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Supporting document 1 (at Approval)

Health effects assessment – Application A1155

2'-FL and LNnT in infant formula and other products

Key findings

FSANZ undertook assessments to determine if the proposed health effects of adding 2'-FL and/or LNnT to infant formula products and formulated supplementary foods for young children (FSFYC) are supported by evidence. As part of the assessment, FSANZ did not identify evidence that would indicate the proposed effects (bifidogenic and anti-infective) would be limited to a particular age group of infants or toddlers. The key findings for each proposed health effect are therefore applicable to all the infant formula products and FSFYC to which this application applies.

Bifidogenic effect

FSANZ's assessment sought to determine if evidence supports the assertion that the addition of 2'-FL and LNnT to infant formula products and FSFYC could have a bifidogenic effect. FSANZ has previously recognised that the presence of *Bifidobacterium* and *Lactobacillus* in the intestinal microflora largely benefit the host^{1,2}.

In vitro bacterial growth/utilisation studies on 2'-FL and LNnT have shown that bifidobacteria and a limited number of other bacteria have the ability to metabolise 2'-FL and LNnT. These studies demonstrate the metabolic mechanism that underpins the bifidogenic effect associated with human milk oligosaccharides (HMO's), including 2'-FL and LNnT and that bifidobacteria such as *Bifidobacterium longum* subsp. *infantis* have a selective growth advantage when 2'-FL and LNnT are present.

Studies of breastfed infants with mothers of known 2'-FL secretor status and studies comparing breastfed infants with infants fed unsupplemented infant formula investigated the microflora composition of faecal samples of infants. These studies corroborate the *in vitro* studies in demonstrating the growth advantage afforded by bifidobacteria when fucosylated oligosaccharides, including 2'-FL, are present in the infant diet. This supports the likelihood that 2'-FL has a bifidogenic effect.

A clinical feeding trial for infants supplied by the applicant provided further evidence that the addition of 2'-FL and LNnT to infant formula products will influence the gut microflora to more closely resemble the gut microflora of breastfed infants with a higher relative abundance of *Bifidobacterium* spp. compared to infants fed unsupplemented formula. This study provides

¹ <http://www.foodstandards.gov.au/code/proposals/Pages/proposalp306addition3639.aspx>

² <http://www.foodstandards.gov.au/code/applications/Pages/applicationa1055shor4991.aspx>

evidence that further supports 2'-FL and LNnT supplementation having a bifidogenic effect and an infant gut microflora composition and metabolic profile more like that of breastfed infants.

FSANZ concludes that the addition of 2'FL and LNnT to infant formula is likely to have a bifidogenic effect in some infants and toddlers.

Anti-infective effect

The evidence assessed by FSANZ supports the likelihood of 2'-FL binding to invasive strains of *Campylobacter jejuni* in the intestinal lumen and inhibiting invasive *C. jejuni* attachment to cellular receptors, thereby having an anti-infective health effect. The mechanism of action by which this occurs was established in an *in vitro* study demonstrating that α 1,2-fucosylated oligosaccharides, including 2'-FL, specifically inhibit attachment of invasive *C. jejuni* to the intestinal H2 antigen binding receptor.

Through this mechanism, 2'-FL mimics the cellular binding site and prevents attachment and subsequent infection and pathogenesis. The plausibility of this anti-infective health effect occurring in infants was established by an *in vivo* murine model demonstrating decreased intestinal infection, invasiveness and disease severity in animals fed 5 g/L 2'-FL and challenged with invasive *C. jejuni* strain 81-176 at a dose of 1×10^8 cfu/mouse.

Evidence from a human study showing a decreased incidence of *Campylobacter* associated diarrhoea in infants of mothers with a higher proportion of 2'-FL in their milk provides additional epidemiological evidence supporting the plausibility of 2'-FL having an anti-infective health effect. This study is consistent with the mechanism of action, whereby intestinal infection with invasive *C. jejuni* and pathogenesis is inhibited in the presence of 2'-FL.

Challenge studies in infants are not feasible, however the evidence assessed by FSANZ establishes a mechanism of action which is supported by an animal study that demonstrates the effect *in vivo*. FSANZ's assessment did not establish an anti-infective dose response effect for 2'-FL and invasive *C. jejuni* infection *in vivo*, although it is possible that higher concentrations of 2'-FL could enhance the inhibitory effect observed in an *in vitro* inhibition assay.

FSANZ concludes that 2'-FL is reasonably likely to have an anti-infective effect against invasive *C. jejuni* strains in infants and toddlers consuming formula supplemented with 2'-FL.

Glossary of terms

2'-FL	2'-O-fucosyllactose
FSFYC	Formulated supplementary foods for young children (or 'toddler milk')
HMO	Human milk oligosaccharide
LNnT	Lacto-N-neotetraose

1 Introduction

Infants are a vulnerable population group. Breastfeeding is the recommended way to feed an infant; however a safe and nutritious substitute for breast milk is required for infants who are not breastfed. Infant formula may provide the sole source of nutrition for formula-fed infants during the first months of life and then serve as the principal liquid source of nourishment in a progressively diversified diet for older infants. Infant formula must be safe for consumption and must also provide all the essential nutrients, in adequate amounts, to support the growth and development of formula-fed infants.

The stated purpose for adding 2'-FL alone or in combination with LNnT to infant formula products or FSFYC is to better reflect the oligosaccharide composition of human milk. These oligosaccharides are stated by the applicant to confer functional benefits to infants and young children, consistent with the oligosaccharide fraction of human milk, with three specified health effects: a bifidogenic effect, an anti-infective effect against pathogens, and a role in immune modulation, improved intestinal barrier function and alleviation of allergic responses.

The primary risk assessment problem to be addressed is whether addition of 2'-FL, alone or in combination with LNnT, would pose a public health and safety risk to the target population when added to infant formula products or FSFYC.

1.1 Scope of consideration

The main purpose of this Supporting Document 1 (SD) is to discuss the approach to the assessment of the evidence and summarise the findings for: 1) the bifidogenic effect; and 2) the anti-infective effect.

FSANZ has undertaken a comprehensive safety, technical and health effects assessment for this application at [1st Call for Submissions](#) and [2nd Call for submissions](#). These assessments concluded there are no public health and safety concerns associated with adding the applicant's 2'-FL and LNnT to infant formula products and FSFYC at the proposed levels, which are consistent with average levels in mature human milk. The assessment of health effects concluded that evidence to support the health effects of improved barrier function, immune modulation and alleviation of allergic responses is inconclusive.

The evidence supports the proposed compositional permission. FSANZ concluded that the requested addition of 2'-FL alone or with LNnT demonstrates two favourable health effects and has the potential to confer beneficial health outcomes in infants and young children. The available evidence indicates that an anti-infective effect against invasive *C. jejuni* is reasonably likely to occur through a mechanism of competitive binding inhibition, thereby preventing invasive *C. jejuni* attaching to the cellular receptors on intestinal epithelial cells. A bifidogenic effect (an increase in the relative abundance of bifidobacteria in the intestinal microflora) is also reasonably likely to occur through a mechanism of selective growth advantage and is supported by evidence from microflora composition studies of breastfed and formula fed infants and a clinical trial of infants supplemented with 2'-FL and LNnT. Other less direct evidence indicates these favourable health effects may be enhanced as concentrations of 2'-FL are increased.

1.2 Approach to assessment

The majority of the safety, technical and health effects assessment report ([SD1 at 2nd CFS](#)) was not amended following the 2nd CFS. However following consideration of the submissions, FSANZ sought advice on our assessment from an expert microbiologist,

Associate Professor Andrew Holmes³. Professor Holmes noted the approach and conclusions reached by FSANZ relating to the anti-pathogenic and bifidogenic effects and concluded that they were appropriate and reasonable. FSANZ also sought advice from a FSANZ Fellow, Professor Seppo Salminen, on the assessment approach; he supported the approach and conclusions of the assessment. On this basis the health effects assessment has been summarised in SD1 to this approval report. The whole assessment is summarised in the following sections.

FSANZ has conducted a comprehensive assessment following the internationally recognised risk analysis framework. The assessment was based on the best available scientific evidence as legislatively required.

Assessment of nutritional safety, tolerance and efficacy also relies on a weight of evidence approach. While infant studies are required, evidence from non-human studies adds weight to the determination of a substance's role, particularly in understanding the mode of action and biological plausibility.

For the current assessment for both safety and favourable health effects, FSANZ considered a body of evidence including *in vitro* studies, animal studies including those in neonatal animals, and human studies including clinical trials. While FSANZ considers that the information submitted by the applicant met the requirements set out in the Application Handbook, a literature review was also undertaken and identified additional studies.

2 Bifidogenic effect

FSANZ's assessment sought to determine if evidence supports the assertion that the addition of 2'-FL and LNnT to infant formula products and FSFYC could have a bifidogenic effect. For the purpose of this assessment, a bifidogenic effect is defined as a proliferation and increase in the relative abundance of *Bifidobacterium* spp. (bifidobacteria) in the intestinal microflora. FSANZ has previously recognised that the presence of *Bifidobacterium* and *Lactobacillus* in the intestinal microflora largely benefit the host^{4,5}.

It is not possible to define a universal standard for a healthy intestinal microbiota, however the composition of exclusively breastfed infants is generally the accepted reference standard for the normal, healthy development of an infant's gut microbiota. Studies have found that the composition of the gut microflora of breastfed infants are more homogeneous and are generally dominated by bifidobacteria compared to formula-fed infants (Bezirtzoglou et al. 2011). The EFSA states that the oligosaccharides of human milk are one of the principal growth factors for bifidobacteria in the infant gut and are responsible for the composition of the gut microbiota found in breastfed infants (EFSA 2014).

2.1 Mechanism of action

The ability of *Bifidobacterium* spp. to metabolise 2'-FL and LNnT is variable within and between species and is dependent on the presence of specific enzymes such as 2'-fucosyllactose. A range of *Bifidobacterium* species have been identified in infant and adult faecal samples. The major species identified in infant faeces include *B. longum* subsp. *infantis*, *B. breve* and *B. bifidum*. In adults, *B. adolescentis* is the major species found in faeces.

³ Associate Professor Holmes specialises in the relationship between nutrition, gut microbiome and health and is the Microbiome Project node leader in the Charles Perkins Centre, and Co-leader of the Food for Health theme of the Centre for Advanced Food Enginomics at the University of Sydney.

⁴ <http://www.foodstandards.gov.au/code/proposals/Pages/proposalp306addition3639.aspx>

⁵ <http://www.foodstandards.gov.au/code/applications/Pages/applicationa1055shor4991.aspx>

Specific adaptations for carbohydrate metabolism of HMOs have been suggested to explain the differences in the *Bifidobacterium* species present in infant and adult faeces (Bunesova et al. 2016). Species such as *B. longum* subsp. *infantis* and *B. bifidum* are hypothesised to have adapted to consume HMOs, while the adult associated species *B. adolescentis* and *B. longum* subsp. *longum* are capable of metabolising plant derived polysaccharides. Other bacterial genera, such as *Bacteroides* can metabolise a variety of polysaccharides from human milk and plant sources.

In vitro bacterial growth/utilisation studies on 2'-FL and LNnT have shown that bifidobacteria and a limited number of other bacteria that are typically a component of the infant microflora, such as *Bacteroides* spp., have the ability to metabolise 2'-FL or LNnT, whereas other bacteria such as *Escherichia coli*, are unable to utilise 2'-FL for growth (Asakuma et al., 2011; Bunesova et al., 2016; Garrido et al., 2015; Garrido et al., 2016; Ruiz-Moyano et al., 2013; Yu et al., 2013a; Yu et al., 2013b).

2.2 Breastfed infants where the mother's secretion status for 2'-FL is known

Two studies (Smith-Brown et al., 2016 and Lewis et al., 2015) investigated the effect of 2'-FL secretor status on the microflora composition of faecal samples of infants. Both studies found that the infants of 2'-FL secretor mothers had greater abundance of bifidobacteria compared to infants with non-secretor mothers. The Lewis et al. (2015) study found that *B. longum* subsp. *infantis* dominated the microflora of the secretor group of infants and that their faeces had a lower percentage of fucosylated oligosaccharides. Furthermore, Lewis et al (2015) found that bifidobacteria were established earlier in secretor-fed infants (60% of infants versus 37.5% at day 6) and more often (80% of infants versus 50% by day 120). These data indicate that 2'-FL and other 2-linked fucosylated oligosaccharides found in the milk of secretor mothers have a bifidogenic effect.

2.3 Breastfed infants and infants fed unsupplemented infant formula

Bezirtzoglou et al. (2011) investigated the development of the gut microflora for exclusively breastfed and unsupplemented infant-formula newborn infants. *Bifidobacterium* genus dominated the gut microflora of the breastfed infants (average 69%) compared with formula-fed infants (average 32%) and at lower numbers. The faecal microflora of the formula-fed infants were found to be more diverse than that of the breastfed infants.

In a similar study, Tannock et al (2013) found that bifidobacteria dominated the microflora of breastfed infants (approximately 80%) compared to goat and cow milk based formulas (approximately 50%).

The Bezirtzoglou et al. (2011) study did not establish the 2'-FL secretor status of the breastfeeding mothers, however an estimated 80% of women world-wide produce human milk that contains 2'-FL (known as 2'-FL secretors) with a mean mature human milk concentration of 2.4-3.0 g/L. Unlike 2'-FL, LNnT is present in the milk of all women with a mean mature human milk concentration of 0.28-0.31 g/L (SD1, 2nd CFS report). The evidence presented in the Bezirtzoglou et al. (2011) and Tannock et al. (2013) studies is consistent with the mechanism of action and the studies of breastfed infants with known secretor status of the mothers, further supporting the likelihood of 2'-FL having a bifidogenic effect in infants.

2.4 Breastfed infants and infants fed either supplemented (2'-FL and LNnT) or unsupplemented infant formula

A clinical study by Puccio et al. (2017) investigated the effect of feeding with 2'-FL in combination with LNnT on the intestinal microflora and stool metabolites of infants. Three groups of infants were included in the study: a group that was breastfed only, a group fed a supplemented infant formula containing 1.0-1.2 g/L of 2'-FL and 0.5-0.6 g/L of LNnT and a group fed an unsupplemented infant formula. The microbial composition and metabolite profiles of the stools were measured. Analysis of microbial diversity in the stools between the groups found that the 2'-FL and LNnT supplemented group was closer in composition to the breastfed group than the unsupplemented infant formula group. Further analysis identified differences in the abundance of *Bifidobacterium*, *Escherichia coli* and unclassified Coprobacillaceae. Analysis of the stool metabolites profile found that the composition of the supplemented formula and breastfed groups were more similar than the unsupplemented formula group.

This study provides evidence that the addition of 2'-FL and LNnT to infant formula products influences the infant gut microflora and stool metabolites to more closely resemble that of breastfed infants and with a higher relative abundance of bifidobacteria compared to infants fed unsupplemented formula.

2.5 Adult study with 2'-FL and/or LNnT

The applicant's 2'-FL and LNnT were evaluated in a parallel, double-blind, randomised, placebo-controlled study in 100 healthy adult volunteers. Participants were randomly assigned to consume either 2'-FL or LNnT at doses of 5, 10 or 20 g/day, a 2:1 mix of 2'-FL and LNnT at 5, 10 or 20 g mixture/day, or glucose (2 g/day) as a placebo control. The duration of the intervention was 14 days. All interventions were provided as a daily bolus dose dissolved in water, which participants were instructed to consume at breakfast. Clinical checks were made on the study participants at entry to the study and again at the end of the intervention. Blood samples for clinical chemistry and haematology analyses, as well as faecal samples for biomarker and microbiota composition analyses, were collected at baseline and at the end of the intervention period. Stool pH was not measured.

The composition of the gut microflora was determined using 16S RNA sequencing analysis. The primary impact of HMO supplementation on the microflora was an increase in the relative abundance of *Actinobacter* and *Bifidobacterium* and reduction in the relative abundance of Firmicutes and Proteobacteria. In total, 77% of all study participants responded to the HMO interventions. A dose response was observed whereby intervention groups given higher HMO concentrations had a greater abundance of *Actinobacter* and *Bifidobacterium*. No statistically significant differences were observed in the concentrations of the short chain fatty acids (SCFA) acetate, butyrate or propionate despite the shifts in the gut microflora.

This study provides evidence that the effect is not restricted to infant populations and that the effect is likely to be dose dependent, whereby higher doses will lead to a greater effect of increasing relative abundance of bifidobacteria in the gut microflora.

A the evidence underpinning the likelihood of 2'-FL and LNnT having a bifidogenic effect are summarised in Appendix 1.

3 Anti-infective effect

The evidence for an anti-infective health effect for 2'-FL and LNnT and toxins and pathogens other than invasive *C. jejuni* was inconclusive and primarily limited to *in vitro* inhibition studies, with no demonstrated mechanism of action identified. The anti-effective effect detailed in this report addresses the anti-infective effect of 2'-FL and invasive strains of *C. jejuni* only.

3.1 Mechanism of action

In cases where individuals develop clinical enteritis after exposure to *C. jejuni*, the bacteria preferentially adhere to the mucus layer of the small intestine. Some strains may attach directly to intestinal epithelial cells, which may lead to bacterial invasion, translocation and bacteraemia (Cooling, 2015). *C. jejuni* can bind to the mucosa and epithelial cells via fucose (Cooling, 2015) and a study by Ruiz-Palacios et al (2003) provides strong evidence that the α 1,2-fucose-containing type 2 chain H-antigen (H2 antigen) expressed on intestinal epithelial cells is the cellular receptor for invasive *C. jejuni* and is essential for infection.

Ruiz-Palacios et al (2003) used Chinese hamster ovary (CHO) cells transfected with genes for α 1,2-fucosyltransferase (CHO-FUT1), α 1,3/4-fucosyltransferase (CHO-FUT3), and α 1,3-fucosyltransferase (CHO-FUT4) to test *C. jejuni* binding. The authors tested binding of four pathogenic adherent *C. jejuni* strains isolated from children and two non-adherent and non-pathogenic strains isolated from healthy children. Cells transfected with the *FUT1* gene and expressing α 1,2-fucose-containing H2 antigen on the cell surface bound the four pathogenic strains but not the non-pathogenic strains. None of the *C. jejuni* strains tested bound to the cells transfected with the *FUT3* and *FUT4* genes. In other experiments, the authors established the specificity of pathogenic *C. jejuni* for the H2 antigen through binding inhibition assays and demonstrated that the neutral oligosaccharide fraction of human breast milk, containing α 1,2-fucosylated oligosaccharides (from secretors), and purified 2'-FL both inhibit binding of pathogenic *C. jejuni* to cells expressing the H2 antigen.

This study provides strong evidence that 2'-FL mimics the cellular binding receptor for invasive *C. jejuni* and that 2'-FL inhibits attachment to cells *in vitro*.

3.2 *In vivo* studies of anti-infective effect of 2'-FL against invasive *C. jejuni*

Murine challenge study with 2'-FL (Glycosyn) and C. jejuni (Yu et al. 2016)

The experimental murine model for *C. jejuni* strain 81-176 infection was optimised at 7 days of antibiotic treatment followed by 3 daily inoculation of 10^8 cfu/mouse in 100 μ L saline gavage. Four week-old C57BL/6 male mice were separated into five treatment arms and received 7 days of an antibiotic treatment to disrupt the microflora and enhance the infection model. Group 1 received no treatment and were uninfected negative controls; Group 2 were uninfected mice administered 5 g/L of 2'-FL in their water for 3 days; Group 3 mice were infected with *C. jejuni* with no further intervention (positive controls); Group 4 mice were infected with an inoculum of *C. jejuni* that also contained 2'-FL; and Group 5 mice were administered 5 g/L of 2'-FL in their drinking water for 3 days before and concurrent with the inoculum containing both *C. jejuni* and 2'-FL. A disease activity index (DAI) schema was applied to infected and control mice which was a composite of weight loss, bleeding, and diarrhoea symptoms. *C. jejuni* was also quantified in faeces and tissues by real-time PCR.

The addition of 5 g/L of 2'-FL concurrently with *C. jejuni* challenge (Group 4), was protective against *C. jejuni* infection and faecal shedding and infection of the intestine, spleen and mesenteric lymph nodes were reduced by 90%, 80%, 96% and 93%, respectively, and DAI

was reduced by 57% compared to infected positive controls. The addition of 5 g/L to drinking water for 3 days before and concurrent with *C. jejuni* challenge (Group 5), was protective against *C. jejuni* infection and infection of the intestine, spleen and mesenteric lymph nodes were reduced by 99%, 97%, 97% and 98%, respectively, and DAI was reduced by 77% compared to infected positive controls.

These results show that 2'-FL added to the diet of mice, is protective against invasive *C. jejuni* infection *in vivo*.

3.3. Anti-infective effect of human milk and *C. jejuni*

With regard to the anti-infective effects of 2'-FL in human milk, Morrow et al. (2004) investigated the protective effects of milk oligosaccharides against diarrhoeal illness in breastfed infants in San Pedro Martir, Mexico City. Ninety three breast-feeding mother-infant pairs were prospectively studied from birth to 2 years from 1988 to 1991 to determine if one or more major 2-linked fucosylated oligosaccharides of human milk are inversely associated with the incidence of diarrhoea caused by *Campylobacter* and calicivirus (norovirus). A single milk sample was analysed by high performance liquid chromatography for each mother; samples were collected between 1 and 5 weeks postpartum. The representativeness of this sample for the course of lactation was analysed using longitudinal data from 11 Mexican secretor mothers. Infant stool samples were collected weekly with additional samples obtained whenever diarrhoea occurred. Diarrhoea samples were routinely tested for *C. jejuni*, pathogenic *Escherichia coli*, *Shigella*, *Salmonella*, *Aeromonas*, and rotavirus. The incidence of *Campylobacter* diarrhoea in infants whose mother's milk contains low levels of 2'-FL (<29% of total HMO) was approximately 8.7 cases per 100 child months, versus an incidence of approximately 1.5 and 1.6 cases per 100 child months for mothers with intermediate (29-36% of total HMO) and high levels of 2'-FL (≥37% of total HMO), respectively ($P=0.0004$). Protective effects associated with the level of 2'-FL in human milk were not reported for other pathogens or moderate-to-severe diarrhoea of any cause. High levels of lacto-N-difucohexaose (>12% of total HMO) in mothers human milk was associated with a decreased incidence of calicivirus-related diarrhoea in infants compared to low levels (<7% of total HMO). High (>77% of total HMO) and intermediate (72-77% of total HMO) levels of all 2-linked fucosyl oligosaccharides were associated with a decreased incidence of moderate-to-severe diarrhoea of any cause, compared to low levels in mother's milk (<72% of total HMO). The concentration of 2'-FL and the total concentration of HMO in human milk was not reported by Morrow et al. (2004) and so it is not possible to infer a protective concentration of 2'-FL in milk. However the evidence from the Morrow et al (2004) study is consistent with the mechanism of action and the animal study described above, and provides additional epidemiological evidence supporting the likelihood of 2'-FL having an anti-infective effect against invasive *C. jejuni* in infants.

4 Summary

FSANZ undertook assessments to determine if the proposed bifidogenic and anti-infective health effects associated with adding 2'-FL and/or LNnT to infant formula products and FSFYC are supported by evidence. FSANZ concludes that the addition of 2'FL and LNnT to infant formula is likely to have a bifidogenic effect in some infants and toddlers through a mechanism of selective growth advantage. The available evidence also demonstrates that an anti-infective effect against invasive *C. jejuni* is likely to occur through a mechanism of competitive binding inhibition, thereby preventing invasive *C. jejuni* attaching to the cellular receptors on intestinal epithelial cells.

5 References

- Alliet P, Puccio G, Janssens E, Cajozzo C, Corsello G, Berger B, Sperisen P, Martin F-P, Sprenger N, Steenhout P (2016) Term infant formula supplemented with human milk oligosaccharides (2'fucosyllactose and lacto-neotetraose) shifts stool microbiota and metabolic signatures closer to that of breastfed infants. *J Pediatr Gastroenterol Nutr* 63:S55. doi: 10.1097/01.mpg.0000489632.17881.57
- 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. *Eur J Clin Nutr* 62:488–494. doi: 10.1038/sj.ejcn.1602738
- Bezirtzoglou E, Tsiotsias A, Welling GW (2011) Microbiota profile in feces of breast- and formula-fed newborns by using fluorescence in situ hybridization (FISH). *Anaerobe* 17:478–482. doi: 10.1016/j.anaerobe.2011.03.009
- Bunesova V, Lacroix C, Schwab C (2016) Fucosyllactose and L-fucose utilization of infant *Bifidobacterium longum* and *Bifidobacterium kashiwanohense*. *BMC Microbiol* 16:248. doi: 10.1186/s12866-016-0867-4
- Cooling L. (2015) Blood groups in infection and host susceptibility. *Clin Microbiol Rev* 28(3):801–870. doi:10.1128/CMR.00109-14.
- EFSA (2014) Scientific Opinion on the essential composition of infant and follow-on formulae. *EFSA Journal* 12(7):3760. doi:10.2903/j.efsa.2014.3760
- Elison E, Vigsnaes LK, Rindom Krogsgaard L, Rasmussen J, Sørensen N, McConnell B, Hennem T, Sommer MOA, Bytzer P (2016) Oral supplementation of healthy adults with 2'-O-fucosyllactose and lacto-N-neotetraose is well tolerated and shifts the intestinal microbiota. *Br J Nutr* 116:1356–1368. doi: 10.1017/S0007114516003354
- Garrido D, Ruiz-Moyano S, Lemay DG, Sela DA, German JB, Mills DA (2015) Comparative transcriptomics reveals key differences in the response to milk oligosaccharides of infant gut-associated bifidobacteria. *Sci Rep* 5:13517. doi: 10.1038/srep13517
- Garrido D, Ruiz-Moyano S, Kirmiz N, Davis JC, Totten SM, Lemay DG, Ugalde JA, German JB, Lebrilla CB, Mills DA (2016) A novel gene cluster allows preferential utilization of fucosylated milk oligosaccharides in *Bifidobacterium longum* subsp. *longum* SC596. *Sci Rep* 6:35045. doi: 10.1038/srep35045
- Lewis ZT, Totten SM, Smilowitz JT, Popovic M, Parker E, Lemay DG, van Tassell ML, Miller MJ, Jin Y-S, German JB, Lebrilla CB, Mills DA (2015) Maternal fucosyltransferase 2 status affects the gut bifidobacterial communities of breastfed infants. *Microbiome* 3:13. doi: 10.1186/s40168-015-0071-z
- Morrow AL, Ruiz-Palacios GM, Altaye M, Jiang X, Guerrero ML, Meinzen-Derr JK, Farkas T, Chaturvedi P, Pickering LK, Newburg DS (2004) Human milk oligosaccharides are associated with protection against diarrhea in breast-fed infants. *J Pediatr* 145:297–303. doi: 10.1016/j.jpeds.2004.04.054
- Puccio G, Alliet P, Cajozzo C, Janssens E, Corsello G, Sprenger N, Wernimont S, Egli D, Gosoni L, Steenhout P (2017) Effects of infant formula with human milk oligosaccharides on growth and morbidity: A Randomized Multicenter Trial. *J Pediatr Gastroenterol Nutr* 64:624–631. doi: 10.1097/MPG.0000000000001520

Smith-Brown P, Morrison M, Krause L, Davies PSW (2016) Mothers secretor status affects development of childrens microbiota composition and function: A Pilot Study. PLoS ONE 11:e0161211. doi: 10.1371/journal.pone.0161211

Steenhout P, Sperisen P, Martin F-P, Sprenger N, Wernimont S, Pecquet S, Berger B (2016) Term infant formula supplemented with human milk oligosaccharides (2'fucosyllactose and lacto-n-neotetraose) shifts stool microbiota and metabolic signatures closer to that of breastfed infants. FASEB J 30:275.7

Tannock GW, Lawley B, Munro K, Gowri Pathmanathan S, Zhou SJ, Makrides M, Gibson RA, Sullivan T, Prosser CG, Lowry D, Hodgkinson AJ (2013) Comparison of the compositions of the stool microbiotas of infants fed goat milk formula, cow milk-based formula, or breast milk. Appl Environ Microbiol 79:3040–3048. doi: 10.1128/AEM.03910-12

Ruiz-Moyano S, Totten SM, Garrido DA, Smilowitz JT, German JB, Lebrilla CB, Mills DA (2013) Variation in consumption of human milk oligosaccharides by infant gut-associated strains of *Bifidobacterium breve*. Appl Environ Microbiol 79:6040–6049. doi: 10.1128/AEM.01843-13

Ruiz-Palacios GM, Cervantes LE, Ramos P, Chavez-Munguia B, Newburg DS (2003) *Campylobacter jejuni* binds intestinal H(O) antigen (Fuc alpha 1, 2Gal beta 1, 4GlcNAc), and fucosyloligosaccharides of human milk inhibit its binding and infection. J. Biol. Chem. 278:14112–14120. doi: 10.1074/jbc.M207744200

Yu Z-T, Chen C, Kling DE, Liu B, McCoy JM, Merighi M, Heidtman M, Newburg DS (2013a) The principal fucosylated oligosaccharides of human milk exhibit prebiotic properties on cultured infant microbiota. Glycobiology 23:169–177. doi: 10.1093/glycob/cws138

Yu Z-T, Chen C, Newburg DS (2013b) Utilization of major fucosylated and sialylated human milk oligosaccharides by isolated human gut microbes. Glycobiology 23:1281–1292. doi: 10.1093/glycob/cwt065

Yu Z-T, Nanthakumar NN, Newburg DS (2016) The Human Milk Oligosaccharide 2'-Fucosyllactose Quenches *Campylobacter jejuni*-Induced Inflammation in Human Epithelial Cells HEp-2 and HT-29 and in Mouse Intestinal Mucosa. J Nutr 146:1980–1990. doi: 10.3945/jn.116.230706

Appendix 1: Summary of the evidence base for bifidogenic effect

Type of studies	Key studies	Main findings	Why this is important
<i>In vitro</i> bacterial growth/utilisation studies on 2'-FL and LNnT	Asakuma et al. (2011) Bunesova et al. (2016) Garrido et al. (2015) Garrido et al. (2016) Ruiz-Moyano et al. (2013) Yu et al. (2013a) Yu et al. (2013b)	These studies show that bifidobacteria and a limited number of other bacteria that are typically a component of the infant microflora, such as <i>Bacteroides</i> spp., have the ability to metabolise 2'-FL or LNnT, whereas other bacteria such as <i>E. coli</i> , are unable to utilise 2'-FL for growth.	These studies demonstrate the metabolic mechanism that underpins the bifidogenic effect associated with 2'-FL and LNnT and that bifidobacteria have a selective growth advantage when 2'-FL and LNnT are present.
Microflora composition in infants	Bezirtzoglou et al. (2011) Tannock et al. (2013) Lewis et al. (2015) Smith-Brown et al. (2016)	These studies show that fucosylated oligosaccharides present in human milk, including 2'-FL, have a bifidogenic effect and that formula-fed infants not only have a lower relative abundance of bifidobacteria but also a faecal microflora that is more diverse.	These studies corroborate the <i>in vitro</i> studies in demonstrating the growth advantage afforded to bifidobacteria when fucosylated oligosaccharides, including 2'-FL, are present in the infant diet. Thereby supporting the biological plausibility of 2'-FL having a bifidogenic effect.
Clinical studies in infants	Puccio et al (2017) Alliet et al. (2016) Steenhout et al. (2016)	This study provides evidence that the addition of 2'-FL and LNnT to infant formula products influences the infant gut microflora to more closely resemble that of breastfed infants and with a higher relative abundance of bifidobacteria compared to infants fed unsupplemented formula.	This study provides evidence further supporting the biological plausibility of 2'-FL and LNnT supplementation having a bifidogenic effect and an infant gut microflora composition and metabolic profile that more closely resembles breastfed infants.
Study in adults	Elison et al. (2016)	Supplementation of an adult study population with either 2'-FL or LNnT or a combination of both HMOs resulted in an increased relative abundance of <i>Bifidobacterium</i> and <i>Actinobacter</i> in a dose dependant manner.	This study provides further evidence supporting the biological plausibility of 2'-FL and LNnT supplementation having a bifidogenic effect and that the effect is not restricted to infant populations and potentially dose dependant.