

# Oligosaccharides and glycoconjugates in bovine milk and colostrum

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Oligosaccharides and glycoconjugates are some of the most important bioactive components in milk. A great deal of information is available on the biological function of the components from human milk. Their primary role seems to be in providing protection against pathogens by acting as competitive inhibitors for the binding sites on the epithelial surfaces of the intestine. Evidence is also available to support the role of some of these components as growth promoters for genera of beneficial microflora in the colon. Compared with human milk, levels of oligosaccharides in bovine milk are very low. Nevertheless, a number of neutral and acidic oligosaccharides have been isolated from bovine milk and characterised. The highest concentration of these molecules is found in early postparturition milk (colostrum). The chemical structure of the oligosaccharides and many of the glycoconjugates from bovine milk are similar to those in human milk. It is likely that bovine oligosaccharides and glycoconjugates can be used in milk products as bioactive components in human nutrition.

## Oligosaccharides: Glycoconjugates: Bovine milk: Colostrum

### Introduction

Milk is nature's designer food to fulfil the nutritional needs for the growth and development of the neonate. The composition of milk progressively changes postparturition to meet the changing and specific requirements of the suckling neonate. During the first few days postparturition, the 'early milk' (colostrum) has a composition quite different from that of 'mature' milk. In the case of bovine milk, the first four days of milk postparturition constitute colostrum. In addition to normal nutrients such as proteins, carbohydrates, fats, vitamins and minerals, colostrum contains many other biologically active constituents. These include growth factors, antimicrobial compounds and immune-enhancing components. The role of milk in these first few days in the life of a newborn calf is not only to provide nutrition, but also to provide protection against infection while the immune system is still developing.

In addition to lactose, the carbohydrate or carbohydrate-containing components of bovine milk and colostrum include oligosaccharides, glycoproteins and glycolipids. In this review we focus on the composition, chemical structure and physiological role of these carbohydrate-rich components. We also explore the role of these compounds as micronutrients in human nutrition, especially with regard to their potential health benefits.

### General composition of bovine and human milk and colostrum

The composition of bovine milk and colostrum, in terms of the major nutritional components, are summarised in Table 1. In comparison with milk, it is difficult to assume a 'typical' composition profile for bovine colostrum. The composition and physical characters vary greatly with individual breeds, feeding, length of dry period of cows and time postparturition. A notable difference between bovine milk or colostrum and their human counterparts is their oligosaccharide content.

### Oligosaccharides in bovine colostrum and milk

Oligosaccharides are strictly defined as carbohydrates which contain between three and ten monosaccharide residues covalently linked through glycosidic bonds. However, disaccharides (which contain only two residues) other than lactose are often also designated as oligosaccharides. Oligosaccharides are divided into two broad classes, neutral and acidic. Neutral oligosaccharides do not contain any charged carbohydrate residues. However, acidic oligosaccharides contain one or more residues of *N*-acetylneuraminic acid (sialic acid) which are negatively charged (hence the term acidic).

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**Table 1.** Composition of bovine and human milk and colostrum (g/100 ml)

	Protein	Lactose	Oligosaccharides	Fat	Ash
Bovine milk*	3.4	4.6	‡	3.7	0.7
Bovine colostrum†	4.1–14.0	2.7–4.6	‡	3.9–4.4	0.5–2
Human milk§	1.0	6.8	1.3	3.9	0.2
Human colostrum§	1.0	5.5	2.4	3.0	–

\* Jenness (1974).

† Kulkarni &amp; Pimpale (1989).

‡ Data not reported.

§ Kunz, Rodriguez-Palmero, Berthold &amp; Jensen (1999).

It was recognised as early as 1933 that human milk contained a major carbohydrate component which was not lactose (Polonosky & Lespagnol, 1933). At the time, the component was termed gynolactose, but it is now known that this fraction consists mainly of oligosaccharides. In human milk, oligosaccharides are the third major component, present at a higher concentration (on a weight per volume basis) than total protein (Table 1). With advances in analytical techniques, approximately 80 acidic and neutral oligosaccharides have been isolated from human milk and their chemical structures have been determined (Newberg & Neubauer, 1995).

In comparison to human breast milk, the oligosaccharide composition of bovine milk is simple: ten sialyl oligosaccharides (Table 3) and eight neutral oligosaccharides (Table 2) have been described in bovine milk and colostrum.

#### Neutral oligosaccharides

The first report on neutral oligosaccharides from bovine milk or colostrum appeared in 1984. Saito *et al.* (1984) described the chemical structure of two neutral disaccharides in colostrum collected 6 h postparturition from Holstein–Friesian cows. These two disaccharides accounted for 74 % of the neutral oligosaccharide fraction (excluding lactose). *N*-Acetyl-lactosamine was by far the major component, accounting for 70 %.

Using liquid chromatography on amino silica gel, Saito *et al.* (1987) isolated three neutral trisaccharides from bovine colostrum, one of which contained fucose. This was the first report describing a fucosylated oligosaccharide from bovine colostrum. Two further trisaccharides and a pentasaccharide from bovine colostrum were subsequently described by Urashima *et al.* (1991). The chemical structure of these molecules in bovine colostrum is summarised in Table 2. In all cases, early colostrum (i.e.

colostrum collected within 6 h postparturition) was used in the studies. It has been reported that the oligosaccharide composition of colostrum changes rapidly postparturition, and Saito *et al.* (1984) showed that two key disaccharides present in early colostrum are completely absent 7 days after parturition.

#### Acidic oligosaccharides

Quantitatively, oligosaccharides are only present in bovine milk in trace amounts; considerably higher concentrations are present in bovine colostrum. As milk production matures postparturition, the concentration of these oligosaccharides declines rapidly. This makes isolation and analysis technically difficult.

The first report on sialyl oligosaccharides from bovine colostrum appeared in 1956 (Kuhn & Brossmer, 1956). Ten years later, Schneir & Rafelson (1966) devised a simpler procedure for extraction of larger quantities of sialyl oligosaccharides from bovine colostrum and reported the isolation of two isomers of *N*-acetylneuraminyl-lactose. Further improvements in the methodology by Veh *et al.* (1981), and later Parkkinen & Finne (1987), resulted in the discovery of a range of sialyl oligosaccharides from bovine colostrum.

Parkkinen & Finne (1985) also demonstrated the presence of two phosphorylated sialyl oligosaccharides in bovine colostrum. One of these compounds was structurally similar to a previously characterised sialyl oligosaccharide found in human urine, namely sialyl-lactosamine-1-phosphate. The second sialyl oligosaccharide was completely novel and contained a phosphate group in position 6, i.e. sialyl-lactosamine-6-phosphate. In total, ten sialylated oligosaccharides have now been described from bovine colostrum. The chemical structures of these oligosaccharides are summarised in Table 3.

The most abundant acidic oligosaccharide reported in

**Table 2.** Neutral oligosaccharides of bovine milk and colostrum

Structure	Name	Reference
Gal β(1–3) Gal β(1–4) Glc	3'-Galactosyl-lactose	Saito <i>et al.</i> 1987
Gal β(1–6) Gal β(1–4) Glc	6'-Galactosyl-lactose	Saito <i>et al.</i> 1987
Gal-β(1–4) Fucα(1–3) GlcNAc	3-Fucosyl <i>N</i> -acetyl-lactosamine	Saito <i>et al.</i> 1987
Gal β(1–4) GlcNAc	<i>N</i> -Acetyl-lactosamine	Saito <i>et al.</i> 1984
Gal NAcβ(1–4) Glc	<i>N</i> -Acetylgalactosaminyl glucose	Saito <i>et al.</i> 1984
GalNAcα(1–3) Gal β(1–4) Glc	<i>N</i> -Acetylgalactosyl-lactose	Urashima <i>et al.</i> 1991
Gal β(1–4) GlcNAc β(1–6) Gal β(1–3) [Galβ(1–4) Glc]	Lacto- <i>N</i> -novopentaose	Urashima <i>et al.</i> 1991
Gal α(1–3) Gal β(1–4) Glc	α-3'-Galactosyl lactose	Urashima <i>et al.</i> 1991

**Table 3.** Acidic oligosaccharides of bovine milk and colostrum

Structure	Name	Reference
NeuAc $\alpha$ (2-3) Gal $\beta$ (1-4) Glc	3-Sialyl-lactose	Schneir & Rafelson, 1966
NeuAc $\alpha$ (2-6) Gal $\beta$ (1-4) Glc	6-Sialyl-lactose	Schneir & Rafelson, 1966
NeuGl $\alpha$ (2-6) Gal $\beta$ (1-4) Glc	6-Glucolyneuraminyllactose	Veh <i>et al.</i> 1981
NeuAc $\alpha$ (2-6) Gal $\beta$ (1-4) GlcNAc	6-Sialyl-lactosamine	Veh <i>et al.</i> 1981
NeuGl $\alpha$ (2-6) Gal $\beta$ (1-4) GlcNAc	6-Glucolyneuraminyllactosamine	Veh <i>et al.</i> 1981
NeuAc $\alpha$ (2-3) Gal $\beta$ (1-3)Gal- $\beta$ (1-4) Glc	3-Sialyl galactosyl-lactose	Parkkinen & Finne, 1987
NeuAc $\alpha$ (2-8) NeuAc $\alpha$ (2-3)Gal $\beta$ (1-4) Glc	Disialyl lactose	Parkkinen & Finne, 1987
NeuAc $\alpha$ (2-6) Gal $\beta$ (1-4) GlcNAc $\alpha$ -1-P	Sialyl-lactosamine-1-phosphate	Parkkinen & Finne, 1987
NeuAc $\alpha$ (2-6) Gal $\beta$ (1-4) GlcNAc $\alpha$ -6-P	Sialyl-lactosamine-6-phosphate	Parkkinen & Finne, 1987
NeuGl $\alpha$ (2-3) Gal $\beta$ (1-4) Glc	3-Glucolyneuraminyllactose	Veh <i>et al.</i> 1981

bovine colostrum is 3-sialyl-lactose followed by sialyl-lactosamine, 6-sialyl-lactose and disialyl-lactose. Together, 3- and 6-sialyl-lactose account for more than 50 % of the total oligosaccharides present in bovine colostrum. Interestingly, 3- and 6-sialyl-lactose are also major components of human milk and have been reported to reach concentrations of 1.0 g/l (McVeagh & Brand Miller, 1997). Table 4 compares the major oligosaccharides of human and bovine milks.

#### Glycolipids, glycopeptides and glycoproteins from bovine milk

In bovine milk, numerous complex carbohydrates are present as glyconjugates, i.e. the carbohydrate chains are attached covalently to the backbone of either proteins or lipids (Kobata, 1977). There has been significant interest in these molecules, especially in their potential value as micronutrients. For instance, infant formulas made with various bovine milk fractions have been assessed for their glycoconjugate content and with respect to benefits of complex carbohydrates for bottle-fed infants (Carlson, 1985; Cleary *et al.* 1985, Sanchez-Diaz *et al.* 1997). In a 1990 study, Neesser *et al.* used two fractionation

procedures to quantitate gangliosides, neutral glycolipids and non-fat complex carbohydrates (glycoproteins and glycopeptides) from bovine milk and bovine milk-based infant formulas. This study concluded that most bovine milk-based infant formulas contained complex carbohydrates in amounts similar to those found in bovine milk. The main ganglioside in bovine milk is reported to be ganglioside containing two sialic acid residues (GD3) (Takamizawa *et al.* 1986).

Mucins are a family of glycoproteins which are heavily glycosylated. The first report on a mucin-like glycoprotein in bovine milk appeared in 1993, when Kanamaru *et al.* (1993) purified a high-molecular-weight glycoprotein from whey by gel-filtration. Similar high-molecular-weight mucin-like glycoproteins have been reported from human milk (Shimazu *et al.* 1986). The amino acid composition and lectin binding properties of the bovine mucin were found to be similar to the human counterpart. In human milk, the mucin-like glycoprotein has been shown to be part of the milk fat globular membrane (MFGM) and its function is believed to provide protection against protease degradation of the MFGM surface. Milk mucins, or their fragments, may also have a role in the inhibition of microbial infection. Schrotten *et al.* (1992) demonstrated

**Table 4.** Major oligosaccharides of human milk and bovine colostrum\*

Oligosaccharide	Human milk† (g/l)	Bovine colostrum† (g/l)	Bovine colostrum‡ (µmol/l)
Lactose	55-70	40-50	27-46§
Lacto-N-tetraose	0.5-1.5	-	-
Lacto-N-fucopentaose I	1.2-1.7	-	-
Lacto-N-fucopentaose II	0.3-1.0	-	-
Lacto-N-fucopentaose III	0.01-0.2	-	-
Lacto-N-difucohexaose	0.1-0.2	-	-
Acidic oligosaccharides			
6-Sialyl-lactose	0.3-0.5	Trace	30
3-Sialyl-lactose	0.1-0.3	Trace	150
Sialyl-lacto-N-tetraose a	0.03-0.2		
Sialyl-lacto-N-tetraose c	0.1-0.6		
Sialyl-lactosamine		Trace	70
Sialyl galactosyl-lactose		Trace	3
Disialyl-lactose		Trace	30
Sialyl-lactose-1-phosphate		Trace	3
Sialyl-lactose-6-phosphate		-	1
3-Glucolyl neuramyl-lactose		-	2
NeuAc2-lacto-N-tetraose	0.2-0.6	-	-

\* Table adapted from Kunz *et al.* (1999).

† Data taken from Montreuil & Mullet (1960), and Kunz & Rudloff (1993).

‡ Data from Parkkinen & Finne (1987).

§ g/l.

that milk mucin components interfere with the adhesion of *Escherichia coli* to epithelial cells of the buccal cavity. However, the exact biological significance of the mucin-like glycoprotein in bovine milk is not known.

### Physiological function and nutritional implications of oligosaccharides and glycoconjugates

One theory on the physiological function of oligosaccharides *in vivo* is that they provide a low osmolar source of energy. This may be true in the case of animals where oligosaccharides are the predominant carbohydrate component of milk, and the gastrointestinal tract of that animal is equipped with appropriate enzymes to hydrolyse and assimilate the oligosaccharides. A good example of this is marsupials, the milk of which, unlike bovine milk, is low in lactose and has high levels of oligosaccharides (Green *et al.* 1987; Messer *et al.* 1987).

Human milk is also rich in oligosaccharides. However, there is enough evidence available in the scientific literature to suggest that most of the human milk oligosaccharides are not digested or absorbed into the small intestine of infants, and are instead delivered intact into the colon (Brand Miller *et al.* 1995; McVeagh & Brand Miller, 1997). It is, therefore, reasonable to assume that oligosaccharides are present in milk for reasons other than nutrition.

#### *Oligosaccharides as prebiotics*

The definition of prebiotics is 'non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon that can improve the host health' (Gibson & Roberfroid, 1995).

Milk oligosaccharides fit this definition because they are neither digested nor absorbed in the upper intestinal tract of humans but are delivered intact into the colon where they can act as nutrients for colonic microflora. There is evidence that neutral oligosaccharides present in human milk act as growth enhancers for bacteria of the genus *Bifidobacterium* in infants. Yoshihama *et al.* (1982) compared the faecal flora of breast-fed infants with that of bottle-fed infants, and showed that levels of bifidobacteria were significantly higher in the former. This study further showed that if the milk of bottle-fed infants was supplemented with 6-galactosyl-lactose, an increase in the faecal population of bifidobacteria was observed. It was postulated that the increased metabolic activity of larger populations of bifidobacteria in the lumen can decrease the intestinal pH, and that this in turn can inhibit proliferation of pathogenic Gram-negative bacteria such as *Shigella flexneri* and *Escherichia coli* (Montreuil, 1994). Bovine colostrum is also known to contain galactosyl-lactose together with other neutral oligosaccharides (Saito *et al.* 1987).

#### *Oligosaccharides as soluble receptors for pathogenic bacteria and viruses*

Oligosaccharides may be viewed as a 'natural' way of

protecting neonates against infection. It is possible that they act as competitive inhibitors against important gut pathogens at the intestinal surface. As discussed above, human milk contains more than 80 oligosaccharides and these molecules do not seem to be digested in the small intestine. Milk bathes the gastrointestinal tract of the neonate and the large range of oligosaccharides may provide a means to inhibit pathogen adhesion to the intestinal epithelium.

A large body of evidence is accumulating to support such a hypothesis (e.g. for reviews see Varki, 1993; Zopf & Roth, 1996; Newburg, 1999). It is believed that oligosaccharides and glycoconjugates in milk and colostrum are soluble receptor analogues of epithelial cell-surface carbohydrates and can therefore compete with virulent bacteria and viruses for attachment sites. Andersson *et al.* (1986) showed that human milk oligosaccharides inhibited adhesion of pneumococci or influenza virus to pharyngeal or buccal epithelial cells. Similarly, sialylated oligosaccharides were shown to inhibit binding of pathogenic strains of *E. coli* in neonates (Parkkinen *et al.* 1983; Korhonen *et al.* 1985). Neutral oligosaccharides from human milk may protect the intestinal tract of neonates from *Vibrio cholera* (Holmgren *et al.* 1983). The role of fucosylated oligosaccharides in the interaction of an enterotoxin with cells has also been reported (Brand Miller *et al.* 1994). More recently Simon *et al.* (1997) demonstrated that adhesion to epithelial cells by the ulcer-causing human pathogen *Helicobacter pylori* was inhibited by sialylated oligosaccharides. In this study the binding of thirteen bacterial isolates to epithelial cell-lines was examined and the effect of pretreatment of the bacteria with oligosaccharides, glycoproteins and glycolipids was determined. The most effective inhibitor of binding was found to be 3-sialyl-lactose which had millimolar 50 % inhibitory concentration (IC<sub>50</sub>).

### Conclusion

In summary, it can be concluded that oligosaccharides and glycoconjugates are important bioactive molecules, present not as nutrients for energy but to provide protection in the first few days in the life of a newborn. They may have a role as micronutrients to meet the specific need of rapidly developing infant, e.g. a source of sialic acid which has a role in the development of the nervous system. Levels of oligosaccharides and glycoconjugates are higher in colostrum than mature milk for both humans and bovines, in bovine milk the level of oligosaccharides drops to trace amounts postparturition. In comparison to human milk oligosaccharides which have a large and diverse array of molecules, the composition of bovine milk oligosaccharides is simple. However, structurally bovine oligosaccharides and glycoconjugates are similar to those found in human milk. It is reasonable to assume that the bioactivity of such molecules would also be similar and therefore can be used in milk products with 'functional attributes' for human nutrition.

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