. 2012, 2017, 2013). Another significant difference between human and dairy-derived 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′-fucosylactose (2′-FL) detected (e.g., 2.01–4.65 g L^{-1} in secretor donor milk) (Asakuma et al. 2008; Chaturvedi et al. 2001; Marriage et al. 2015). Dairy-derived milk also contains higher levels of sialylated oligosaccharides containing Neu5Ac or Neu5Gc unlike human milk which is dominated by neutral oligosaccharides (Urashima et al. 2001, 2013). The acidic oligosaccharides from the milk of cows contains mainly Neu5Ac (97%), while Neu5Gc contributes 64% and 94% of the total sialic acid content in those from the milk of goats and sheep, respectively (Albrecht et al. 2014). Neu5Gc-containing saccharides had not been observed in HMO, but recently, Quin et al. (2020) detected these types of HMO in low abundance. Since humans are not able to synthesize this sialic acid, the authors hypothesized that the Neu5Gc originated from the diet of the lactating women. Also, unlike HMO, BMOs and GMOs are known to contain α-linked Gal or GalNAc structures, sialyl derivatives of β3′-GL (Galβ1-3Galβ1-4Glc) or 6′-GL (Galβ1-6Galβ1-4Glc), disialyllactose (Neu5Acα2-8Neu5Acα2-3Galβ1-4Glc), and, less commonly, ganglio-type oligosaccharides. Added to this, only about 40-50 BMO and GMO structures have been identified to date (Albrecht et al. 2014; Tao et al. 2008). However, despite these differences, structurally identical oligosaccharides are found in human and dairy-derived milk oligosaccharide pools. The quantities, where known, of neutral and acidic BMOs and GMOs identified to date are presented in Tables 7.1 and 7.2, respectively.

Table 7.2 Quantities of acidic oligosaccharides found in human, bovine, and goat milks

Oligosaccharide (abbreviation)	Human (g/L)	Bovine (g/L)	Goat (g/L)
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′-Sialyl-lactosamine (6′SLN)	–	0.009–0.176	Trace
Disialyl-lactose (DSL)	–	0.002–0.07	0.001–0.005
Disialyllactose-*N*-tetraose (DSLNT)	0.2–0.6	Trace	–

3′-SL; Neu5Acα2-3Galβ1-4Glc, 6′-SL; Neu5Acα2-6Galβ1-4Glc, LST a; Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc, LST b; Galβ1-3(Neu5Acα2-6)GlcNAcβ1-3Galβ1-4Glc, LST c; Neu5Acα2-6Galβ1-4GlcNAcβ1-3Galβ1-4Glc, 6′SLN; Neu5Acα2-6Galβ1-4GlcNAc, DSL; Neu5Acα2-8Neu5Acα2-3Galβ1-4Glc, DSLNT; Neu5Acα2-3Galβ1-3(Neu5Acα2-6)GlcNAcβ1-3Galβ1-4Glc. The ranges shown reflect differences due to variations in the analytical methods used in the different studies and reflect changes in abundance over lactation, i.e., from colostrum to mature milk. Compiled data from: Kunz et al. (2000), Gopal and Gill (2000), Wang et al. (2001), Nakamura et al. (2003), Nakamura and Urashima (2004), Fong et al. (2011), Oliveira et al. (2012), Meyrand et al. (2013), Aldredge et al. (2013), Albrecht et al. (2014), Oliveira et al. (2015), Austin et al. (2016), Sprenger et al. (2017), Thurl et al. (2017), Ma et al. (2018), Tonon et al. (2019), Samuel et al. (2019), Sousa et al. (2019), van Leeuwen et al. (2020)

7.4 Biosynthesis of Milk Oligosaccharides

Despite the intense interest in HMO in recent decades, many details of HMO biosynthesis remain unclear. While the many possible monosaccharide addition events are known, the order of the biosynthetic steps and many of the enzymes involved are less well characterized. For example, the lactose core is extended by alternating actions of β-1,3-*N*-acetylglucosaminyltransferases (b3GnT) and β-1,4-galactosaminyltransferases (b4GalT) or β-1,3-galactosyltransferase (b3GalT) while β-galactoside sialyltransferases (SGalT) and α-1,2-fucosyltransferases (including the FUT2 "secretor" locus) are responsible for some sialylation and fucosylation of a terminal galactose, respectively (Kellman et al. 2020a). However, each enzymatic activity in HMO extension and branching can potentially be catalyzed by multiple isozymes in the respective gene family. Direct evidence of the specific isozymes performing each reaction in vivo is extremely limited. Kellman et al. (2020b) recently used a systems biology framework that integrated glycan and RNA expression data to construct an HMO biosynthetic network and predict the glycosyltransferases involved. To accomplish this, models were constructed describing the most likely pathways for the synthesis of the oligosaccharides accounting for >95% of the HMO content in human milk. Through these models, the authors proposed candidate genes for elongation, branching, fucosylation, and sialylation of HMOs. The study further explored selected enzyme activities through kinetic assay and their co-regulation through transcription factor analysis. This type of knowledge can provide insights for advancements in large-scale synthesis of HMOs as ingredients.

A number of recent studies have also shed more light on BMO synthesis. Wickramasinghe et al. (2011) examined the genes coding for enzymes involved in BMO metabolism by comparing the oligosaccharide profiles in 32 milk samples across lactation with the expression of glycosylation-related genes. Ninety-two glycosylation-related genes were found to be expressed in milk somatic cells. Recently, Liu et al. (2019) measured the abundance of 12 major BMO in the milk of 360 cows, which had high density single nucleotide polymorphism (SNP) marker genotypes. Most of the BMO were found to be highly heritable. A genome-wide association study (GWAS) allowed the group to fine-map several quantitative trait loci (QTL) and identify candidate genes with major effects on five of the BMO [3′-SL (Neu5Acα2-3Galβ1-4Glc), *N*-acetylgalactosaminyllactose (GNL), sialyl-3′-galactosyllactose (Neu5Acα2-3Galβ1-3Galβ1-4Glc, S3′-GL), lacto-*N*-novopentaose 1 (Galβ1-3(Galβ1-4GlcNAcβ1-6)Galβ1-4Glc, *novo*-LNP-I), and lacto-*N*-neotetraose (Galβ1-4GlcNAcβ1-3Galβ1-4Glc, LN*n*T)]. Among them, a putative causal mutation close to the *ABO* gene (encoding ABO blood group glycosyltransferases) on Chromosome 11 accounted for approximately 80% of genetic variance for two BMO, GNL and LN*n*T. This mutation lies very close to a variant associated with the expression levels of ABO. A third QTL mapped close to *ST3GAL6* (that codes for α-2-3-sialyltransferase) on Chromosome 1 explaining 33% of genetic variation of 3′-SL. The presence of major gene effects suggests that targeted marker-assisted selection could lead to a significant increase in the level of these BMO in milk.

In a similar study, also using GWAS, Poulsen et al. (2019) aimed to estimate genetic parameters in order to examine whether BMO in Danish Holstein and Danish Jersey milk are heritable. The group also aimed to identify underlying SNP markers affecting BMO variation in the dairy breeds. In total, 15 different BMO were monitored. The GWAS identified in total 1770 SNPs for five different BMO in Danish Holstein and 6913 SNPs for 11 BMO in Danish Jersey cows. In Danish Holstein cows, a major overlapping QTL was identified on BTA1 for lacto-*N*-hexaose (Galβ1-3GlcNAcβ1-3(Galβ1-4GlcNAcβ1-6)Galβ1-4Glc, LNH) and lacto-*N*-tetraose (Galβ1-3GlcNAcβ1-3Galβ1-4Glc, LNT) explaining 24% of the variation in these BMOs. The most significant SNPs were associated with *B3GNT5*, a gene encoding a glycosyltransferase involved in glycan synthesis. In Danish Jersey cows, a very strong QTL was detected for the BMO with composition 2 Hex 1 HexNAc on BTA11. The most significant SNP was assigned to *ABO*. This SNP has been reported to be a missense mutation and explains 56% of the BMO variation. Other candidate genes of interest identified for BMO synthesis were *ALG3*, *B3GALNT2*, *LOC520336*, *PIGV*, *MAN1C1*, *ST6GALNAC6*, *GLT6D1*, *GALNT14*, *GALNT17*, *COLGALT2*, *LFNG*, and *SIGLEC*.

A number of studies have also investigated the synthesis of oligosaccharides in goat milk. Crisà et al. (2016) sequenced and assembled the goat milk transcriptome at the colostrum stage and at 120 days of lactation. The analysis of 144 different oligosaccharide metabolism-related genes showed that most of these (64%) were more expressed in colostrum than in mature milk, with eight expressed at very high levels including the sugar transporters, *SLC2A3* and *SLC2A1*, a fucose synthesis gene, *GMDS*, a lactose synthesis gene, *NME2*, the galactosyltransferase, *B4GALT1*, the *N*-acetylglucosaminyl transferase, *B3GNT2*, a sialic acid synthesis gene, *NANS*, and the glycosidase, *HEXB*. More recently, this group (Crisà et al. 2019) identified the complete cDNAs of candidate genes implicated in sialylated oligosaccharide biosynthesis, namely β-1,4-galactosyltransferase 1 (*B4GALT1*), α-lactalbumin (*LALBA*) related to lactose synthesis (the precursor molecule for 3′-SL and 6′-SL biosynthesis), β-galactoside α-2,3-sialyltransferase 5 (*ST3GAL5*) related to 3′-SL biosynthesis, and β-galactoside α-2,6-sialyltransferase 1 (*ST6GAL1*) related to 6′-SL (Neu5Acα2-6Galβ1-4Glc) biosynthesis. The group then analyzed their expression during lactation in Garganica and Maltese goat breeds and measured the levels of 3′-SL, 6′-SL and disialyllactose (Neu5Acα2-3Galβ1-3(Neu5Acα2-6)GlcNAcβ1-3Galβ1-4Glc, DSL) in their milk to make correlations between expressed genes and phenotype. Gene expression analysis demonstrated that *LALBA* and *ST6GAL1* had the highest and lowest expression in both the breeds, respectively. The interaction effects of the breeds and sampling times were associated with higher levels of *B4GALT1* and *ST3GAL5* gene expression in Garganica when compared to the Maltese goats at kidding. *B4GALT1*, *LALBA*, and *ST3GAL5* gene expression changed from kidding to 60 and 120 days in Maltese goats, while in Garganica goats, a difference was observed only for the *LALBA* gene. Breed and lactation effects were also found to influence the sialylated oligosaccharide profile. Positive correlations of *B4GALT1*, *LALBA*, *ST3GAL5*, and *ST6GAL1* with 3′-SL/6′-SL and DSL were found. These types of studies provide information of specific candidate

Table 7.3 Functional properties associated with bovine milk oligosaccharides (BMOs)

Activity	Potential benefits to health	Reference
Prebiotic	Produced a microbiota-dependent promotion of growth and metabolic changes indicative of improved nutrient use in germ-free mice and new-born piglets which had been colonized with a consortium of cultured bacterial strains isolated from the fecal microbiota of a severely stunted Malawian infant	Charbonneau et al. (2016)
	Supplementation of infant formulas with *Bifidobacterium lactis* (CNCM I-3446) and BMOs induced a shift toward bifidobacteria-dominated stools, resembling those of breastfed infants	Radke et al. (2017), Simeoni et al. (2016)
	Supported the growth of *Bifidobacterium longum* ssp. *longum* and *Parabacteroides distasonis*, while at the same time inhibiting the growth of *Clostridium perfringens* and *Escherichia coli*	Jakobsen et al. (2019)
	Modulated microbiota composition and volatile fatty acids profiles in piglet neonatal model	Wang et al. (2021)
Anti-infective	Inhibition of *E. coli* hemagglutination	Martín et al. (2002)
	Inhibition of *Salmonella fyris* adhesion to Caco-2 cells	Coppa et al. (2006)
	Reduced the cellular invasion and translocation of *Campylobacter jejuni* in HT-29 cells, in a concentration-dependent manner	Lane et al. (2012)
	Adherence inhibition of enteric pathogens, such as *Escherichia coli*, *Cronobacter sakazakii*, and *Salmonella enterica* serovar Typhymurium	Maldonado-Gomez et al. (2015)
	Inhibited the adhesion of *Salmonella enterica* IID604 to Caco-2 cells	Urakami et al. (2018)
	Reduced *Streptococcus pneumoniae* adhesion to pharynx and lung cells in vitro when tested at physiological concentrations	Ryan et al. (2018)
	Reduced adhesion of *Staphylococcus aureus* to Caco-2 cells	Yue et al. (2020)
Barrier function	Modulated host–bacterial crosstalk, leading to enhanced epithelial barrier function, as measured by paracellular ion flux through transepithelial electrical resistance following *Clostridium difficile* toxin A challenge	Duncan et al. (2020)
Immunomodulation/ inflammation	Oligosaccharides from bovine colostrum influenced the expression of various cytokines, chemokines, and cell surface receptors in HT-29 cells	Lane et al. (2013)
	Effects of a high-fat diet such as liver abnormalities, steatosis, and inflammation can be eliminated via regulating lipid and glucose metabolism through the consumption of BMOs and *Bifidobacterium longum* subsp. *infantis* in genetically predisposed animal models	Jena et al. (2018)

(continued)

Table 7.3 (continued)

Activity	Potential benefits to health	Reference
Obesity and intestinal permeability	Significantly reduced weight gain and intestinal permeability that is induced in mice consuming a high-fat diet	Hamilton et al. (2017)
	In combination with a weekly gavage of the probiotic *Bifidobacterium longum* subsp. *infantis*, increases in intestinal permeability associated with the high-fat diet were prevented	Boudry et al. (2017)
Brain function	A whey preparation enriched in BMOs improved spatial cognition, with effects on hippocampal genes related to sialic acid metabolism, myelination, and ganglioside biosynthesis in preterm pigs	Obelitz-Ryom et al. (2019)
	BMOs (derived from bovine whey and composed primarily of galacto-oligosaccharides and trace amounts of 3′-SL and 6′-SL) were found to have distinct effects on brain structure and cognitive performance in pigs	Fleming et al. (2020)

Table 7.4 Functional properties associated with goat milk oligosaccharides (GMOs)

Activity	Potential benefits to health	Reference
Prebiotic	Utilization of oligosaccharides by bifidobacteria and *Bacteriodes* spp. and the capacity for short-chain fatty acid (SCFA) production	Oliveira et al. (2012)
	Increased numbers of *Bifidobacterium* spp. have been demonstrated using in vitro fermentation models	Thum et al. (2015), Barnett et al. (2018)
	Consumption of GMOs 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	Thum et al. (2016)
	Significantly enhanced the growth of bifidobacteria and lactobacilli in vitro	Leong et al. (2019)
Anti-infective	Inhibited the adhesion of *Salmonella enterica* IID604 to Caco-2 cells	Urakami et al. (2018)
	Inhibited the adhesion of *E. coli* NCTC 10418 and *Salmonella typhimurium* to Caco-2 cells	Leong et al. (2019)
	Reduced adhesion of *Staphylococcus aureus* to Caco-2 cells	Yue et al. (2020)
	In combination with *Bifidobacterium longum* subsp. *infantis* ATCC 15697, GMOs show synergism in vitro as anti-infectives against *Campylobacter jejuni*	Quinn et al. (2020b)
Effect on inflammation	Intestinal anti-inflammatory effect in a trinitrobenzenesulfonic acid-induced colitis in rats	Daddaoua et al. (2006)
	Reduction of intestinal inflammation in a rat model of dextran sodium sulfate-induced colitis	Lara-Villoslada et al. (2006)
Barrier function	Enhanced transepithelial electrical resistance, mucin gene expression, and mucin protein abundance in epithelial co-cultures	Barnett et al. (2016, 2018)

genes related to milk oligosaccharide synthesis and have the potential to guide breeding strategies to achieve production of milk with higher diversity and concentrations of oligosaccharides.

7.5 Gastrointestinal Digestion and Absorption of Milk Oligosaccharides

Milk oligosaccharides are typically considered indigestible by human gastrointestinal enzymes. Ingested HMOs can be found to be intact and non-metabolized in infant feces. Nonetheless, there are several reports of HMO existing in urine of exclusively breastfed infants (De Leoz et al. 2013; Dotz et al. 2014; Rudloff et al. 2012, 1996) as well as in preterm infants. Underwood et al. (2015) suggested that portions of HMO are absorbed into plasma. Moreover, experiments have detected the presence of some milk oligosaccharides, specifically sialylated oligosaccharides, in plasma from formula-fed and partially breastfed infants (Ruhaak et al. 2014), and direct evidence of HMO in the circulation of breastfed infants has been established (Goehring et al. 2014). Vazquez et al. (2017) administered a single oral dose of the HMOs, 2′-FL, 6′-SL and LN*n*T at different concentrations to adult rats. The time course of absorption of HMO into the bloodstream and their appearance in urine was studied. The results showed that rats, similar to human infants, effectively absorb a portion of HMO from the intestine into plasma and excrete them in urine. A specific kinetic absorption study with 2′-FL, was then performed in 9- to 11-day-old rat pups and confirmed that a significant amount of 2′-FL was absorbed into the systemic circulation and subsequently excreted in urine during lactation in rats in a dose-dependent manner. Basal levels of these HMO were found in the plasma and urine of adult rats as well as rat pups as a natural result of nursing. Hirschmugl et al. (2019) provided direct evidence of HMOs in cord blood and suggested that the placenta transfers HMOs from the maternal to the fetal circuit. In the study, the researchers analyzed HMO concentration and composition in cord blood in comparison to maternal serum HMOs at delivery in a small pregnancy/birth cohort. Maternal-to-fetal 2′-FL transfer across the human placenta, using an ex vivo placental perfusion approach was also investigated and after 180 min perfusion, 22% of maternally offered 2′-FL was found in the fetal circuit without reaching equilibrium.

These studies collectively confirm that, although most HMO are excreted in feces, a portion of HMO is absorbed into plasma and may modulate or contribute to systemic functions. The degree of absorption appears to vary substantially by structure, and the biological implications of this absorption have yet to be fully elucidated. It has been hypothesized, however, that absorbed oligosaccharides can prevent urinary tract infections in infants and that consumption of 3′-SL and 6′-SL increases brain ganglioside-bound sialic acid content, suggesting that these carbohydrates make an important contribution to brain development and immune

function (discussed below). Milk oligosaccharides that are not absorbed are available for consumption by the gut microbiota. Human milk has long been known to influence the development of the infant gut microbiota in ways that confer health benefits to the infant, and more recent studies have determined that the milk oligosaccharides are key to providing this prebiotic functionality (discussed below). The studies providing evidence that BMOs and GMOs can mimic specific properties associated with HMOs are summarized in Tables 7.3 and 7.4, respectively, and discussed in detail in the following sections.

7.6 Brain-Stimulating Activity by Milk Oligosaccharides

HMOs have been associated with increased delivery of sialic acid for the developing brain (Wang 2009). The concentration of sialic acid in saliva and brain tissue is known to be higher in breastfed versus formula-fed infants (Tram et al. 1997; Wang et al. 2003, 2001). Animal studies have investigated the functional brain effects of sialic acid using rats of varying age (Morgan et al. 1981; Morgan and Winick 1980; Oliveros et al. 2018; Sakai et al. 2006), mice (Kikusui et al. 2012), and full-term neonatal piglets (Wang et al. 2007). Sialic acid supplementation has been shown to increase the sialic acid concentration in brain gangliosides and improve cognition in term newborn pigs. BMOs consist of a high proportion of sialylated structures and may therefore confer similar effects (Wang et al. 2007; Jacobi et al. 2016; Obelitz-Ryom et al. 2019). Hobbs et al. (2021) recently reviewed the effects of sialylated milk oligosaccharides on the brain and gut health of newborns. 3′-SL and 6′-SL have been found to support normal microbial communities and behavioral responses in mice during stress by modulating the gut–brain axis (Tarr et al. 2015). Similarly, these oligosaccharides have been shown to increase ganglioside sialic acid concentrations in the corpus callosum and cerebellum and modulate the colonic microbiota of formula-fed piglets (Jacobi et al. 2016). Recently, Wang et al. (2019) provided in vivo evidence that milk 3′-SL, 6′-SL and 6′-sialyllactosamine (Neu5Acα2-6Galβ1-4GlcNAc, 6′-SLN) can alter many important brain metabolites and neurotransmitters required for optimizing neurodevelopment in piglets using in vivo magnetic resonance spectroscopic (MRS) approaches. Dietary sialyllactose (3′-SL or 6′-SL) was also shown to influence sialic acid concentrations in the prefrontal cortex and magnetic resonance imaging measures in the corpus callosum of young pigs (Mudd et al. 2017). The structural and functional neurodevelopmental outcomes in preterm pigs with or without supplementation of an oligosaccharide-enriched whey with sialyllactose during the first 19 days after preterm birth was also investigated (Obelitz-Ryom et al. 2019). The whey preparation improved spatial cognition, with effects on the expression of hippocampal genes related to sialic acid metabolism, myelination, and ganglioside biosynthesis in the preterm pigs.

Hauser et al. (2021) recently investigated the long-term consequences of a selective lactational deprivation of 6′-SL in knock-out mice. To test whether lactational 6′-SL deprivation affects cognitive capabilities in adulthood, the researchers

assessed attention, perseveration and memory. To detail the associated endophenotypes, they investigated hippocampal electrophysiology, plasma metabolomics, and gut microbiota composition. To investigate the underlying molecular mechanisms, gene expression (at eye-opening and in adulthood) in two brain regions mediating executive functions and memory (hippocampus and prefrontal cortex) was assessed. Compared to control mice, offspring deprived of 6′-SL during lactation exhibited consistent alterations in all cognitive functions addressed, hippocampal electrophysiology, and in pathways regulating the serotonergic system (identified through gut microbiota and plasma metabolomics). These alterations were associated with reduced expression of genes involved in central nervous system development. Moreover, the reduced expression was site- (prefrontal cortex) and time-specific (eye-opening). The data suggest that 6′-SL in maternal milk adjusts cognitive development through a short-term upregulation of genes modulating neuronal patterning in the prefrontal cortex.

Apart from sialylated oligosaccharides, other oligosaccharides such as LN*n*T and 2′-FL have also been implicated in enhancing cognition during development. Docq et al. (2020) recently summarized the reported observations regarding the effects of HMOs on memory and cognition in rats, mice, and piglets. The impact of both BMOs and HMOs on cognition, brain development, and hippocampal gene expression in pigs was also recently assessed. HMOs (LN*n*T and 2′-FL) and BMOs (derived from bovine whey and composed primarily of galacto-oligosaccharides and trace amounts of 3′-SL and 6′-SL) were found to have distinct effects on brain structure and cognitive performance (Fleming et al. 2020). Pigs were tested on the novel object recognition task using delays of 1 or 48 h at postnatal Day 22. At postnatal Day 32–33, magnetic resonance imaging procedures were used to assess structural brain development, and hippocampal tissue was collected for analysis of mRNA expression. Pigs consuming only HMOs exhibited recognition memory after a delay of 1 h, and those consuming BMOs and HMOs exhibited recognition memory after a delay of 48 h. Both absolute and relative volumes of cortical and subcortical brain regions were altered by varying oligosaccharides in the diet. Hippocampal mRNA expression of GABRB2, SLC1A7, CHRM3, and GLRA4 were most strongly affected. The authors concluded that the HMOs and BMOs had distinct effects on brain structure and cognitive performance. A recent paper associated levels of 2′-FL in human milk at 1 month with cognitive function at 24 months in human infants (Berger et al. 2020a).

7.7 Effects of Milk Oligosaccharides on the Gut Microbiota

The human gut lacks glycoside hydrolases, other than lactase, and intestinal membrane transporters which can degrade milk oligosaccharides; therefore, HMOs are not digested in the upper part of the gastrointestinal tract of infants. As a result, the majority of HMOs reach the colon, where they act as a substrate for specific

bacteria, influencing the composition of the gastrointestinal microbiota. HMOs are specifically known to influence populations of beneficial bacteria such as *Bifidobacterium* (Akkerman et al. 2019), a dominant genus in the intestine of breast-fed infants, thereby acting as prebiotics. Bifidobacteria are involved in modulating the immune system, inducing anti-inflammatory and antioxidant responses, producing antimicrobial substances, as well as competitively excluding pathogens. These bacteria have the ability to use HMOs with dedicated enzymes (glycoside hydrolases), transporters, and other molecules that contribute to degradation. Genomic analysis of a prototypical infant-derived bifidobacterial strain, *Bifidobacterium longum* subsp. *infantis*, which grows well on HMOs, revealed a cluster of genes coding for enzymes dedicated to the degradation of HMOs, named HMO-1 cluster, suggesting co-evolution of this strain with human milk (Sela and Mills 2010). *Bifidobacterium bifidum* can extracellularly release monosaccharides from type 2 HMO and lacto-*N*-biose from type 1 HMOs, allowing them to be utilized by other bifidobacterial species (Sakanaka et al. 2019). In addition, several strains of *Bifidobacterium breve* and *B. longum* subsp. *infantis* have metabolic pathways, specific for fucosyllactose (Matsuki et al. 2016; Sakanaka et al. 2019).

In order to elucidate the prebiotic molecular mechanism for degradation of HMOs, several bacteria have been tested for their ability to grow on individual or total HMOs as the sole carbon source in vitro. A vast literature on the ability of bifidobacteria to metabolize HMO exists, and these studies have been summarized in recent review articles (Cheng et al. 2020; Hundshammer and Minge 2020; Walsh et al. 2020a). Also, other individual strains such as *Bacteroides* (Yu et al. 2013), *Enterococcus* (Yu et al. 2013), *Akkermansia* (Kostopoulos et al. 2020), *Lactobacillus* (Yu et al. 2013), *Streptococcus*, and *Clostridium* cluster IV/XIVa (Pichler et al. 2020) have been shown in vitro to have the ability to utilize oligosaccharides. However, few longitudinal studies exist which investigate the establishment of the infant gut microbiota in relation to changes in breastmilk HMO composition. Borewicz et al. (2020) followed 24 mother–infant pairs to investigate the associations between concentrations of selected HMOs in breastmilk, infant feces, and the fecal microbiota composition in healthy, breastfed infants at 2, 6, and 12 weeks of age. Lactation duration was found to have a significant effect on the HMO content of breastmilk, which decreased with time, except for 3-fucosylactose (Galβ1-4(Fucα1-3)Glc, 3-FL) and lacto-*N*-fucopentaose III (Galβ1-4(Fucα1-3) GlcNAcβ1-3Galβ1-4Glc LNFP III). The group confirmed that microbiota composition was strongly influenced by infant age and was associated with the mode of delivery and concentration of LNFP III in breastmilk at 2 weeks, infant sex, delivery mode, and concentrations of 3′-SL in milk at 6 weeks, and infant sex and levels of lacto-*N*-hexaose (LNH) in milk at 12 weeks of age. Correlations between levels of individual breastmilk HMOs and relative abundance of operational taxonomic units (OTUs) found in infant feces, including the most predominant *Bifidobacterium* OTUs, were weak and varied with age. The fecal concentration of HMOs decreased with age and was strongly and negatively correlated with relative abundance of OTUs within genera *Bifidobacterium*, *Parabacteroides*, *Escherichia-Shigella*,

Bacteroides, *Actinomyces*, *Veillonella*, *Lachnospiraceae Incertae Sedis*, and *Erysipelotrichaceae Incertae Sedis*, indicating the likely importance of these taxa for HMO metabolism in vivo.

While the role of HMOs as prebiotics is well characterized, the use of BMOs as prebiotics is less well investigated, and only a limited number of in vitro studies have been documented (Jakobsen et al. 2020, 2019; Perdijk et al. 2019). The ability of BMOs to modulate the gut microbiota in vivo has been the subject of some recent studies (reviewed by Quinn et al. 2020a). A study by Charbonneau et al. (2016) used animal models (gnotobiotic mice and piglets) of infant undernutrition to show that dietary supplementation with BMOs provides a microbially mediated increase in lean body mass and bone growth and generates metabolite profiles indicative of improved nutrient utilization. BMOs, derived from demineralized whey permeate and also containing galactooligosaccharides (GOS), were proven to be beneficial in two clinical trials. The supplementation of infant formulas with *B. lactis* (CNCM I-3446) and BMOs induced a shift toward bifidobacteria-dominated stools, resembling those of breastfed infants (Radke et al. 2017; Simeoni et al. 2016). In order to discriminate the prebiotic effects of BMOs from the probiotic effects of *B. lactis* and synbiotic effects of their combination, all the three conditions were tested separately by Marsaux et al. (2020). BMOs alone significantly induced acetate and lactate production (leading to pH decrease) and stimulated bifidobacterial growth in ten donors. A further in-depth study on two different donors proved the ability of *B. lactis* to colonize the infant microbiota, regardless of the competitiveness of the environment. BMOs further enhanced this engraftment, suggesting a strong synbiotic effect. In another study by Jena et al. (2018), BMO supplementation was also found to significantly increase the expression of butyrate-generating bacterial genes in mice fed a western diet, which is of importance as butyrate can have anti-inflammatory effects in the liver and colon. A recent study by Wang et al. (2021) demonstrated that supplementation of BMOs and HMO (as described for the study of Fleming et al. 2020, above) were found to modulate the microbiota composition and volatile fatty acid profiles in a neonatal piglet model. HMOs alone did not affect overall microbial composition, but increased the relative proportion of specific taxa, including *Blautia*, compared to other groups. Abundance of *Bacteroides* was increased in the ascending colon by BMOs and synergistically by BMOs and HMOs in the feces. Similar in vivo studies on oligosaccharides from goat milk are limited. Thum et al. (2015) found that consumption of GMOs 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 suggest that HMOs and dairy oligosaccharides exert distinct actions on the gut microbiota and formula-fed infants could benefit from formula containing a variety of oligosaccharides.

7.8 Effects of Milk Oligosaccharides on Obesity

Feeding newborn infants human milk is known to temper weight gain compared to formula (Gillman et al. 2001; Kramer and Kakuma 2012) and is most beneficial in the first 6 months. Animal studies provide evidence that exposure to oligosaccharides may diminish weight gain, adiposity, and caloric intake (Hamilton et al. 2017). Alderete et al. (2015) conducted a small pilot study in non-Hispanic white mother–infant pairs ($n = 25$), which revealed that individual fucosylated and sialylated oligosaccharides were related to infant adiposity at 6 months. This was in line with more recent findings (Larsson et al. 2019) in a separate but similar cohort and sample size ($n = 30$). A subsequent study by Berger et al. (2020b) aimed to determine whether HMOs at 1 month predicted infant weight gain at 6 months, and if associations varied by HMO secretor status. The participants were 157 Hispanic mother–infant pairs and human milk samples were collected at 1 month. Nineteen individual HMOs were analyzed using high-performance liquid chromatography, and secretor status was determined by the presence of 2′-FL or lacto-*N*-fucopentaose I (Fucα1-2Galβ1-3GlcNAcβ1-3Galβ1-4Glc, LNFPI). Infant weight was measured at 1 and 6 months. Path analysis was used to test the effects of HMO composition on infant weight gain, adjusting for maternal age, pre-pregnancy BMI, infant age, sex, and birthweight. The results suggested that higher lacto-*N*-fucopentaose II [Galβ1-3(Fucα1-4) GlcNAcβ1-3Galβ1-4Glc, LNFPII] in human milk may decrease obesity risk across all infants, whereas higher LN*n*T and disialyl lacto-*N*-tetraose (DSLNT) may increase obesity risk in infants of non-secretors only. To determine if HMOs are associated with eating behavior in the Hispanic infants, cross-sectional analysis of the cohort of Hispanic mother–infant dyads was performed. Several HMOs were both positively and negatively associated with infant food responsiveness, which is a measure of drive to eat (Plows et al. 2020).

Maternal obesity has also been associated with changes in HMO concentrations. Lagström et al. (2020) investigated the association between maternal HMO composition and child growth during the first 5 years of life. In addition, the association between maternal pre-pregnancy BMI and HMO composition was assessed. Human milk samples from 802 mothers were obtained from a prospective population-based birth cohort study in Finland. Maternal HMO composition 3 months after delivery was associated with height and weight during the first 5 years of life in children of secretor mothers. Specifically, HMO diversity and the concentration of LN*n*T were inversely associated and that of 2′-FL was directly associated with child height and weight *z* scores in a model adjusted for maternal pre-pregnancy BMI, mode of delivery, birthweight *z* score, sex, and time. Maternal pre-pregnancy BMI was associated with HMO composition. The authors concluded that the association between maternal HMO composition and childhood growth may imply a causal relation, and altered HMO composition may mediate the impact of maternal pre-pregnancy BMI on childhood obesity, both of which warrant further investigation. In a study by Saben et al. (2021), maternal obesity was associated with lower concentrations of several fucosylated and sialylated HMOs and infants born to obese women had

lower intakes of these HMOs. Maternal BMI was positively associated with LN*n*T, 3-FL, 3′-SL, and 6′-SL and negatively associated with DSLNT, disialyllacto-*N*-hexaose (DSLNH), fucodisialyllacto-*N*-hexaose (FDSLNH), and total acidic HMOs concentrations at 2 months. Infant intakes of 3-FL, 3′-SL and 6′-SL, DSLNT, DSLNH, and total acidic HMOs were positively associated with infant growth over the first 6 months of life.

Related to this, HMOs may be important modulators of gut–brain axis development and homeostasis. The brain reward system, specifically the mesolimbic dopamine (DA) projections from the ventral tegmental area (VTA) to nucleus accumbens (NAc) is involved in the motivation and preference for food. A recent study by Tuplin et al. (2021) aimed to determine if HMO fortified diets given during the critical period of reward system development (21 days after birth) could affect the structure of the reward system. At weaning (Day 21), Sprague-Dawley rats were randomized to one of four fortified diet groups: control, 3′-SL, 2′-FL, or a combination of 3′-SL and 2′-FL. Messenger RNA (mRNA) expression was quantified for DA and appetite associated markers in the VTA and NAc, and Western blots measured the immediate early gene FosB and its isoform ΔFosB. Females fed the 3′SL + 2′FL fortified diet displayed a decrease in DAT expression in the VTA and an increase in leptin expression in the NAc. Females displayed an overall lower expression of NAc D2, VTA ghrelinR, and VTA leptin. In males, VTA DAT and FosB were negatively correlated with body weight and systemic leptin. The authors concluded that sex differences in the expression of DA markers underscore the need to investigate this phenomenon and understand the functional significance in preventing or treating obesity.

In relation to BMOs, dietary supplementation was associated with reduced weight gain and adiposity in mice fed a high-fat diet. The study by Hamilton et al. (2017) showed that consumption of BMOs by mice could prevent the deleterious effect of a high-fat diet on the gut microbiota and intestinal permeability in addition to attenuating the development of an obese phenotype. Gut microbiota and intestinal permeability were assessed in the ileum, cecum, and colon. Addition of BMOs to the high-fat diet significantly attenuated weight gain, decreased adiposity, and decreased caloric intake. BMOs completely abolished the high-fat diet-induced increase in paracellular and transcellular permeability in the small and large intestines. BMOs also increased the abundance of beneficial microbes such as *Bifidobacterium* and *Lactobacillus* in the ileum.

7.9 Anti-Pathogenic Effect of Milk Oligosaccharides

Milk oligosaccharides are considered to be soluble receptor analogs of epithelial cell surface carbohydrates, because they are generated by the action of similar enzymes. 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 receptors. We refer the reader to the expansive literature that exists describing the action of HMOs against a variety of bacterial and viral pathogens as it is beyond the scope of this chapter to cover all anti-infective studies associated with HMOs (Hickey 2012; Hundshammer and Minge 2020; Laucirica et al. 2017; Li et al. 2014; Manthey et al. 2014; Morozov et al. 2018). In terms of in vitro studies, HMOs have been shown to interfere with the lectin–glycan association for pathogens such as enterohaemorrhagic, entereopathogenic, enterotoxic, uropathogenic *Escherichia coli*, *Entamoeba histolytica*, *Campylobacter jejuni*, *Clostridium difficile*, *Helicobacter pylori*, *Listeria monocytogenes*, *Neisseria meningitidis* C, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella enterica*, group B *Streptococcus*, *Vibrio cholerae*, human immunodeficiency virus, norovirus, influenza virus, and respiratory syncytial virus. Indeed, a recent study by Yue et al. (2020) demonstrated that HMOs, BMOs, and GMOs were found to reduce the adhesion of *Staphylococcus aureus* to Caco-2 cells in comparison to the lactose control. Oligosaccharides isolated and purified from the colostrum of Holstein-Friesians were found to dramatically reduce the cellular invasion and translocation of *Campylobacter jejuni* in HT-29 intestinal cells, in a concentration-dependent manner (Lane et al. 2012). Similarly, Maldonado-Gomez et al. (2015) demonstrated that oligosaccharides from bovine colostrum could prevent the adhesion of enteropathogenic *E. coli*, *Cronobacter sakazakii*, and *Salmonella enterica* serovar Typhimurium to HEp-2 cell monolayers cultured in vitro. Recently, neutral and acidic oligosaccharides also isolated from 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). The oligosaccharides inhibited the adhesion of *S. enterica* to Caco-2 cells at concentrations ranging from 2.5 to 5.0 mg mL^{-1}. Another recent study by Ryan et al. (2018) examined BMOs extracted from demineralized whey, using a combination of membrane filtration and chromatography. The authors found that the BMOs were capable of reducing *Streptococcus pneumoniae* adhesion to pharynx and lung cells in vitro when tested at physiological concentrations. Two recent studies also investigated the direct anti-adhesive capacity of isolated GMOs. One study observed reduced adhesion of *Escherichia coli* and *Salmonella typhimurium* to Caco-2 cells when pre-incubated with GMOs (Leong et al. 2019). This was observed independent of beneficial microbiota. The second study showed the same results with *Salmonella* by green fluorescent antibodies against the *Salmonella* strain used (Urakami et al. 2018). Quinn et al. (2020b) examined the synergistic effect of GMO-treated *Bifidobacterium infantis* on preventing the attachment of a highly invasive strain of *Campylobacter jejuni* to intestinal HT-29 cells. The combination decreased the adherence of *C. jejuni* to the HT-29 cells by an average of 42% compared to the control (non-GMO treated *B. infantis*). Taken together, these studies highlight the significant antimicrobial activity associated with milk oligosaccharides and their potential as novel substitutes for antibiotics in preventing infection.

7.10 Immunomodulating Effect of Milk Oligosaccharides

HMOs are known to affect immune cell populations and cytokine secretion. HMOs are also absorbed into the blood, where they affect binding of monocytes, lymphocytes, and neutrophils to endothelial cells and the formation of platelet–neutrophil complexes. The immunological effects of HMO have been reviewed (Ayechu-Muruzabal et al. 2018; Plaza-Díaz et al. 2018; Triantis et al. 2018). Less is known about the role that BMOs and GMOs play in modulating the immunological system. Lane et al. (2013) compared the transcriptional response of colonic HT-29 epithelial cells to the entire pool of HMOs and bovine colostrum oligosaccharides (BCOs). RT-PCR analysis revealed that HMOs and BCOs influenced the expression of various cytokines, chemokines, and cell surface receptors, suggesting that BMOs may result in an intestinal immune response similar to that of HMOs. In a recent study, Cowardin et al. (2019) colonized germ-free mice with cultured bacterial strains from a 6-month-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. GMOs have shown to be anti-inflammatory in a rat model of hapten-induced colitis (Daddaoua et al. 2006). When compared with the control group, the GMO group showed decreased anorexia and body weight loss, reduced bowel wall thickening and longitudinal extension of necrotic lesions, and downregulated colonic expression of interleukin 1β, inducible nitric oxide synthase, cyclooxygenase 2, and mucin 3; and increased trefoil factor 3. Lara-Villoslada et al. (2006) also studied the effect of GMOs on colon inflammation in rats induced by dextran sodium sulfate (DSS). The GMO-treated rats showed less severe colonic lesions and a more favorable intestinal microbiota. After DSS treatment, histological analysis showed that the GMO-treated rats had no ulceration and recovered from inflammation, while the DSS control rats had significant ulceration and inflammation. Also, blood granulocyte levels were reduced in GMO-fed rats compared to control rats. In GMO-fed rats, the levels of myeloperoxidase activity, a proxy for neutrophil infiltration, did not increase upon DSS treatment, while in control rats, a five-fold activity increase is observed upon DSS treatment. These studies suggest that GMOs reduce intestinal inflammation and contributed to the recovery of damaged colonic mucosa, indicating they may have potential for the treatment of inflammatory bowel disease.

7.11 Influence of Milk Oligosaccharides on Intestinal Cell Properties

Beginning in the perinatal period and extending through the first year of life, the gastrointestinal tract undergoes numerous morphological changes that influence its physiological functions. Human milk provides trophic factors that influence maturation of the GI tract, an important developmental process in all infants but of particular importance in pre- and early-term infants. Necrotizing enterocolitis (NEC) is a leading cause of morbidity and death in preterm infants, occurring more often in formula-fed than in breastfed infants. In preclinical studies using a newborn rat model of NEC, pups fed with a formula containing DSLNT demonstrated a reduction in NEC severity and decreased mortality (Jantscher-Krenn et al. 2012). Wu et al. (2019) suggested that the mechanism for the prevention of NEC by HMOs is related to recovery of the colonic barrier function. The authors demonstrated that a HMO-gavage given to rat pups increases Muc2 levels and decreases intestinal permeability to macromolecular dextran. In vitro experiments showed that HMO-treated cells have increased Muc2 expression, decreased bacterial attachment and dextran permeability during challenge by enteric pathogens.

The effect of dairy-derived oligosaccharides on intestinal properties has also been explored. Kuntz et al. (2019) identified that BMOs from different cattle breeds were able to induce growth arrest and differentiation of non-transformed human intestinal cells by modulating the epidermal growth factor receptor (EGFR) signal pathways, and cell cycle associated gene expression in a similar way as was shown for HMOs (Kuntz et al. 2009, 2008). Studies investigating the ability of BMOs to modulate intestinal permeability have also shown promising results. The study of Hamilton et al. (2017) mentioned above showed that consumption of BMOs can significantly reduce the intestinal permeability that is induced in mice consuming a high-fat diet. In a similar study, introduction of BMOs to the diet of high-fat-fed mice, in combination with a weekly gavage of the probiotic *Bifidobacterium longum* subsp. *infantis*, prevented increases in intestinal permeability otherwise associated with the high-fat diet (Boudry et al. 2017). Duncan et al. (2020) recently explored the synbiotic action of BMOs and galactooligosaccharides (GOS) with *Lactobacillus rhamnosus* in imparting protection of epithelial barrier function against *Clostridium difficile* enterotoxicity, in a simplified in vitro host–epithelial barrier function model system. The authors found that the BMO-enriched preparation modulated host–bacterial crosstalk, leading to enhanced epithelial barrier function, as measured by paracellular ion flux through transepithelial electrical resistance following challenge with *C. difficile* toxin A. Interestingly, recent studies examining the impact of a GMOs on barrier function of epithelial cell co-cultures found that the GMOs at the maximum concentration tested (4.0 mg mL^{-1}) enhanced trans-epithelial 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. 2018, 2016).

7.12 Separation, Detection, and Quantification of Milk Oligosaccharides

Purifying milk oligosaccharides prior to analysis is sometimes challenging due to the similarity in physical properties between the oligosaccharides and lactose. The smallest oligosaccharides have a degree of polymerization of three and will often behave similarly to lactose when oligosaccharides are enriched by polarity or size-based methods. The general scheme for obtaining oligosaccharides from milk is first to precipitate fat and protein using different reagents. The defatting step is often performed simply by centrifugation or by solvent extraction. Protein precipitation is usually completed with organic solvents, such as ethanol, chloroform/methanol, acetone, or acetonitrile. In several studies, membrane separation has been applied to eliminate milk proteins. For more detail on the extraction and fractionation of milk carbohydrates, we refer the reader to a review by Nagaraj et al. (2018) where methods such as pressurized liquid extraction (PLE), supercritical fluid extraction (SFE), and solid-phase extraction (SPE) are discussed. Oligosaccharides can be separated from lactose by gel filtration using various resins (e.g., Sephadex G25, Toyopearl HW50, and Biogel P2) or by stepwise elution from a column of activated charcoal using ethanol. High pH anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) is often used to separate milk oligosaccharides. Reversed-phase high-performance liquid chromatography (RP-HPLC) is another widely used method for oligosaccharides analysis, although it requires sample derivatization since native milk oligosaccharides are typically polar and are not retained by the column. Derivatization refers to the addition of a chromophore or fluorophore to the carbohydrate analyte in order to provide a greater degree of sensitivity and often enhance the selectivity of the detection system. Milk oligosaccharides have also been separated by hydrophilic interaction chromatography HPLC (HILIC) which provides good isomer separation but requires oligosaccharide labeling. Capillary electrophoresis (CE) is suitable for both derivatized and underivatized carbohydrates and is also considered a powerful and adaptable separation method.

Recent advances in the analysis of human milk oligosaccharides by liquid phase separation methods have been reviewed by Porfirio et al. (2020) and Auer et al. (2020). To detect the oligosaccharides after separation, there is a choice between derivatized (labeled) and label-free detection, using e.g., refractive index (RI), evaporative light scattering detection (ELSD), pulsed-amperometric detection (PAD), and mass spectrometry (MS). RI has limited sensitivity and is limited to isocratic separation conditions and therefore this detection approach is not suitable for complex mixtures. Likewise, ELSD has limited compatibility with common oligosaccharide separation gradients. This means that label-free detection is essentially limited to PAD and MS. Identification of the individual oligosaccharide structures in milk has come with numerous analytical challenges, many of which have been resolved in recent years.

Over the past 10 years, a number of studies have documented the detection of oligosaccharides from dairy sources. Mariño et al. (2011) employed an analytical

scheme based on fluorescent labeling of BMOs from colostrum, pre-fractionation by weak anionic exchange chromatography and separation by HILIC-HPLC. Structural assignments for 37 free oligosaccharides including 20 sialylated species were obtained by combining HILIC-HPLC, exoglycosidase digestion and offline negative-ion mode MS/MS. Aldredge et al. (2013) also fractionated oligosaccharides from bovine colostrum pool by high-performance liquid chromatography, incubated each BMO with glycosidases of known specificity, and analyzed the changes produced with LC-MS. This labor-intensive approach determined a variety of glycosidic linkages and specific monosaccharide types for numerous BMOs. Albrecht et al. (2014) performed a study to obtain a comprehensive overview of oligosaccharides present in the milk of a number of domestic animals including cows and goats. A combination of weak anion-exchange chromatography, ultra-performance liquid chromatography–hydrophilic interaction liquid chromatography with fluorescence detection (UPLC-HILIC-FLD) and complementary quadrupole time-of-flight MS as well as exoglycosidase sequencing allowed for the determination of the oligosaccharide sequences and linkages as well as their relative quantification. A total of 35 BMO structures were identified of which five were novel, 12 neutral, three fucosylated, 21 acidic with two phosphorylated. In relation to the GMOs, 40 were identified of which 19 were novel, 16 neutral, three fucosylated, 23 acidic with one phosphorylated. Mehra et al. (2014) employed concentration techniques on dairy streams at pilot scale combined with advanced mass spectrometry, to discover numerous high-molecular weight fucose-containing oligosaccharides in a whey stream of bovine milk. Among the BMOs identified, 18 have high-molecular weight and corresponded in size to the most abundant oligosaccharides present in human milk. Lee et al. (2016) then went on to use a nanoLC separation coupled to a high-resolution and sensitive quadrupole time-o-flight (Q-ToF) MS system to detect over 30 BMOs that were previously elucidated. Martín-Ortiz et al. (2016) analyzed GMOs from colostrum using nanoflow liquid chromatography–quadrupole time-of-flight MS (Nano-LC-Chip–Q-TOF MS). Up to 78 oligosaccharides containing hexose, hexosamine, fucose, *N*-acetylneuraminic acid, or *N*-glycolylneuraminic acid monomeric units were identified in the samples, some of them detected for the first time in goat colostra. Also, in relation to goat milk, Lu et al. (2020) identified and quantified oligosaccharides by using UPLC-MS/MS. The elution conditions of the UPLC was optimized leading to successful identification of 64 oligosaccharides in Guanzhong and Saanen goat milk. Recently, Sunds et al. (2021) characterized oligosaccharides in the milk of native Nordic cattle breeds, to reveal whether such breeds hold unique oligosaccharide distribution and variation. The study involved 80 milk samples collected from eight native breeds originating from Norway (Norwegian Doela cattle and Norwegian Telemark cattle), Sweden (Swedish Mountain cattle), Denmark (Danish Red anno 1970), Iceland (Icelandic cattle), Lithuania (native Lithuanian Black and White), and Finland (Western Finncattle and Eastern Finncattle). The analysis was conducted using high-performance liquid chromatography chip/quadrupole time-of-flight mass spectrometry (HPLC-Chip/Q-TOF MS) and thereby created comprehensive libraries for each breed based on tandem MS, as well as a relative quantification of

all the BMOs identified. Eighteen unique monosaccharide compositions and a multitude of isomers were identified. No *N*-glycolylneuraminic acid was identified among the breeds. Western Finncattle milk was found to be the most abundant in neutral, acidic, and fucosylated oligosaccharides. Eastern Finncattle milk had significantly higher levels of acidic oligosaccharides, and Icelandic cattle milk had significantly higher levels of fucosylated oligosaccharides, compared to the mean (the average stage of lactation and the average parity per breed). Such studies are of interest for future exploitation of milk oligosaccharides via selective breeding strategies.

Considering the multitude of applications which may be assigned to milk oligosaccharides, and often to specific structures, it is very important to have robust methods to accurately determine the levels of these structures in milk, dairy streams, and infant formulas. Fong et al. (2011) developed a method for the quantitation of different BMOs using HILIC-HPLC with high-resolution selected reaction monitoring-MS. Concentrations of five BMOs (3′-sialyllactose (3′-SL), 6′-sialyllactose (6′-SL), 6′-sialyllactosamine (6′-SLN), disialyllactose (DSL), and *N*-acetylgalactosaminyllactose (GNL)) in bovine mature defatted milk, homogenized mature milk, non-pasteurized mature milk, bovine colostrum, and infant formula were determined. Liu et al. (2014) subsequently improved the quantitative analysis of 3′-SL, 6′-SL, and 6′-SLN in bovine mature milk using a method based on HILIC coupled to MS in negative ion mode. Milk from commercial dairy and beef cows in early lactation has also been compared for oligosaccharide content (Sischo et al. 2017). Early lactation multiparous cows (5–12 days into milking) from five commercial Holstein dairy herds and five Angus or Angus hybrid beef herds were sampled once. Oligosaccharide diversity and abundance within and between samples was assessed using LC-MS and principal component analysis. Overall, oligosaccharide relative abundance was consistently greater in the cluster dominated by beef cows.

Liu et al. (2017) performed a systematic survey on seasonal variation of 14 major oligosaccharides with 19 cows over the entire milking season using a LC-MS technique. This study revealed a number of significant correlations between structurally related and structurally nonrelated oligosaccharides and a substantial individual animal difference for all 14 oligosaccharides. In another study, relative quantities of oligosaccharides in a large collection of milk samples were recently measured using LC-MS and isobaric labeling (the use of mass tags which have an identical overall mass but vary in terms of the distribution of heavy isotopes around their structure), an analytical technique that improves instrumental throughput for large sample sets by allowing samples to be multiplexed prior to analysis by mass spectrometry (Robinson et al. 2018). In a subsequent study, Robinson et al. (2019) applied a high-throughput isobaric labeling technique to measure oligosaccharide abundances in 634 milk samples collected from Danish Holstein-Friesian and Jersey dairy cattle by LC-MS. Thirteen oligosaccharides that vary significantly by breed were identified, with most structures being more abundant in the milk of Jersey cattle. The abundances of several oligosaccharides were increased in second-parity cows, and correlations between the abundances of oligosaccharide pairs were identified,

potentially indicating similarities in their synthetic pathways. Fucosylated oligosaccharide structures were also widely identified among both breeds. Fischer-Tlustos et al. (2020) recently characterized the oligosaccharide profile of colostrum and transition milk from ten primiparous and ten multiparous Holstein cows. Samples were analyzed for oligosaccharide concentrations using LC-MS. The results demonstrated that colostrum and transition milk contain elevated concentrations of certain BMOs compared with mature milk. Parity differences were also detected for levels of 3′-SL, 6′-SL, and 6′-SLN, with multiparous cows having greater concentrations than primiparous cows over the first 7 days of milking.

The use of HPAEC-PAD has been reported in a number of studies to quantify BMOs and is advantageous because it requires minimal sample preparation and achieves good chromatographic separation of oligosaccharide isomers within 30 min. Lee et al. (2015) used the method to measure 3′-SL, 6′-SL, and 6′-SLN in colostrum whey permeate. Similarly, Quinn et al. (2020c) used HPAEC-PAD to monitor the impact of days post-parturition and parity on the oligosaccharide profile of cow's milk. Colostrum and milk samples were obtained from 18 cows 1–5 days after parturition. Three distinct phases were identified: colostrum (Day 0), transitional milk (Days 1–2) and mature milk (Days 3–5). LS-tetrasaccharide c (Neu5Ac α2-6Galβ1-4GlcBAcβ1-3Galβ1-4Glc, LST c), lacto-*N*-neotetraose (LN*n*T), disialyllacto-*N*-tetraose (DSLNT), 3′-sialyl-*N*-acetyllactosamine (Neu5Acα2-3Galβ1-4GlcNAc, 3′-SLN), 3′-SL, lacto-*N*-neohexaose [Galβ1-4GlcNAcβ1-3{Galβ1-4GlcNAcβ1-6}Galβ1-4Glc, LN*n*H], and DSL were found to be highly affiliated with colostrum. The cow's parity was also shown to have a significant effect on the oligosaccharide profile. CE has also been applied to quantify 3′-SL, 6′-SL, and DSLNT (Monti et al. 2015) and was used recently for the rapid characterization of the relative abundances of 33 BMOs in milk collected from exclusively grass-fed or grain/corn-fed cows at matched time points during the first week of lactation (Vicaretti et al. 2018). Sousa (2019) and van Leeuwen et al. (2020) as part of their reviews discuss the quantitative studies performed on GMOs. Claps et al. (2014) evaluated the influence of two goat breeds (Garganica and Maltese) on the oligosaccharide content in colostrum and observed a higher concentration of 3′-SL and 6′-SL in milk of the Garganica breed compared to Maltese in the period after parity. It was also observed that there was an increase in the 3′-SL and 6′-SL levels found in the breeds 24 h after parity, with the 3′-SL concentration always being higher when compared to 6′-SL in both the periods.

7.13 Industrial-Scale Strategies to Produce Milk Oligosaccharides

Four different approaches have been investigated regarding current commercial HMO production: chemical synthesis, whole-cell biotransformation (fermentation), enzymatic, and chemo-enzymatic routes (Craft and Townsend 2017; Fang et al.

2018; Prudden et al. 2017). Currently, biocatalytic methods are considered the most efficient in terms of HMO production yields reviewed by Pérez-Escalante et al. (2020). Only 2′-FL and LN*n*T are currently commercially available for addition to infant formula despite the existence of over 150 HMO structures. Microbial engineering has recently made it possible to produce these two compounds at industrial scale by fermentation using genetically modified *Escherichia coli* (Bode et al. 2016; Bych et al. 2019). Recent commercialization and regulatory approval of synthesized HMOs have now paved the way for expanding the HMO portfolio as future ingredients in foods other than infant formula. 2′-FL was an obvious starting point for HMO production given its simple structure and as the most abundant HMO in breastmilk (Thurl et al. 2017). In contrast, LN*n*T is less abundant in human milk when compared to lacto-*N*-tetraose (LNT) for example, but is easier to synthesize at large scale (Baumgärtner et al. 2015) and was therefore marketed first. Indeed, for more complex and larger structures, fermentation yields are often low (Sprenger et al. 2017; Faijes et al. 2019). Despite this, 42 HMO structures (including building blocks) have been produced using the cell factory approach to date (Faijes et al. 2019). The regulatory landscape surrounding HMO production was recently summarized by Bych et al. (2019) and Walsh et al. (2020b).

Considering the wide availability of dairy side streams from which oligosaccharides can be isolated, BMOs and GMOs show promise as future therapeutics that could be used to provide HMO-associated health benefits to infants (when breastfeeding is not possible) and adults on a large scale. Studies show that BMOs and GMOs can be isolated and purified from whey, permeate, and mother liquor following lactose crystallization, which provides abundant raw materials for their industrial production (Barile et al. 2009; Martinez-Ferez et al. 2006; Mehra et al. 2014; Wang and Yu 2021). Whey, the liquid part of milk that separates from the curd during cheese production, is a particularly attractive source of oligosaccharides. The average BMO concentration in whey is approximately 0.2 g/L, and similar levels of GMOs are found in goat cheese whey (Bode et al. 2016; Thum et al. 2015). Whey permeate is obtained from the process of whey ultrafiltration and is disposed of or used to produce food-grade lactose by crystallization. Milk oligosaccharides pass through the ultrafiltration membranes, ending up in the whey permeate (Barile et al. 2009; Mehra et al. 2014). In terms of oligosaccharide content in whey permeate, de Moura Bell et al. (2018) found that the concentration of BMOs in bovine whey permeate was approximately 0.21 g/L while Thum et al. (2015) found that the concentration of GMOs in goat whey permeate was approximately 0.2 g/L. As mentioned, the permeate can then be used to produce lactose by crystallization, thus improving the economic value and in turn reducing the lactose content. 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. Mehra et al. (2014) found that bovine mother liquor contained approximately 170 mg/L of sialyllactose for a similar concentration of lactose (49 g/L) in bovine whey permeate. Moreover, the concentration ratio of sialyllactose/lactose in bovine mother liquor was approximately 2.5- to 3.5-fold more concentrated than that in bovine whey permeate. To the best of our knowledge, concentrations of oligosaccharides in goat mother liquor is

unknown. The enrichment of oligosaccharides from bovine and goat milk was reviewed recently by Quinn et al. (2020a) and Wang and Yu (2021).

Martinez-Ferez et al. (2006) were among the first to describe the use of membrane technology for the isolation of oligosaccharides from domestic animal milk. A two-stage tangential filtration process was used on pasteurized skimmed goat milk. At the end of the process, 80% of the oligosaccharides were obtained in the final retentate. Oliveira et al. (2012) also used ultrafiltration on goat whey to remove proteins and fat globules, and then the ultrafiltered permeate was further processed using a 1 kDa "tight" membrane. The final retentate was fractionated to yield 28 oligosaccharide-rich fractions using preparative-scale molecular size exclusion chromatography. Mehra et al. (2014) concentrated BMOs from mother liquor using membrane filtration. A combination of HPLC and accurate mass spectrometry allowed the identification of optimal processing conditions for the production of kilogram quantities of BMO-enriched powders. Among the BMOs identified in the powder, 18 had high-molecular weights and corresponded in size to the most abundant oligosaccharides present in human milk. Six oligosaccharides identified contained fucose, which until then had rarely been found in bovine milk (Mehra et al. 2014).

A combination of lactose hydrolysis and membrane filtration is more commonly used to isolate oligosaccharides from milk. de Moura Bell et al. (2018) developed a novel pilot-scale approach for the recovery of highly pure oligosaccharides from colostral bovine whey permeate. Given that the concentration of BMOs in colostrum is much higher than in mature milk (Fong et al. 2011; Nakamura et al. 2003), this represents a possible source from which to separate BMOs at large scale. The method is based on the integration of optimized processing conditions that favor maximum lactose hydrolysis and monosaccharide fermentation prior to oligosaccharide concentration using selective membrane filtration. After complete lactose hydrolysis and fermentation of the monosaccharides by yeast, nanofiltration of fermented whey permeate 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 generation of GMOs at pilot scale with a 75% recovery of oligosaccharides (Aquino et al. 2017). Recently, the enzymatic hydrolysis used in the above study was further optimized by Thum et al. (2019), through maximizing the specificity of the β-galactosidase from *Aspergillus oryzae* which was used for lactose hydrolysis. Overall, processing conditions using temperatures ≤40 °C and an enzyme concentration of ≤0.25% resulted in a higher preservation/formation of GMOs from the whey. Martín-Ortiz et al. (2019) were also successful in the selective removal of lactose, and its constituent monosaccharides, from pooled goat colostrum using a procedure based on the combined use of β-galactosidase from *Kluyveromyces lactis* to hydrolyze lactose and *Saccharomyces cerevisiae* to remove the released galactose and glucose through fermentation.

Sousa et al. (2019) investigated the characterization and concentration of oligosaccharides naturally present in goat cheese whey obtained from two types of goat milk. The goat cheese whey was processed by a two-step cross-flow filtration process. 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 63–96% of oligosaccharides was obtained when compared with the original whey. Although membrane filtration is the most commonly investigated technique for producing dairy-derived oligosaccharides at large scale, there has been some recent success using scalable chromatography approaches to produce BMOs from whey streams (Marotta and Hickey, 2018). However, the advantages of membrane technology over chromatographic separation technology include the lower energy cost, easy modification of critical operational parameters, reduction of the processing time, scaling-up, and reduction of environmental pollution (Wang and Yu 2021).

7.14 Concluding Remarks

The valuable effects of HMOs in breastmilk are largely lost to formula-fed infants. Substitution of infant formula with BMOs and GMOs to impart HMO functions is a potential solution, in addition to the benefits already observed by supplementation of formulas with 2′-fucosyllactose. The safety and tolerability of dairy-derived oligosaccharides for human consumption were recently evaluated and showed promising results (Smilowitz et al. 2017). Such studies pave the way for dairy oligosaccharides to be evaluated further in human trials, including in infant formula production. The development and optimization of industrial scale methods to isolate and enrich oligosaccharides from bovine and goat milk will be important going forward if they are to be used as health-promoting ingredients. Knowledge of the genes related to oligosaccharides synthesis and the influence of genetics, environment, breed, parity, diet, and seasonality on their expression should have potential to guide breeding strategies in cows and goats. Such information should allow the production of milk with a higher diversity and concentration of oligosaccharides and ultimately facilitate their large-scale production.

References

Akkerman, R., Faas, M. M., & de Vos, P. (2019). Non-digestible carbohydrates in infant formula as substitution for human milk oligosaccharide functions: Effects on microbiota and gut maturation. *Critical Reviews in Food Science and Nutrition, 59*(9), 1486–1497.

Albrecht, S., Lane, J. A., Marino, K., Al Busadah, K. A., Carrington, S. D., Hickey, R. M., & Rudd, P. M. (2014). A comparative study of free oligosaccharides in the milk of domestic animals. *The British Journal of Nutrition, 111*(7), 1313–1328.

Alderete, T. L., Autran, C., Brekke, B. E., Knight, R., Bode, L., Goran, M. I., & Fields, D. A. (2015). Associations between human milk oligosaccharides and infant body composition in the first 6 mo of life. *The American Journal of Clinical Nutrition, 102*(6), 1381–1388.

Aldredge, D. L., Geronimo, M. R., Hua, S., Nwosu, C. C., Lebrilla, C. B., & Barile, D. (2013). Annotation and structural elucidation of bovine milk oligosaccharides and determination of novel fucosylated structures. *Glycobiology, 23*(6), 664–676.

Aquino, L. F., de Moura Bell, J. M., Cohen, J. L., Liu, Y., Lee, H., de Melo Silva, V. L., Domizio, P., Junior, C. A. C., & Barile, D. (2017). Purification of caprine oligosaccharides at pilot-scale. *Journal of Food Engineering, 214*, 226–235.

Asakuma, S., Urashima, T., Akahori, M., Obayashi, H., Nakamura, T., Kimura, K., Watanabe, Y., Arai, I., & Sanai, Y. (2008). Variation of major neutral oligosaccharides levels in human colostrum. *European Journal of Clinical Nutrition, 62*(4), 488–494.

Auer, F., Jarvas, G., & Guttman, A. (2020). Recent advances in the analysis of human milk oligosaccharides by liquid phase separation methods. *Journal of Chromatography B, 122497*.

Austin, S., De Castro, C. A., Bénet, T., Hou, Y., Sun, H., Thakkar, S. K., Vinyes-Pares, G., Zhang, Y., & Wang, P. (2016). Temporal change of the content of 10 oligosaccharides in the milk of Chinese urban mothers. *Nutrition, 8*(6), 346.

Ayechu-Muruzabal, V., van Stigt, A. H., Mank, M., Willemsen, L. E., Stahl, B., Garssen, J., & Van't Land, B. (2018). Diversity of human milk oligosaccharides and effects on early life immune development. *Frontiers in Pediatrics, 6*, 239.

Barile, D., Tao, N., Lebrilla, C. B., Coisson, J.-D., Arlorio, M., & German, J. B. (2009). Permeate from cheese whey ultrafiltration is a source of milk oligosaccharides. *International Dairy Journal, 19*(9), 524–530.

Barnett, A. M., Roy, N. C., McNabb, W. C., & Cookson, A. L. (2016). Effect of a semi-purified oligosaccharide-enriched fraction from caprine milk on barrier integrity and mucin production of co-culture models of the small and large intestinal epithelium. *Nutrients, 8*(5), 267.

Barnett, M. L., Song, Z., Bitton, A., Rose, S., & Landon, B. E. (2018). Gatekeeping and patterns of outpatient care post healthcare reform. *The American Journal of Managed Care, 24*(10), e312–e318.

Baumgärtner, F., Jurzitza, L., Conrad, J., Beifuss, U., Sprenger, G. A., & Albermann, C. (2015). Synthesis of fucosylated lacto-*N*-tetraose using whole-cell biotransformation. *Bioorganic & Medicinal Chemistry, 23*(21), 6799–6806.

Berger, P. K., Plows, J. F., Jones, R. B., Alderete, T. L., Yonemitsu, C., Poulsen, M., Ryoo, J. H., Peterson, B. S., Bode, L., & Goran, M. I. (2020a). Human milk oligosaccharide 2'-fucosyllactose links feedings at 1 month to cognitive development at 24 months in infants of normal and overweight mothers. *PLoS One, 15*(2), e0228323.

Berger, P. K., Plows, J. F., Jones, R. B., Alderete, T. L., Yonemitsu, C., Ryoo, J. H., Bode, L., & Goran, M. I. (2020b). Human milk oligosaccharides and Hispanic infant weight gain in the first 6 months. *Obesity, 28*(8), 1519–1525.

Bode, L. (2019). Human milk oligosaccharides: Next-generation functions and questions. In S. M. Donovan, J. B. German, B. Lonnerdahl, & A. Lucas (Eds.), *Human milk: Composition, clinical benefits and future opportunities* (Vol. 90, pp. 191–201). Basel: Karger Publishers.

Bode, L., Contractor, N., Barile, D., Pohl, N., Prudden, A. R., Boons, G.-J., Jin, Y.-S., & Jennewein, S. (2016). Overcoming the limited availability of human milk oligosaccharides: Challenges and opportunities for research and application. *Nutrition Reviews, 74*(10), 635–644.

Borewicz, K., Gu, F., Saccenti, E., Hechler, C., Beijers, R., de Weerth, C., van Leeuwen, S. S., Schols, H. A., & Smidt, H. (2020). The association between breastmilk oligosaccharides and faecal microbiota in healthy breastfed infants at two, six, and twelve weeks of age. *Scientific Reports, 10*(1), 1–12.

Boudry, G., Hamilton, M. K., Chichlowski, M., Wickramasinghe, S., Barile, D., Kalanetra, K. M., Mills, D. A., & Raybould, H. E. (2017). Bovine milk oligosaccharides decrease gut permeability and improve inflammation and microbial dysbiosis in diet-induced obese mice. *Journal of Dairy Science, 100*(4), 2471–2481.

Bych, K., Mikš, M. H., Johanson, T., Hederos, M. J., Vigsnæs, L. K., & Becker, P. (2019). Production of HMOs using microbial hosts—From cell engineering to large scale production. *Current Opinion in Biotechnology, 56*, 130–137.

Charbonneau, M. R., O'Donnell, D., Blanton, L. V., Totten, S. M., Davis, J. C., Barratt, M. J., Cheng, J., Guruge, J., Talcott, M., & Bain, J. R. (2016). Sialylated milk oligosaccharides promote microbiota-dependent growth in models of infant undernutrition. *Cell, 164*(5), 859–871.

Chaturvedi, P., Warren, C. D., Altaye, M., Morrow, A. L., Ruiz-Palacios, G., Pickering, L. K., & Newburg, D. S. (2001). Fucosylated human milk oligosaccharides vary between individuals and over the course of lactation. *Glycobiology, 11*(5), 365–372.

Cheng, L., Akkerman, R., Kong, C., Walvoort, M. T., & de Vos, P. (2020). More than sugar in the milk: Human milk oligosaccharides as essential bioactive molecules in breast milk and current insight in beneficial effects. *Critical Reviews in Food Science and Nutrition, 61*(7), 1–17.

Claps, S., Di Napoli, M., Sepe, L., Caputo, A., Rufrano, D., Di Trana, A., Annicchiarico, G., & Fedele, V. (2014). Sialyloligosaccharides content in colostrum and milk of two goat breeds. *Small Ruminant Research, 121*(1), 116–119.

Coppa, G. V., Zampini, L., Galeazzi, T., Facinelli, B., Ferrante, L., Capretti, R., & Orazio, G. (2006). Human milk oligosaccharides inhibit the adhesion to Caco-2 cells of diarrheal pathogens: *Escherichia coli*, *Vibrio cholerae*, and *Salmonella fyris*. *Pediatric Research, 59*(3), 377–382.

Cowardin, C. A., Ahern, P. P., Kung, V. L., Hibberd, M. C., Cheng, J., Guruge, J. L., Sundaresan, V., Head, R. D., Barile, D., & Mills, D. A. (2019). Mechanisms by which sialylated milk oligosaccharides impact bone biology in a gnotobiotic mouse model of infant undernutrition. *Proceedings of the National Academy of Sciences of the United States of America, 116*(24), 11988–11996.

Craft, K. M., & Townsend, S. D. (2017). Synthesis of lacto-N-tetraose. *Carbohydrate Research, 440*, 43–50.

Crisà, A., Ferrè, F., Chillemi, G., & Moioli, B. (2016). RNA-Sequencing for profiling goat milk transcriptome in colostrum and mature milk. *BMC Veterinary Research, 12*(1), 264. https://doi.org/10.1186/s12917-016-0881-7

Crisà, A., Claps, S., Moioli, B., & Marchitelli, C. (2019). Identification of the complete coding cDNAs and expression analysis of B4GALT1, LALBA, ST3GAL5, ST6GAL1 in the colostrum and milk of the Garganica and Maltese goat breeds to reveal possible implications for oligosaccharide biosynthesis. *BMC Veterinary Research, 15*(1), 1–14.

Daddaoua, A., Puerta, V., Requena, P., Martínez-Férez, A., Guadix, E., Sánchez de Medina, F. N., Zarzuelo, A., Suárez, M. A. D., Boza, J. J., & Martínez-Augustin, O. (2006). Goat milk oligosaccharides are anti-inflammatory in rats with hapten-induced colitis. *The Journal of Nutrition, 136*(3), 672–676.

De Leoz, M. L. A., Wu, S., Strum, J. S., Niñonuevo, M. R., Gaerlan, S. C., Mirmiran, M., German, J. B., Mills, D. A., Lebrilla, C. B., & Underwood, M. A. (2013). A quantitative and comprehensive method to analyze human milk oligosaccharide structures in the urine and feces of infants. *Analytical and Bioanalytical Chemistry, 405*(12), 4089–4105.

de Moura Bell, J. M., Cohen, J. L., de Aquino, L. F., Lee, H., de Melo Silva, V. L., Liu, Y., Domizio, P., & Barile, D. (2018). An integrated bioprocess to recover bovine milk oligosaccharides from colostrum whey permeate. *Journal of Food Engineering, 216*, 27–35.

Docq, S., Spoelder, M., Wang, W., & Homberg, J. R. (2020). The protective and long-lasting effects of human milk oligosaccharides on cognition in mammals. *Nutrients, 12*(11), 3572.

Dotz, V., Rudloff, S., Blank, D., Lochnit, G., Geyer, R., & Kunz, C. (2014). 13C-labeled oligosaccharides in breastfed infants' urine: Individual-, structure-and time-dependent differences in the excretion. *Glycobiology, 24*(2), 185–194.

Duncan, P. I., Aitio, O., Heiskanen, A., Niemelä, R., Saarinen, J., Helin, J., Porta, N., Fiaux, M., Moënnoz, D., & Golliard, M. (2020). Structure and function of bovine whey derived oligosaccharides showing synbiotic epithelial barrier protective properties. *Nutrients, 12*(7), 2007.

European Union. (2017). Commission Implementing Regulation (EU) 2017/2470 of 20 December 2017 establishing the Union list of novel foods in accordance with Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods. *Official Journal of the European Union, 60*.

Faijes, M., Castejón-Vilatersana, M., Val-Cid, C., & Planas, A. (2019). Enzymatic and cell factory approaches to the production of human milk oligosaccharides. *Biotechnology Advances, 37*(5), 667–697.

Fang, J. L., Tsai, T. W., Liang, C. Y., Li, J. Y., & Yu, C. C. (2018). Enzymatic synthesis of human milk fucosides α1, 2-Fucosyl *para*-lacto-*N*-hexaose and its isomeric derivatives. *Advanced Synthesis and Catalysis, 360*(17), 3213–3219.

Fischer-Tlustos, A., Hertogs, K., van Niekerk, J., Nagorske, M., Haines, D., & Steele, M. (2020). Oligosaccharide concentrations in colostrum, transition milk, and mature milk of primi-and multiparous Holstein cows during the first week of lactation. *Journal of Dairy Science, 103*(4), 3683–3695.

Fleming, S. A., Mudd, A. T., Hauser, J., Yan, J., Metairon, S., Steiner, P., Donovan, S. M., & Dilger, R. N. (2020). Human and bovine milk oligosaccharides elicit improved recognition memory concurrent with alterations in regional brain volumes and hippocampal mRNA expression. *Frontiers in Neuroscience, 14*, 770.

Fong, B., Ma, K., & McJarrow, P. (2011). Quantification of bovine milk oligosaccharides using liquid chromatography–selected reaction monitoring–mass spectrometry. *Journal of Agricultural and Food Chemistry, 59*(18), 9788–9795.

Gillman, M. W., Rifas-Shiman, S. L., Camargo, C. A., Jr., Berkey, C. S., Frazier, A. L., Rockett, H. R., Field, A. E., & Colditz, G. A. (2001). Risk of overweight among adolescents who were breastfed as infants. *Journal of the American Medical Association, 285*(19), 2461–2467.

Goehring, K. C., Kennedy, A. D., Prieto, P. A., & Buck, R. H. (2014). Direct evidence for the presence of human milk oligosaccharides in the circulation of breastfed infants. *PLoS One, 9*(7), e101692.

Gopal, P. K., & Gill, H. S. (2000). Oligosaccharides and glycoconjugates in bovine milk and colostrum. *British Journal of Nutrition, 84*(S1), 69–74.

Hamilton, M. K., Ronveaux, C. C., Rust, B. M., Newman, J. W., Hawley, M., Barile, D., Mills, D. A., & Raybould, H. E. (2017). Prebiotic milk oligosaccharides prevent development of obese phenotype, impairment of gut permeability, and microbial dysbiosis in high fat-fed mice. *American Journal of Physiology. Gastrointestinal and Liver Physiology, 312*(5), G474–G487.

Hauser, J., Pisa, E., Vásquez, A. A., Tomasi, F., Traversa, A., Chiodi, V., Martin, F.-P., Sprenger, N., Lukjancenko, O., & Zollinger, A. (2021). Sialylated human milk oligosaccharides program cognitive development through a non-genomic transmission mode. *Molecular Psychiatry, 26*(3), 1–18.

Hickey, R. M. (2012). The role of oligosaccharides from human milk and other sources in prevention of pathogen adhesion. *International Dairy Journal, 22*(2), 141–146.

Hirschmugl, B., Brandl, W., Csapo, B., van Poppel, M., Köfeler, H., Desoye, G., Wadsack, C., & Jantscher-Krenn, E. (2019). Evidence of human milk oligosaccharides in cord blood and maternal-to-fetal transport across the placenta. *Nutrients, 11*(11), 2640.

Hobbs, M., Jahan, M., Ghorashi, S. A., & Wang, B. (2021). Current perspective of sialylated milk oligosaccharides in mammalian milk: Implications for brain and gut health of newborns. *Foods, 10*(2), 473. https://doi.org/10.3390/foods10020473

Hundshammer, C., & Minge, O. (2020). In love with shaping you—Influential factors on the breast milk content of human milk oligosaccharides and their decisive roles for neonatal development. *Nutrients, 12*(11), 3568.

Jacobi, S. K., Yatsunenko, T., Li, D., Dasgupta, S., Yu, R. K., Berg, B. M., Chichlowski, M., & Odle, J. (2016). Dietary isomers of sialyllactose increase ganglioside sialic acid concentrations in the corpus callosum and cerebellum and modulate the colonic microbiota of formula-fed piglets. *The Journal of Nutrition, 146*(2), 200–208.

Jakobsen, L. M., Sundekilde, U. K., Andersen, H. J., Nielsen, D. S., & Bertram, H. C. (2019). Lactose and bovine milk oligosaccharides synergistically stimulate *B. longum* subsp. *longum* growth in a simplified model of the infant gut microbiome. *Journal of Proteome Research, 18*(8), 3086–3098.

Jakobsen, L., Maldonado-Gómez, M. X., Sundekilde, U. K., Andersen, H. J., Nielsen, D. S., & Bertram, H. C. (2020). Metabolic effects of bovine milk oligosaccharides on selected commensals of the infant microbiome—Commensalism and postbiotic effects. *Metabolites, 10*(4), 167.

Jantscher-Krenn, E., Zherebtsov, M., Nissan, C., Goth, K., Guner, Y. S., Naidu, N., Choudhury, B., Grishin, A. V., Ford, H. R., & Bode, L. (2012). The human milk oligosaccharide disialyllacto-*N*-tetraose prevents necrotising enterocolitis in neonatal rats. *Gut, 61*(10), 1417–1425.

Jena, P. K., Sheng, L., Nagar, N., Wu, C., Barile, D., Mills, D. A., & Wan, Y.-J. Y. (2018). Synbiotics *Bifidobacterium infantis* and milk oligosaccharides are effective in reversing cancer-prone non-alcoholic steatohepatitis using western diet-fed FXR knockout mouse models. *The Journal of Nutritional Biochemistry, 57*, 246–254.

Kellman, B. P., Zhang, Y., Logomasini, E., Meinhardt, E., Godinez-Macias, K. P., Chiang, A. W., Sorrentino, J. T., Liang, C., Bao, B., & Zhou, Y. (2020a). A consensus-based and readable extension of Linear Code for Reaction Rules (LiCoRR). *Beilstein Journal of Organic Chemistry, 16*(1), 2645–2662.

Kellman, B. P., Richelle, A., Yang, J.-Y. E., Chapla, D. G., Chiang, A. W.-T., Najera, J., Bao, B., Koga, N., Mohammad, M. A., & Bruntse, A. B. (2020b). Elucidating human milk oligosaccharide biosynthetic genes through network-based multi-omics integration. *bioRxiv*. https://doi.org/10.1101/2020.09.02.278663

Kikusui, T., Shimozawa, A., Kitagawa, A., Nagasawa, M., Mogi, K., Yagi, S., & Shiota, K. (2012). *N*-Acetylmannosamine improves object recognition and hippocampal cell proliferation in middle-aged mice. *Bioscience, Biotechnology, and Biochemistry, 76*(12), 2249–2254.

Kostopoulos, I., Elzinga, J., Ottman, N., Klievink, J. T., Blijenberg, B., Aalvink, S., Boeren, S., Mank, M., Knol, J., & de Vos, W. M. (2020). *Akkermansia muciniphila* uses human milk oligosaccharides to thrive in the early life conditions in vitro. *Scientific Reports, 10*(1), 1–17.

Kramer, M. S., & Kakuma, R. (2012). Optimal duration of exclusive breastfeeding. *Cochrane Database of Systematic Reviews, 8*.

Kuntz, S., Rudloff, S., & Kunz, C. (2008). Oligosaccharides from human milk influence growth-related characteristics of intestinally transformed and non-transformed intestinal cells. *The British Journal of Nutrition, 99*(3), 462–471.

Kuntz, S., Kunz, C., & Rudloff, S. (2009). Oligosaccharides from human milk induce growth arrest via G2/M by influencing growth-related cell cycle genes in intestinal epithelial cells. *The British Journal of Nutrition, 101*(9), 1306–1315.

Kuntz, S., Rudloff, S., & Kunz, C. (2019). Milk oligosaccharides from different cattle breeds influence growth-related characteristics of intestinal cells. *Frontiers in Nutrition, 6*, 31. https://doi.org/10.3389/fnut.2019.00031

Kunz, C., Rudloff, S., Baier, W., Klein, N., & Strobel, S. (2000). Oligosaccharides in human milk: Structural, functional, and metabolic aspects. *Annual Review of Nutrition, 20*(1), 699–722.

Lagström, H., Rautava, S., Ollila, H., Kaljonen, A., Turta, O., Mäkelä, J., Yonemitsu, C., Gupta, J., & Bode, L. (2020). Associations between human milk oligosaccharides and growth in infancy and early childhood. *The American Journal of Clinical Nutrition, 111*(4), 769–778.

Lane, J. A., Mariño, K., Naughton, J., Kavanaugh, D., Clyne, M., Carrington, S. D., & Hickey, R. M. (2012). Anti-infective bovine colostrum oligosaccharides: *Campylobacter jejuni* as a case study. *International Journal of Food Microbiology, 157*(2), 182–188.

Lane, J. A., O'Callaghan, J., Carrington, S. D., & Hickey, R. M. (2013). Transcriptional response of HT-29 intestinal epithelial cells to human and bovine milk oligosaccharides. *The British Journal of Nutrition, 110*(12), 2127–2137.

Lara-Villoslada, F., Debras, E., Nieto, A., Concha, A., Gálvez, J., López-Huertas, E., Boza, J., Obled, C., & Xaus, J. (2006). Oligosaccharides isolated from goat milk reduce intestinal inflammation in a rat model of dextran sodium sulfate-induced colitis. *Clinical Nutrition, 25*(3), 477–488.

Larsson, M. W., Lind, M. V., Laursen, R. P., Yonemitsu, C., Larnkjær, A., Mølgaard, C., Michaelsen, K. F., & Bode, L. (2019). Human milk oligosaccharide composition is associated with excessive weight gain during exclusive breastfeeding—An explorative study. *Frontiers in Pediatrics, 7*, 297. https://doi.org/10.3389/fped.2019.00297

Laucirica, D. R., Triantis, V., Schoemaker, R., Estes, M. K., & Ramani, S. (2017). Milk oligosaccharides inhibit human rotavirus infectivity in MA104 cells. *The Journal of Nutrition, 147*(9), 1709–1714.

Lee, H., de MeloSilva, V. L., Liu, Y., & Barile, D. (2015). Quantification of carbohydrates in whey permeate products using high-performance anion-exchange chromatography with pulsed amperometric detection. *Journal of Dairy Science, 98*(11), 7644–7649.

Lee, H., Cuthbertson, D. J., Otter, D. E., & Barile, D. (2016). Rapid screening of bovine milk oligosaccharides in a whey permeate product and domestic animal milks by accurate mass database and tandem mass spectral library. *Journal of Agricultural and Food Chemistry, 64*(32), 6364–6374.

Leong, A., Liu, Z., Almshawit, H., Zisu, B., Pillidge, C., Rochfort, S., & Gill, H. (2019). Oligosaccharides in goats' milk-based infant formula and their prebiotic and anti-infection properties. *The British Journal of Nutrition, 122*(4), 441–449.

Li, M., Monaco, M. H., Wang, M., Comstock, S. S., Kuhlenschmidt, T. B., Fahey, G. C., Jr., Miller, M. J., Kuhlenschmidt, M. S., & Donovan, S. M. (2014). Human milk oligosaccharides shorten rotavirus-induced diarrhea and modulate piglet mucosal immunity and colonic microbiota. *The ISME Journal, 8*(8), 1609–1620.

Liu, Z., Moate, P., Cocks, B., & Rochfort, S. (2014). Simple liquid chromatography–mass spectrometry method for quantification of major free oligosaccharides in bovine milk. *Journal of Agricultural and Food Chemistry, 62*(47), 11568–11574.

Liu, Z., Auldist, M., Wright, M., Cocks, B., & Rochfort, S. (2017). Bovine milk oligosaccharide contents show remarkable seasonal variation and intercow variation. *Journal of Agricultural and Food Chemistry, 65*(7), 1307–1313.

Liu, Z., Wang, T., Pryce, J. E., MacLeod, I. M., Hayes, B. J., Chamberlain, A. J., Vander Jagt, C., Reich, C. M., Mason, B. A., & Rochfort, S. (2019). Fine-mapping sequence mutations with a major effect on oligosaccharide content in bovine milk. *Scientific Reports, 9*(1), 1–12.

Lu, J., Zhang, Y., Song, B., Zhang, S., Pang, X., Sari, R. N., Liu, L., Wang, J., & Lv, J. (2020). Comparative analysis of oligosaccharides in Guanzhong and Saanen goat milk by using LC–MS/MS. *Carbohydrate Polymers, 235*, 115965.

Ma, L., McJarrow, P., Mohamed, H. J. J., Liu, X., Welman, A., & Fong, B. Y. (2018). Lactational changes in the human milk oligosaccharide concentration in Chinese and Malaysian mothers' milk. *International Dairy Journal, 87*, 1–10.

Maldonado-Gomez, M. X., Lee, H., Barile, D., Lu, M., & Hutkins, R. W. (2015). Adherence inhibition of enteric pathogens to epithelial cells by bovine colostrum fractions. *International Dairy Journal, 40*, 24–32.

Manthey, C. F., Autran, C. A., Eckmann, L., & Bode, L. (2014). Human milk oligosaccharides protect against enteropathogenic *Escherichia coli* attachment *in vitro* and EPEC colonization in suckling mice. *Journal of Pediatric Gastroenterology and Nutrition, 58*(2), 165–168.

Mariño, K., Lane, J. A., Abrahams, J. L., Struwe, W. B., Harvey, D. J., Marotta, M., Hickey, R. M., & Rudd, P. M. (2011). Method for milk oligosaccharide profiling by 2-aminobenzamide labeling and hydrophilic interaction chromatography. *Glycobiology, 21*(10), 1317–1330.

Marotta, M. M., & Hickey, R. M. (2018). Patent P12672EP00 'A process to enrich oligosaccharides from whey streams and a composition thereof'.

Marriage, B. J., Buck, R. H., Goehring, K. C., Oliver, J. S., & Williams, J. A. (2015). Infants fed a lower calorie formula with 2′ FL show growth and 2′ FL uptake like breast-fed infants. *Journal of Pediatric Gastroenterology and Nutrition, 61*(6), 649–658.

Marsaux, B., Van den Abbeele, P., Ghyselinck, J., Prioult, G., Marzorati, M., & Bogićević, B. (2020). Synbiotic effect of *Bifidobacterium lactis* CNCM I-3446 and bovine milk-derived oligosaccharides on infant gut microbiota. *Nutrients, 12*(8), 2268.

Martín, M. J., Martín-Sosa, S., & Hueso, P. (2002). Binding of milk oligosaccharides by several enterotoxigenic *Escherichia coli* strains isolated from calves. *Glycoconjugate Journal, 19*(1), 5–11.

Martinez-Ferez, A., Rudloff, S., Guadix, A., Henkel, C. A., Pohlentz, G., Boza, J. J., Guadix, E. M., & Kunz, C. (2006). Goats' milk as a natural source of lactose-derived oligosaccharides: Isolation by membrane technology. *International Dairy Journal, 16*(2), 173–181.

Martín-Ortiz, A., Salcedo, J., Barile, D., Bunyatratchata, A., Moreno, F. J., Martin-García, I., Clemente, A., Sanz, M. L., & Ruiz-Matute, A. I. (2016). Characterization of goat colostrum oligosaccharides by nano-liquid chromatography on chip quadrupole time-of-flight mass spectrometry and hydrophilic interaction liquid chromatography-quadrupole mass spectrometry. *Journal of Chromatography, 1428*, 143–153.

Martín-Ortiz, A., Moreno, F. J., Ruiz-Matute, A. I., & Sanz, M. L. (2019). Selective biotechnological fractionation of goat milk carbohydrates. *International Dairy Journal, 94*, 38–45.

Matsuki, T., Yahagi, K., Mori, H., Matsumoto, H., Hara, T., Tajima, S., Ogawa, E., Kodama, H., Yamamoto, K., & Yamada, T. (2016). A key genetic factor for fucosyllactose utilization affects infant gut microbiota development. *Nature Communications, 7*, 11939. https://doi.org/10.1038/ncomms11939

Mehra, R., Barile, D., Marotta, M., Lebrilla, C. B., Chu, C., & German, J. B. (2014). Novel high-molecular weight fucosylated milk oligosaccharides identified in dairy streams. *PLoS One, 9*(5), e96040. https://doi.org/10.1371/journal.pone.0096040

Meli, F., Puccio, G., Cajozzo, C., Ricottone, G. L., Pecquet, S., Sprenger, N., & Steenhout, P. (2014). Growth and safety evaluation of infant formulae containing oligosaccharides derived from bovine milk: A randomized, double-blind, noninferiority trial. *BMC Pediatrics, 14*(1), 1–11.

Meyrand, M., Dallas, D., Caillat, H., Bouvier, F., Martin, P., & Barile, D. (2013). Comparison of milk oligosaccharides between goats with and without the genetic ability to synthesize αs1-casein. *Small Ruminant Research, 113*(2-3), 411–420.

Monti, L., Cattaneo, T. M. P., Orlandi, M., & Curadi, M. C. (2015). Capillary electrophoresis of sialylated oligosaccharides in milk from different species. *Journal of Chromatography, 1409*, 288–291.

Morgan, B. L., & Winick, M. (1980). Effects of environmental stimulation on brain *N*-acetylneuraminic acid content and behavior. *The Journal of Nutrition, 110*(3), 425–432.

Morgan, B. L., Oppenheimer, J., & Winick, M. (1981). Effects of essential fatty acid deficiency during late gestation on brain *N*-acetylneuraminic acid metabolism and behaviour in the progeny. *The British Journal of Nutrition, 46*(2), 223–230.

Morozov, V., Hansman, G., Hanisch, F. G., Schroten, H., & Kunz, C. (2018). Human milk oligosaccharides as promising antivirals. *Molecular Nutrition & Food Research, 62*(6), 1700679.

Mudd, A. T., Fleming, S. A., Labhart, B., Chichlowski, M., Berg, B. M., Donovan, S. M., & Dilger, R. N. (2017). Dietary sialyllactose influences sialic acid concentrations in the prefrontal cortex and magnetic resonance imaging measures in corpus callosum of young pigs. *Nutrients, 9*(12), 1297.

Nagaraj, V., Upadhyay, N., Nath, B. S., & Singh, A. K. (2018). Advances in fractionation and analysis of milk carbohydrates. In V. Nagaraj, N. Upadhyay, B. S. Nath, & A. K. Singh (Eds.), *Technological approaches for novel applications in dairy processing* (p. 127). London: IntechOpen.

Nakamura, T., & Urashima, T. (2004). The milk oligosaccharides of domestic farm animals. *Trends in Glycoscience and Glycotechnology, 16*(88), 135–142.

Nakamura, T., Kawase, H., Kimura, K., Watanabe, Y., Ohtani, M., Arai, I., & Urashima, T. (2003). Concentrations of sialyloligosaccharides in bovine colostrum and milk during the prepartum and early lactation. *Journal of Dairy Science, 86*(4), 1315–1320.

Obelitz-Ryom, K., Bering, S. B., Overgaard, S. H., Eskildsen, S. F., Ringgaard, S., Olesen, J. L., Skovgaard, K., Pankratova, S., Wang, B., & Brunse, A. (2019). Bovine milk oligosaccharides with sialyllactose improves cognition in preterm pigs. *Nutrients, 11*(6), 1335.

Oliveira, D., Wilbey, R. A., Grandison, A., Duarte, L. C., & Roseiro, L. (2012). Separation of oligosaccharides from caprine milk whey, prior to prebiotic evaluation. *International Dairy Journal, 24*(2), 102–106.

Oliveira, D. L., Wilbey, R. A., Grandison, A. S., & Roseiro, L. B. (2015). Milk oligosaccharides: A review. *International Journal of Dairy Technology, 68*(3), 305–321.

Oliveros, E., Vázquez, E., Barranco, A., Ramírez, M., Gruart, A., Delgado-García, J. M., Buck, R., Rueda, R., & Martín, M. J. (2018). Sialic acid and sialylated oligosaccharide supplementation during lactation improves learning and memory in rats. *Nutrients, 10*(10), 1519.

Perdijk, O., Van Baarlen, P., Fernandez-Gutierrez, M. M., Van Den Brink, E., Schuren, F. H., Brugman, S., Savelkoul, H. F., Kleerebezem, M., & Van Neerven, R. (2019). Sialyllactose and galactooligosaccharides promote epithelial barrier functioning and distinctly modulate microbiota composition and short chain fatty acid production *in vitro*. *Frontiers in Immunology, 10*, 94.

Pérez-Escalante, E., Alatorre-Santamaría, S., Castañeda-Ovando, A., Salazar-Pereda, V., Bautista-Ávila, M., Cruz-Guerrero, A. E., Flores-Aguilar, J. F., & González-Olivares, L. G. (2020). Human milk oligosaccharides as bioactive compounds in infant formula: Recent advances and trends in synthetic methods. *Critical Reviews in Food Science and Nutrition, 62*, 181–214. https://doi.org/10.1080/10408398.2020.1813683

Pichler, M. J., Yamada, C., Shuoker, B., Alvarez-Silva, C., Gotoh, A., Leth, M. L., Schoof, E., Katoh, T., Sakanaka, M., & Katayama, T. (2020). Butyrate producing colonic *Clostridiales* metabolise human milk oligosaccharides and cross feed on mucin via conserved pathways. *Nature Communications, 11*(1), 1–15.

Plaza-Díaz, J., Fontana, L., & Gil, A. (2018). Human milk oligosaccharides and immune system development. *Nutrients, 10*(8), 1038.

Plows, J. F., Berger, P. K., Jones, R. B., Yonemitsu, C., Ryoo, J. H., Alderete, T. L., Bode, L., & Goran, M. I. (2020). Associations between human milk oligosaccharides (HMOs) and eating behaviour in Hispanic infants at 1 and 6 months of age. *Pediatric Obesity, 15*(12), e12686. https://doi.org/10.1371/journal.pone.0228323

Porfirio, S., Archer-Hartmann, S., Moreau, G. B., Ramakrishnan, G., Haque, R., Kirkpatrick, B. D., Petri, W. A., Jr., & Azadi, P. (2020). New strategies for profiling and characterization of human milk oligosaccharides. *Glycobiology, 30*(10), 774–786.

Poulsen, N. A., Robinson, R. C., Barile, D., Larsen, L. B., & Buitenhuis, B. (2019). A genome-wide association study reveals specific transferases as candidate loci for bovine milk oligosaccharides synthesis. *BMC Genomics, 20*(1), 1–15.

Prudden, A. R., Liu, L., Capicciotti, C. J., Wolfert, M. A., Wang, S., Gao, Z., Meng, L., Moremen, K. W., & Boons, G.-J. (2017). Synthesis of asymmetrical multiantennary human milk oligosaccharides. *Proceedings of the National Academy of Sciences of the United States of America, 114*(27), 6954–6959.

Puccio, G., Alliet, P., Cajozzo, C., Janssens, E., Corsello, G., Sprenger, N., Wernimont, S., Egli, D., Gosoniu, L., & Steenhout, P. (2017). Effects of infant formula with human milk oligosaccharides on growth and morbidity: A randomized multicenter trial. *Journal of Pediatric Gastroenterology and Nutrition, 64*(4), 624–631.

Quin, C., Vicaretti, S. D., Mohtarudin, N. A., Garner, A. M., Vollman, D. M., Gibson, D. L., & Zandberg, W. F. (2020). Influence of sulfonated and diet-derived human milk oligosaccharides on the infant microbiome and immune markers. *The Journal of Biological Chemistry, 295*(12), 4035–4048.

Quinn, E. M., Joshi, L., & Hickey, R. M. (2020a). Symposium review: Dairy-derived oligosaccharides—Their influence on host-microbe interactions in the gastrointestinal tract of infants. *Journal of Dairy Science, 103*(4), 3816–3827.

Quinn, E. M., Slattery, H., Walsh, D., Joshi, L., & Hickey, R. M. (2020b). *Bifidobacterium longum* subsp. *infantis* ATCC 15697 and goat milk oligosaccharides show synergism *in vitro* as anti-infectives against *Campylobacter jejuni*. *Foods, 9*(3), 348.

Quinn, E. M., O'Callaghan, T. F., Tobin, J. T., Murphy, J. P., Sugrue, K., Slattery, H., O'Donovan, M., & Hickey, R. M. (2020c). Changes to the oligosaccharide profile of bovine milk at the onset of lactation. *Dairy, 1*(3), 284–296.

Radke, M., Picaud, J.-C., Loui, A., Cambonie, G., Faas, D., Lafeber, H. N., de Groot, N., Pecquet, S. S., Steenhout, P. G., & Hascoet, J.-M. (2017). Starter formula enriched in prebiotics and probiotics ensures normal growth of infants and promotes gut health: A randomized clinical trial. *Pediatric Research, 81*(4), 622–631.

Robinson, R. C. (2019). Structures and metabolic properties of bovine milk oligosaccharides and their potential in the development of novel therapeutics. *Frontiers in Nutrition, 6*, 50.

Robinson, R. C., Poulsen, N. A., & Barile, D. (2018). Multiplexed bovine milk oligosaccharide analysis with aminoxy tandem mass tags. *PLoS One, 13*(4), e0196513. https://doi.org/10.1371/journal.pone.0196513

Robinson, R. C., Poulsen, N. A., Colet, E., Duchene, C., Larsen, L. B., & Barile, D. (2019). Profiling of aminoxy TMT-labeled bovine milk oligosaccharides reveals substantial variation in oligosaccharide abundance between dairy cattle breeds. *Scientific Reports, 9*(1), 1–10.

Rudloff, S., Pohlentz, G., Diekmann, L., Egge, H., & Kunz, C. (1996). Urinary excretion of lactose and oligosaccharides in preterm infants fed human milk or infant formula. *Acta Paediatrica, 85*(5), 598–603.

Rudloff, S., Pohlentz, G., Borsch, C., Lentze, M. J., & Kunz, C. (2012). Urinary excretion of *in vivo* 13 C-labelled milk oligosaccharides in breastfed infants. *The British Journal of Nutrition, 107*(7), 957–963.

Ruhaak, L. R., Stroble, C., Underwood, M. A., & Lebrilla, C. B. (2014). Detection of milk oligosaccharides in plasma of infants. *Analytical and Bioanalytical Chemistry, 406*(24), 5775–5784.

Ryan, J. T., Slattery, H., Hickey, R. M., & Marotta, M. (2018). Bovine milk oligosaccharides as anti-adhesives against the respiratory tract pathogen *Streptococcus pneumoniae*. *International Dairy Journal, 81*, 87–94.

Saben, J. L., Sims, C. R., Abraham, A., Bode, L., & Andres, A. (2021). Human milk oligosaccharide concentrations and infant intakes are associated with maternal overweight and obesity and predict infant growth. *Nutrients, 13*(2), 446.

Sakai, F., Ikeuchi, Y., Urashima, T., Fujihara, M., Ohtsuki, K., & Yanahira, S. (2006). Effects of feeding sialyllactose and galactosylated-*N*-acetylneuraminic acid on swimming learning ability and brain lipid composition in adult rats. *Journal of Applied Glycoscience, 53*(4), 249–254. https://doi.org/10.5458/jag.53.249

Sakanaka, M., Hansen, M. E., Gotoh, A., Katoh, T., Yoshida, K., Odamaki, T., Yachi, H., Sugiyama, Y., Kurihara, S., & Hirose, J. (2019). Evolutionary adaptation in fucosyllactose uptake systems supports bifidobacteria-infant symbiosis. *Science Advances, 5*(8), eaaw7696. https://doi.org/10.1126/sciadv.aaw7696

Samuel, T. M., Binia, A., de Castro, C. A., Thakkar, S. K., Billeaud, C., Agosti, M., Al-Jashi, I., Costeira, M. J., Marchini, G., Martínez-Costa, C., Picaud, J.-C., Stiris, T., Stoicescu, S.-M., Vanpeé, M., Domellöf, M., Austin, S., & Sprenger, N. (2019). Impact of maternal characteristics on human milk oligosaccharide composition over the first 4 months of lactation in a cohort of healthy European mothers. *Scientific Reports, 9*, 11767.

Sela, D. A., & Mills, D. A. (2010). Nursing our microbiota: Molecular linkages between bifidobacteria and milk oligosaccharides. *Trends in Microbiology, 18*(7), 298–307.

Simeoni, U., Berger, B., Junick, J., Blaut, M., Pecquet, S., Rezzonico, E., Grathwohl, D., Sprenger, N., Brüssow, H., & Team, S. (2016). Gut microbiota analysis reveals a marked shift to bifidobacteria by a starter infant formula containing a synbiotic of bovine milk-derived oligosaccharides and *Bifidobacterium animalis* subsp. *lactis* CNCM I-3446. *Environmental Microbiology, 18*(7), 2185–2195.

Sischo, W. M., Short, D. M., Geissler, M., Bunyatratchata, A., & Barile, D. (2017). Comparative composition, diversity, and abundance of oligosaccharides in early lactation milk from commercial dairy and beef cows. *Journal of Dairy Science, 100*(5), 3883–3892.

Smilowitz, J. T., Lemay, D. G., Kalanetra, K. M., Chin, E. L., Zivkovic, A. M., Breck, M. A., German, J. B., Mills, D. A., Slupsky, C., & Barile, D. (2017). Tolerability and safety of the intake of bovine milk oligosaccharides extracted from cheese whey in healthy human adults. *Journal of Nutritional Science, 6*, e6.

Sousa, H. M. O. (2019). *ECG compression and QRS detection: An IoT approach*. Thesis. Faculdade de Ciencias, Universadade do Porto, Portugal.

Sousa, Y. R., Araújo, D. F., Pulido, J. O., Pintado, M. M. E., Martínez-Férez, A., & Queiroga, R. C. (2019). Composition and isolation of goat cheese whey oligosaccharides by membrane technology. *International Journal of Biological Macromolecules, 139*, 57–62.

Sprenger, N., Lee, L. Y., De Castro, C. A., Steenhout, P., & Thakkar, S. K. (2017). Longitudinal change of selected human milk oligosaccharides and association to infants' growth, an observatory, single center, longitudinal cohort study. *PLoS One, 12*(2), e0171814.

Sumiyoshi, W., Urashima, T., Nakamura, T., Arai, I., Nagasawa, T., Saito, T., Tsumura, N., Wang, B., Brand-Miller, J., Watanabe, Y., & Kimura, K. (2004). Galactosyllactoses in the milk of Japanese women: Changes in concentration during the course of lactation. *Journal of Applied Glycoscience, 51*, 341–344.

Sunds, A. V., Bunyatratchata, A., Robinson, R., Glantz, M., Paulsson, M., Leskauskaite, D., Pihlanto, A., Inglingstad, R., Devold, T. G., & Vegarud, G. E. (2021). Comparison of bovine milk oligosaccharides in native North European cattle breeds. *International Dairy Journal, 114*, 104917.

Tao, N., DePeters, E., Freeman, S., German, J., Grimm, R., & Lebrilla, C. B. (2008). Bovine milk glycome. *Journal of Dairy Science, 91*(10), 3768–3778.

Tarr, A. J., Galley, J. D., Fisher, S. E., Chichlowski, M., Berg, B. M., & Bailey, M. T. (2015). The prebiotics 3′ sialyllactose and 6′ sialyllactose diminish stressor-induced anxiety-like behavior and colonic microbiota alterations: Evidence for effects on the gut–brain axis. *Brain, Behavior, and Immunity, 50*, 166–177.

Thum, C., Cookson, A., McNabb, W. C., Roy, N. C., & Otter, D. (2015). Composition and enrichment of caprine milk oligosaccharides from New Zealand Saanen goat cheese whey. *Journal of Food Composition and Analysis, 42*, 30–37.

Thum, C., McNabb, W. C., Young, W., Cookson, A. L., & Roy, N. C. (2016). Prenatal caprine milk oligosaccharide consumption affects the development of mice offspring. *Molecular Nutrition & Food Research, 60*(9), 2076–2085.

Thum, C., Weinborn, V., Barile, D., McNabb, W. C., Roy, N. C., & de Moura Bell, M. L. N. (2019). Understanding the effects of lactose hydrolysis modeling on the main oligosaccharides in goat milk whey permeate. *Molecules, 24*(18), 3294.

Thurl, S., Munzert, M., Boehm, G., Matthews, C., & Stahl, B. (2017). Systematic review of the concentrations of oligosaccharides in human milk. *Nutrition Reviews, 75*(11), 920–933.

Tonon, K. M., de Morais, M. B., Abrão, A. C. F. V., Miranda, A., & Morais, T. B. (2019). Maternal and infant factors associated with human milk oligosaccharides concentrations according to secretor and Lewis phenotypes. *Nutrition, 11*(6), 1358.

Tram, T., Miller, J. B., McNeil, Y., & McVeagh, P. (1997). Sialic acid content of infant saliva: Comparison of breast fed with formula fed infants. *Archives of Disease in Childhood, 77*(4), 315–318.

Triantis, V., Bode, L., & van Neerven, R. J. (2018). Immunological effects of human milk oligosaccharides. *Frontiers in Pediatrics, 6*, 190.

Tuplin, E. W. N., Chleilat, F., Alukic, E., & Reimer, R. A. (2021). The effects of human milk oligosaccharide supplementation during critical periods of development on the mesolimbic dopamine system. *Neuroscience, 459*, 166–178.

Underwood, M. A., Gaerlan, S., De Leoz, M. L. A., Dimapasoc, L., Kalanetra, K. M., Lemay, D. G., German, J. B., Mills, D. A., & Lebrilla, C. B. (2015). Human milk oligosaccharides in premature infants: Absorption, excretion, and influence on the intestinal microbiota. *Pediatric Research, 78*(6), 670–677.

Urakami, H., Saeki, M., Watanabe, Y., Kawamura, R., Nishizawa, S., Suzuki, Y., Watanabe, A., & Ajisaka, K. (2018). Isolation and assessment of acidic and neutral oligosaccharides from goat milk and bovine colostrum for use as ingredients of infant formulae. *International Dairy Journal, 83*, 1–9.

Urashima, T., Saito, T., Nakamura, T., & Messer, M. (2001). Oligosaccharides of milk and colostrum in non-human mammals. *Glycoconjugate Journal, 18*(5), 357–371.

Urashima, T., Asakuma, S., Leo, F., Fukuda, K., Messer, M., & Oftedal, O. T. (2012). The predominance of type I oligosaccharides is a feature specific to human breast milk. *Advances in Nutrition, 3*(3), 473S–482S.

Urashima, T., Taufik, E., Fukuda, K., & Asakuma, S. (2013). Recent advances in studies on milk oligosaccharides of cows and other domestic farm animals. *Bioscience, Biotechnology, and Biochemistry, 77*(3), 455–466.

Urashima, T., Messer, M., & Oftedal, O. T. (2017). Oligosaccharides in the milk of other mammals. In M. McGuire, M. McGuire, & L. Bode (Eds.), *Probiotics and probiotics in human milk. Origins and functions of milk-borne oligosaccharides and bacteria* (pp. 45–139). London: Academic Press.

Urashima, T., Hirabayashi, J., Sato, S., & Kobata, A. (2018). Human milk oligosaccharides as essential tools for basic and application studies on galectins. *Trends in Glycoscience and Glycotechnology, 30*(172), SE51–SE65.

van Leeuwen, S. S., Te Poele, E. M., Chatziioannou, A. C., Benjamins, E., Haandrikman, A., & Dijkhuizen, L. (2020). Goat milk oligosaccharides: Their diversity, quantity, and functional properties in comparison to human milk oligosaccharides. *Journal of Agricultural and Food Chemistry, 68*(47), 13469–13485.

Vazquez, E., Santos-Fandila, A., Buck, R., Rueda, R., & Ramirez, M. (2017). Major human milk oligosaccharides are absorbed into the systemic circulation after oral administration in rats. *The British Journal of Nutrition, 117*(2), 237–247.

Vicaretti, S. D., Mohtarudin, N. A., Garner, A. M., & Zandberg, W. F. (2018). Capillary electrophoresis analysis of bovine milk oligosaccharides permits an assessment of the influence of diet and the discovery of nine abundant sulfated analogues. *Journal of Agricultural and Food Chemistry, 66*(32), 8574–8583.

Walsh, C., Lane, J. A., van Sinderen, D., & Hickey, R. M. (2020a). Human milk oligosaccharides: Shaping the infant gut microbiota and supporting health. *Journal of Functional Foods, 72*, 104074.

Walsh, C., Lane, J. A., van Sinderen, D., & Hickey, R. M. (2020b). From lab bench to formulated ingredient: Characterization, production, and commercialization of human milk oligosaccharides. *Journal of Functional Foods, 72*, 104052.

Wang, B. (2009). Sialic acid is an essential nutrient for brain development and cognition. *Annual Review of Nutrition, 29*, 177–222.

Wang, Y., & Yu, J. (2021). Membrane separation processes for enrichment of bovine and caprine milk oligosaccharides from dairy byproducts. *Comprehensive Reviews in Food Science and Food Safety, 20*, 3667–3689. https://doi.org/10.1111/1541-4337.12758

Wang, B., Miller, J. B., Sun, Y., Ahmad, Z., McVeagh, P., & Petocz, P. (2001). A longitudinal study of salivary sialic acid in preterm infants: Comparison of human milk–fed versus formula-fed infants. *The Journal of Pediatrics, 138*(6), 914–916.

Wang, B., McVeagh, P., Petocz, P., & Brand-Miller, J. (2003). Brain ganglioside and glycoprotein sialic acid in breastfed compared with formula-fed infants. *The American Journal of Clinical Nutrition, 78*(5), 1024–1029.

Wang, B., Yu, B., Karim, M., Hu, H., Sun, Y., McGreevy, P., Petocz, P., Held, S., & Brand-Miller, J. (2007). Dietary sialic acid supplementation improves learning and memory in piglets. *The American Journal of Clinical Nutrition, 85*(2), 561–569.

Wang, H. X., Chen, Y., Haque, Z., de Veer, M., Egan, G., & Wang, B. (2019). Sialylated milk oligosaccharides alter neurotransmitters and brain metabolites in piglets: An *in vivo* magnetic resonance spectroscopic (MRS) study. *Nutritional Neuroscience, 24*, 885–895. https://doi.org/10.1080/1028415X.2019.1691856

Wang, M., Monaco, M. H., Hauser, J., Yan, J., Dilger, R. N., & Donovan, S. M. (2021). Bovine milk oligosaccharides and human milk oligosaccharides modulate the gut microbiota composition and volatile fatty acid concentrations in a preclinical neonatal model. *Microorganisms, 9*(5), 884. https://doi.org/10.3390/microorganisms9050884

Wickramasinghe, S., Hua, S., Rincon, G., Islas-Trejo, A., German, J. B., Lebrilla, C. B., & Medrano, J. F. (2011). Transcriptome profiling of bovine milk oligosaccharide metabolism genes using RNA-sequencing. *PLoS One, 6*(4), e18895.

Wu, R. Y., Li, B., Koike, Y., Määttänen, P., Miyake, H., Cadete, M., Johnson-Henry, K. C., Botts, S. R., Lee, C., & Abrahamsson, T. R. (2019). Human milk oligosaccharides increase mucin expression in experimental necrotizing enterocolitis. *Molecular Nutrition & Food Research, 63*(3), 1800658.

Yu, Z.-T., Chen, C., & Newburg, D. S. (2013). Utilization of major fucosylated and sialylated human milk oligosaccharides by isolated human gut microbes. *Glycobiology, 23*(11), 1281–1292.

Yue, H., Han, Y., Yin, B., Cheng, C., & Liu, L. (2020). Comparison of the antipathogenic effect toward *Staphylococcus aureus* of *N*-linked and free oligosaccharides derived from human, bovine, and goat milk. *Journal of Food Science, 85*(8), 2329–2339.