Oligosaccharides in goat’s milk-based infant formula and their prebiotic and anti-infection properties

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Abstract

Human milk contains an abundant supply and diverse array of oligosaccharides (HMOs) that are known to impart significant health benefits to the nursing infant including establishment and maintenance of a healthy gut microflora, immune development and protection against gastrointestinal infections. When breast-feeding is not possible or insufficient, infant formulas are commonly used as an alternative. However, limited information is available about the presence of naturally occurring oligosaccharides in these infant formulas and their likely health benefits. The present study examined the presence of naturally occurring oligosaccharides in commercial goat’s milk-based Stage 1 and Stage 2 infant formulas and their prebiotic and anti-infection properties. Liquid chromatography/mass spectrometry (LC/MS) was used to detect and quantify oligosaccharides and their prebiotic potential was assessed by their ability, at concentrations present in reconstituted ready-to-use infant formula, to promote the growth of *Bifidobacterium animalis* subsp. *lactis* BB12, *Bifidobacterium longum* BB536, *Lactobacillus acidophilus* 4461 and *Lactobacillus casei* 2607 in vitro. For anti-infection properties, the ability of goat milk oligosaccharides to prevent the adhesion of *Escherichia coli* NCTC 10418 and a *Salmonella typhimurium* isolate to Caco-2 cells was investigated. The results showed the presence of 14 quantifiable oligosaccharides in stage-1 and stage-2 goat’s milk-based infant formula. This was similar to the numbers of oligosaccharides detected in the fresh goat’s milk. Of these, five were structurally similar to those found in human milk. These oligosaccharides were shown to significantly enhance the growth of bifidobacteria and lactobacilli and reduce the adhesion of *E. coli* NCTC 10418 and *S. typhimurium* to Caco-2 cells. Together, these results suggest that oligosaccharides naturally present in goat’s milk-based infant formula exhibit strong prebiotic and anti-pathogen adhesion properties and may confer gut health benefits to infants.
Introduction

Breast milk is considered a complete food for the newborn and breastfeeding is known to provide a wide range of health benefits to both mother and the baby. The World Health Organization (WHO) recommends exclusive breastfeeding for the first six months of an infant’s life, and continued breastfeeding with complementary foods for up to two years of age (1). However, when a mother’s milk is not available, infant milk formula is considered as an effective alternative. Cow milk-based infant formulas are widely used, but goat milk-based products are becoming increasingly popular as goat’s milk is considered to be more similar to human milk compared with cow’s milk. This includes higher levels of oligosaccharides in goat’s milk relative to milk of other mammalian species (2) and lower levels of α-S1 casein (3). Goat’s milk is also reported to exhibit significant homology in lactoferrin N-glycans with human milk” (4).

Human milk is known to contain high concentrations (5-20g/L in mature milk) and diversity of oligosaccharides (5, 6). Several studies have shown that oligosaccharides, particularly HMOs, are effective in selectively promoting the growth of bifidobacteria and shaping the composition of intestinal microbiota, preventing adhesion of pathogens to intestinal mucosa and reducing the risk of bacterial, viral and parasitic infections, modulating immune and intestinal immune cell function, lowering the risk of necrotizing enterocolitis and providing nutrients (sialic acid) for brain development and cognition (7 - 12). Especially, 2’-fucosylactose (2’-FL) has been reported to be important in protecting infants against gut infections (13, 14). This has stimulated efforts by infant formula manufacturers to produce infant formulas that closely resemble human milk in composition and performance (15). However, this process is challenging given that oligosaccharides in goat’s or cow’s milk exhibit limited diversity and are present at significantly lower concentrations compared with those in human milk (16 - 20). Importantly, the complexity of HMOs makes it almost impossible for these to be duplicated in infant formulas (21). To overcome this challenge, modern infant formulas are commonly fortified with fructooligosaccharides (FOS) and galactooligosaccharides (GOS) (22). However, their effectiveness in conferring health benefits similar to those of breast milk remains to be proven (23; 24).
It has previously been shown that high heat treatment could cause degradation or change to the structure of some oligosaccharides \(^{(25, 26)}\). How the heat treatments applied during manufacture of infant formula (milk pasteurization and exposure to temperatures of 180-220°C during spray drying \(^{(27)}\)) affect the presence and functionality of naturally present or added oligosaccharides in infant formula remains unknown. Only a handful of studies to date have attempted to quantify the amount and types of milk oligosaccharides present in infant formula products. The primary objective of this study was to investigate the diversity and concentration of oligosaccharides present in goat’s milk and goat milk-based infant formula and their prebiotic and anti-pathogen adhesion properties.

**Materials and Methods**

**Oligosaccharide Standards**

Oligosaccharide standards were purchased from Dextra Laboratories, Reading, UK. These included: 3’-Sialyllactose (3’-SL), 6’-Sialyllactose (6’-SL), 3’-Sialyl-N-acetyllactosamine (3’-SLN), 6’-Sialyl-N-acetyllactosamine (6’-SLN), 2’-Fucosyllactose (2’-FL), 3’-Fucosyllactose (3’-FL), Lacto-N-hexaose (LNH), 3’-Galactosyllactose (3’-GSL), 4’-Galactosyllactose (4’-GSL), 6’-Galactosyllactose (6’-GSL), and Disialyllactose (DSL). Oligosaccharide standards which were received in powder form were reconstituted with Milli-Q water (22±2°C) to make a stock solution at a concentration of 1 g/L. Stock solutions were further diluted with Milli-Q water to give a final concentration range of 0.1 g/L to 0.001 g/L.

**Preparation of oligosaccharides**

Oli6 Stage 1 (S1-GIF) and Stage 2 goat’s milk infant formulas (S2-GIF) and raw goat milk (pooled milk from a group of ten Saanen goats) were obtained from Nuchev Pty Ltd, Melbourne, Australia. Galactooligosaccharide mixture (GOS) was purchased from New Francisco (Yunfu City) Biotechnology Corporation Ltd, China (King-Prebiotics® GOS-700-P, Batch number 17003). Goat’s milk infant formula was reconstituted according to manufacturer’s instructions. GOS was reconstituted to 20g/L, whilst concentrations of milk oligosaccharides extracted from infant formula were adjusted
to match typical levels in reconstituted goat’s milk infant formula. Milk samples were centrifuged at 4000 xg for 30 min at 4°C to remove remaining milk lipids. Equal volumes of MilliQ water were added to defatted milk and subsequently filtered through a 10kDa molecular weight cut-off filter (Amicon® Centrifugal Filters, Merck, Australia) in a centrifuge (4000 xg for 30 min). The clear filtrate was assumed to contain all oligosaccharides. Extracts were filtered with Millipore 0.45µm syringe filter.

**Oligosaccharide Quantification by LC-MS**

Analysis of oligosaccharides was performed based on the protocols of Liu et al. (28), this method was found to be highly accurate and sensitive in detecting oligosaccharides (limit of detection of <0.1 ng and accuracy of 95 to 105% for a variety of spiked oligosaccharides). Chromatographic separation of milk oligosaccharides was achieved via a Kinetex hydrophilic Interation liquid chromatography (HILIC) column (150 x 4.6 mm, 2.8µm, Phenomenex) on an Agilent 1290 Infinity HPLC system (Agilent, Walbron, Germany). Components of the LC-MS included a degasser, binary pump, temperature controlled auto-sampler (maintained at 4°C), and column compartment (maintained at 30°C). The mobile phase (A+B) consisted of water containing 5mM ammonium acetate (A) and acetonitrile with 0.1% formic acid (B). The flow rate was 0.6mL/min with a gradient elution of 3-50% of solution (A) over 35 min. Analyte detection was performed via mass spectrometry LTQ Orbitrap Velos (Thermo Scientific, Waltham, MA) with a heated electrospray ionization (HESI) source maintained at 270°C by a heating source of 350°C. The sheath, auxiliary and sweep gases were at 40, 15 and 8 units respectively. Voltage source was fixed at 3.2kV in negative mode. Data were collected in profile data acquisition mode over the mass range from 200 to 2000 mass/charge (m/z) in negative Fourier transform (FT) mode (resolution of 60000) and processed using the Xcalibur software package (Thermo Scientific).

**Evaluation of Prebiotic Properties**

Two Bifidobacterium strains (Bifidobacterium animalis subsp. lactis BB12, Bifidobacterium longum BB536) and two Lactobacillus strains (Lactobacillus casei 2607 and Lactobacillus acidophilus 4461) were used in these experiments (29 - 31). Strains were grown anaerobically in MRS broth (32).
with filter-sterilised cysteine (0.05%, final concentration) added for the *Bifidobacterium* strains. For growth experiments, modified MRS (mMRS) was used in which glucose was omitted unless otherwise indicated. Lactose (5g/L) was also included as a positive control. Bacterial suspensions of log-phase cells in 2× mMRS were adjusted to an OD$_{600}$ nm of 0.11, and then 100μL of the cell suspension was added to wells in a sterile 96-well plate. Each well was pre-filled with an equal volume of milk oligosaccharide extract and gently mixed after addition of cells. The final oligosaccharide concentration was adjusted to match that of the reconstituted goat’s milk-based infant formula (ready-to-use as per the manufacturer’s instructions) in order to maintain the same concentration and relative proportions of the oligosaccharides. Plates were incubated anaerobically at 37°C for 24 h. OD$_{600}$ nm readings of the suspensions were taken before and after growth using a plate reader. The extent of growth was determined by the increase in OD$_{600}$ nm from t=0. Each strain/substrate combination and controls in wells were assayed in triplicate.

Sterile GOS (20g/L) was included as the positive control, while negative controls contained only bacteria and the medium without added oligosaccharides. Dextrose and lactose were used as comparison benchmarks for simple sugar utilization by the bacteria.

**Cell culture**

Caco-2 cells, human colorectal epithelial adenocarcinoma cells which exhibit high cellular differentiation, were selected as a human gut adhesion model. The Caco-2 cells were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 10% foetal bovine serum (FBS) and 1% of an antibiotic-antimycotic solution (10,000 units/mL of penicillin, 10,000µg/mL of streptomycin and 25µg/mL of Gibco® Amphotericin B; Life Technologies, Australia). Cells were incubated at 37°C, 95% humidity in an atmosphere containing 5% carbon dioxide. At 80% confluence, cells were passaged as follows: after aspiration of culture media, cells were washed with 5mL pre-warmed phosphate-buffered saline at pH 7 (Life Technologies, Australia), 5mL of trypsin-EDTA (0.25%, Life Technologies, Australia) was added and then the cells incubated for 5 to 10 minutes. Cells were washed with fresh media (10mL) and centrifuged at 815 ×g for 5 minutes to remove trypsin and EDTA. The cell pellet was reconstituted with 1mL fresh media. A cell count was
performed and the cells re-cultured in a sterile flask pre-filled with fresh media (density of $10^5$ cells/mL). For anti-adhesion studies, 24-well plates were seeded with $5 \times 10^5$ cells per well and incubated as before to obtain confluent monolayers. One day prior to experimentation, the media was replaced with fresh media without any antibiotic-antimycotic.

**Anti-adhesion study with Caco-2 cell monolayer**

The protocol used was based on those of earlier studies with slight modifications ($^{33,34}$). *Escherichia coli* NCTC 10418 and *Salmonella typhimurium* were from RMIT University’s culture collection. A single colony isolate of each strain was inoculated into 9 mL Nutrient Broth (Amyl Media, Dandenong, Australia) in a sterile tube and incubated at 37°C for two hours with shaking for cells to reach exponential growth phase. Each suspension was then adjusted to an OD600nm of 0.3 and oligosaccharides (prepared as described previously) added at equal volumes (final oligosaccharide concentration equivalent to that of the ready-to-use reconstituted goat’s milk-based infant formula) and the cell suspensions incubated at 37°C for 120 minutes. In addition, sterilised GOS (20g/L) and Milli-Q water were used as positive and negative controls, respectively. Lactose, in non-growth-limiting concentrations (5g/L), was also included as one of the treatments to distinguish its effect from oligosaccharides. The bacteria-oligosaccharide suspension was added to each well in a sterile 24-well tissue culture plate containing a Caco-2 monolayer and incubated aerobically at 37°C for 90 mins. Post-incubation, the Caco-2 cells were washed three times with PBS and then lysed with cold 0.1% Triton-X to release adherent bacterial cells. Serial dilutions of lysates were prepared and plated onto Nutrient Agar plates which were incubated anaerobically for 24 hours or until colonies were evident. Numbers of colonies were then counted. Each experimental treatment was conducted in triplicate. Anti-adhesion percentage was calculated as:

$$\text{anti-adhesion (\%) = } \frac{\text{bacteria count (control)} - \text{sample bacteria count (sample)}}{\text{bacteria count (control)}} \times 100$$
Statistical analysis

Results for LC/MS analyses, and growth and adhesion assays are expressed as mean ± standard error. For experiments involving bacterial cultures, the difference between each treatment group was tested using one-way ANOVA and Tukey’s multiple comparison tests. Statistical significance was defined as a $p$ value of <0.05. All statistical analyses were performed using GraphPad Prism (GraphPad Prism version 8 for Windows, GraphPad Software).

Results

LC-MS Quantification of Milk Oligosaccharides

The high throughput analytical method used in this study was effective in identifying oligosaccharides present in significant quantities without requiring sample concentration. A total of 14 oligosaccharides were found to be present in S1-GIF and S2-GIF. This included seven major (predominant) and seven minor (present in small amounts) oligosaccharides (Table 1; Figures 1-3). 3’-GSL and 6’-GSL were grouped together as triose sugars due to their complex chromatograph profiles (Figure 4). Of the 14 oligosaccharides, five (2’-FL, 3’-SL, 6’-SL, LNT and LNH) were structurally similar to those found in human breast milk (Table 1). There was no difference in the chromatographic profiles of S1-GIF and S2-GIF (data not shown).

The oligosaccharide profile of goat’s milk was similar in diversity to that of S1-GIF and S2-GIF with a few minor differences. One of the minor milk oligosaccharides, $N$-acetyl-glucosaminyl-lactose (NAL, $m/z$ 544.18) was found to be absent in raw goat’s milk (Table 1, Figure 3), and S2-GIF had slightly higher abundance of 6’-glycolyl-neuraminyl-lactose (NGL, $m/z$ 648.19). Furthermore, the chromatographic profile of GOS was dominated by 3’-GSL and 6’-GSL that were also detected in goat’s milk (Figure 4). It also implies that the total concentration of triose sugars identified in this study included GOS. For both GIFs prepared according to the manufacturer’s instructions, oligosaccharide concentrations were similar to those found in normal unpasteurized goat’s milk (Table 1).
Goat Milk Oligosaccharides Promote Bifidobacterium and Lactobacillus Growth

As shown in Figure 5, the growth rate of *B. animalis* BB12 and *B. longum* BB536 was significantly enhanced, compared to controls, when strains were co-cultured in the presence of oligosaccharides derived from S1-GIF and S2-GIF. This growth-promoting effect was also significantly greater (P<0.5), to that observed with GOS. Oligosaccharides purified from S1-GIF and S2-GIF were also effective at enhancing the growth of *L. casei* 2607 when compared to controls, lactose and GOS but not dextrose. In contrast, *L. acidophilus* 4461 exhibited similar growth rate when cultured in the presence of GOS, lactose, as well as S1-GIF- and S2-GIF-derived oligosaccharides. These data suggest that milk oligosaccharides derived from goat’s milk-based infant formula are effective at selectively promoting the growth of health-promoting bacteria in the gut. Nonetheless, clinical trials are needed (currently being planned) to confirm these benefits in human subjects.

Goat Milk Oligosaccharides Prevent *E. coli* and *S. typhimurium* Adhesion to Caco-2 monolayer

Adhesion of *E. coli* NCTC 10418 and *S. typhimurium* were found to be inhibited by oligosaccharides present in S1-GIF and S2-GIF (Figure 6) and no statistically significant differences were observed between S1 and S2 formulas. Purified GOS was also effective at preventing adhesion of pathogens to Caco-2 cells, to at least the same degree. Interestingly, oligosaccharides purified from S1-GIF, S2-GIF and GOS appeared to be slightly more efficient at inhibiting *E. coli* NCTC 10418 adhesion to Caco-2 cells compared with *S. typhimurium*, at least for the isolates we examined here. In our study, lactose had no significant protective effect.

Discussion

The diverse array of highly complex oligosaccharides present in human breast milk (HMOs) is considered to be of vital importance in promoting the growth of beneficial bacteria in the infant gut, conferring protection against intestinal infections and promoting the development and maturation of the immune system (9; 14). Compared to this, oligosaccharides found in bovine and caprine milk exhibit lesser diversity and complexity, and are found in relatively smaller amounts. As a result, the
amount and nature of natural milk oligosaccharides present in either caprine or bovine milk-based infant formulas, and their effectiveness in conferring gut and immune health benefits on the infant, similar to those of breast milk, remains to be determined and is of significant research interest.

The results of the present study show that goat’s milk-based infant formula contains significant amounts and a diverse array of oligosaccharides, many of which are structurally similar to HMOs. To our knowledge, this is the first such report showing such richness of oligosaccharides in goat’s milk-based infant formula and their similarity to HMOs. Fourteen oligosaccharides, including seven major oligosaccharides were detected in goat’s milk-based infant formula. Of these, five oligosaccharides are also found in human breast milk (Table 1). Goat’s milk was also found to contain a similar number of oligosaccharides. Notably, the number of oligosaccharides detected in this study is significantly lower than that reported previously in goat’s milk (20; 35-41). This is probably due to major differences in the methods used in these studies. The present study used a high throughput method that was aimed at detecting only quantifiable oligosaccharides; the samples used in this study involved the reconstitution of powders according to manufacturer’s instructions for infant use and involved no sample concentration steps. In contrast, all earlier studies have employed methods involving significant enrichment of oligosaccharides with the aim to detect all oligosaccharides, including those present in trace amounts. Using a similar method, we detected 37 oligosaccharides in goat’s milk in earlier studies (Leong et al., unpublished). Similar profile and diversity of oligosaccharides detected in raw goat’s milk and goat’s milk-based infant formula suggests that oligosaccharides are not affected by heat treatment used during manufacture of infant formula.

As in human breast milk, fucosylated and sialylated oligosaccharides were found to be the dominant oligosaccharides present in goat’s milk-based infant formula. 2’-FL is the most abundant oligosaccharide in human milk (35, 42) and is the focus of significant commercial and regulatory interest. It has been shown to play a significant role in anti-infection properties of breast-milk; for example, infants with high levels of 2’FL have been shown to exhibit greater resistance to
ST-E coli and Campylobacter-associated diarrhoea (43). Oligosaccharides resist digestion in the small intestine and reach the colon intact where they are known to influence the structure and function of gut microbiota. For example, several studies have shown that HMOs are effective in selectively promoting the growth of beneficial bacteria, such as Bifidobacterium bifidum that dominate the gut microflora of breast fed infants (44-47). The ability of HMOs and a mixture of FOS/GOS to promote the growth of bifidobacteria and lactobacilli in-vitro also has been demonstrated (48). In the present study, goat milk oligosaccharides were found to exhibit similar prebiotic properties. They were found to be effective in promoting the growth of both Bifidobacterium and Lactobacillus species. Furthermore, oligosaccharides derived from both stage 1 and stage 2 goat’s milk-based infant formula appeared to be more efficient at promoting the growth of B. longum BB536 and L casei 2607 than GOS. Whether this suggests a stronger prebiotic activity of goat milk oligosaccharides compared with GOS generally or is simply a reflection of bacterial species and/or strain-selectivity in substrate utilisation is not clear (49).

Differences in the ability of bifidobacteria and lactobacilli in their ability to utilize carbohydrates such as lactose GOS and lactulose has also been previously reported (50). Lactose is highly likely to be present in the goat’s milk infant formula oligosaccharide fractions, however the relatively insignificant growth-promoting effect of lactose, especially for bifidobacteria, compared with that for S1 and S2 fractions, suggests that the growth enhancement observed with S1 and S2 was largely due to oligosaccharides. Furthermore, the goat’s milk infant formula was found in our study to be rich in 3’-SL, 6’-SL and 2’- FL, and the ability of B. longum and B. infantis to selectively ferment these oligosaccharides together with similarities in the gut microbiota composition of infants fed goat’s milk-based infant formula compared with breast-fed babies, has also been recently reported (51).

The results of our study further showed that oligosaccharide enriched fractions prepared from both stage 1 and stage 2 goat’s milk-based infant formula were effective in reducing the adherence of E coli and S. typhimurium to Caco-2 cells, as similarly reported for HMOs (41, 46). Lower incidence of gastrointestinal infections in breast-fed infants compared with formula-fed infants (52) and a role for
HMOs in this protection has been reported \(^{(53)}\). We found differences in the anti-adhesion efficacy against two different enteric pathogens to Caco-2 cells when pre-incubated and co-cultured with the same GIF oligosaccharide fractions, although further strains or isolates of these pathogens need to be tested before making a definitive conclusion. However, it is significant in this regard that Coppa \textit{et al.} \(^{(34)}\) found species differences between \textit{Escherichia coli}, \textit{Vibrio cholerae}, and \textit{Salmonella} fyris, in experiments using HMOs and (like us) Caco-2 cells. Whilst some recent studies have reported that oligosaccharides from human, cow and goat’s milk \(^{(54, 55)}\) are effective in inhibiting the adhesion of \textit{E. coli} to gut cells, these experiments were conducted using much higher concentrations of oligosaccharides than we used in our study, suggesting the possibility of a dose-dependent effect. In our study GOS was at least equally as effective as goat milk oligosaccharides in preventing the adhesion of \textit{E. coli} and \textit{S. typhimurium} (by 52.4\% and 35\% respectively compared with the negative control; see Figure 6).

Attachment of pathogens to specific receptors on the gut epithelial cells is a critical step in host colonisation and infection. Carbohydrate chains form a major component of cell surface membrane, and lactose being a core saccharide of glycolipids represents a major binding site for bacteria \(^{(56, 57)}\). Some pathogens also bind to lectins in attempt to colonize gut epithelial surfaces and the lectin binding requires either sialyl or fucose groups to be present on the cognate glycan binding partners \(^{(14)}\). The resemblance of certain GOS to saccharide-containing glycoproteins used by many pathogens to attach to intestinal cells has also been reported \(^{(58)}\). Due to structural similarities between goat milk oligosaccharides and gut cell surface carbohydrate groups, these compounds, especially sialylated and fucosylated oligosaccharides, may reduce pathogen adhesion to gut epithelial cells by acting as soluble analogues of host cell receptors and/or changing the expression of such structures \(^{(59 - 61)}\). In addition, oligosaccharides have also been shown to mediate protection against intestinal pathogens by enhancing immune function \(^{(11)}\). \textit{T o g e t h e r, t h e s e r e s u l t s c o m b i n e d} with the results of our study show that oligosaccharides present in goat milk-based infant formula have strong prebiotic and anti-infection properties, and may confer protection against gastrointestinal infections to the infant. Further studies are required to confirm if this is so.
Acknowledgements

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Author Contributions

Conceptualization: HG, BZ, AL; data curation: AL; Formal Analysis: AL, ZL; Investigation: AL; Methodology: AL, ZL, CP, HG, BZ, HA; Resources: HG; Supervision: HG, BZ; validation: AL; Writing original draft: AL; Writing - review & editing: HG, CP, BZ

Conflict of Interest

The authors declare no conflict of interest. HG and BZ had received funding from Nuchev Pty Ltd in the past to review nutritional composition and analyse oligosaccharides in goat’s milk. The research costs were covered by RMIT University funding for PhD student (AL) research. Nuchev Pty Ltd had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.
References


### Table 1: Concentration of major and minor oligosaccharides detected in goat’s milk-based infant formula and their presence in human milk.

<table>
<thead>
<tr>
<th>Oligo</th>
<th>Neutral mass (m/z)</th>
<th>S1-GIF*</th>
<th>S2-GIF</th>
<th>Goat’s Milk</th>
<th>Reported in human milk (42), (62)</th>
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</thead>
<tbody>
<tr>
<td><strong>Major milk oligosaccharides (μg/mL and relative percentage)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>3'-SL</td>
<td>633.212</td>
<td>7.67 (36.8%)</td>
<td>0.084</td>
<td>6.33 (32.7%)</td>
<td>0.087</td>
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<tr>
<td>6'-SL</td>
<td>633.212</td>
<td>4.45 (21.4%)</td>
<td>0.026</td>
<td>4.90 (25.3%)</td>
<td>0.014</td>
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<tr>
<td>2'-FL</td>
<td>488.174</td>
<td>0.88 (4.22%)</td>
<td>0.039</td>
<td>1.38 (7.14%)</td>
<td>0.042</td>
</tr>
<tr>
<td>6'-SLN</td>
<td>674.238</td>
<td>0.12 (0.58%)</td>
<td>0.000</td>
<td>0.15 (0.78%)</td>
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<tr>
<td>DSL</td>
<td>924.307</td>
<td>0.42 (2.02%)</td>
<td>0.004</td>
<td>0.44 (2.28%)</td>
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<tr>
<td>LNH</td>
<td>1072.381</td>
<td>0.06 (0.29%)</td>
<td>0.004</td>
<td>0.10 (0.52%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Triose†</td>
<td>504.169</td>
<td>7.24 (34.7%)</td>
<td>0.272</td>
<td>6.03 (31.2%)</td>
<td>0.087</td>
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<td><strong>Minor milk oligosaccharides (peak area)</strong></td>
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<tr>
<td>NAL</td>
<td>544.18</td>
<td>1609364</td>
<td>6706</td>
<td>1134549</td>
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<tr>
<td>NGL‡</td>
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<td>23527858</td>
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<tr>
<td>LNT</td>
<td>706.23</td>
<td>158050</td>
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<td>167765</td>
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<tr>
<td>3'-SHL</td>
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</table>

*Abbreviations: S1-GIF, Stage 1 Goat Milk Infant Formula; S2-GIF, Stage 2 Goat Milk Infant Formula

†Total putative concentration reported for 3'-GSL and 6'-GSL. ‡Relative abundance reported as a total sum of two isomers (ND, not detecte
Figure 1: Ion chromatograms of extracted oligosaccharide standards (abbreviations are as described in the Experimental Methods section) and their respective retention times (shown on the X-axis). The m/z of each deprotonated ion was calculated to ±0.01.

A  Goat’s milk

B  S1-GIF

Figure 2: Ion chromatogram of major oligosaccharides extracted from (A) goat’s milk, (B) S1-GIF (Stage 1 goat’s milk infant formula)
Figure 3: Ion chromatogram of oligosaccharides (minor oligosaccharides, without standards) extracted from (A) goat’s milk, (B) S1-GIF (goat’s milk infant formula).
Figure 4: Ion chromatograms of extracted oligosaccharides: (A) GOS, (B) S1-GIF, (C) goat's milk, and (D) oligosaccharide standards (3'-GSL and 6'-GSL). Abbreviations are as described in the Experimental Methods section.
Figure 5: Extent of growth of (A) *B. animalis* BB12, (B) *B. longum* BB536, (C) *L. acidophilus* 4461, and (D) *L. casei* 2607 after 24 hours of co-incubation with milk oligosaccharides at 37°C anaerobically. Abbreviations: D, dextrose ("++" indicates presence of dextrose, whilst "-" indicates absence of dextrose in bacteria culture media; L, lactose; GOS, galactooligosaccharide; S1-GIF, Stage 1 goat’s milk infant formula; S2-GIF, Stage 2 goat’s milk infant formula. Values with different superscripts are significantly different (p > 0.05).
**Figure 6:** Bacterial counts of (A) *Escherichia coli* NCTC 10418 and (B) *Salmonella typhimurium* adhered to Caco-2 monolayers. Pathogens were co-cultured with purified oligosaccharide for 2 hours aerobically at 37°C prior being co-incubated with Caco-2 cells for 90 minutes at 37°C. Abbreviations: L, lactose; S1-GIF, Stage 1 goat’s milk infant formula; S2-GIF, Stage 2 goat’s milk infant formula.; GOS, galactooligosaccharide. Values with different superscripts are significantly different ($p > 0.05$).