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## **Supporting document 2**

# Assessment of beneficial effects – Review of Application A1155 2'-FL and LNnT in infant formula and other products

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## **Executive summary**

### *Overall summary of assessment*

FSANZ has reassessed the evidence for a bifidogenic effect of 2'-fucosyllactose (2'-FL) and lacto-*N*-neotetraose (LNnT) and an inhibitory effect of 2'-FL on the binding of pathogenic strains of *Campylobacter* to the infant gastrointestinal epithelium.

FSANZ notes that the low number of relevant clinical trials constrains the assessment of beneficial health effects of 2'-FL and LNnT and the estimation of the magnitude of those effects. Acknowledging the limitations in the body of evidence, FSANZ concludes that for infants:

- a) the addition of synthetic 2'-FL and LNnT to infant formula leads to a *Bifidobacterium*-enriched microbiota that is more similar to that observed in breastfed infants than in those fed unsupplemented formula. However, the size of the effect of 2'-FL and LNnT on bacterial populations is difficult to estimate. Evidence for a link between the presence of 2'-FL and/or LNnT in human milk or formula and any specific health outcome is limited to secondary outcomes of one randomised control trial and observational studies of lower quality.
- b) there is a consistent body of indirect evidence to demonstrate a credible mechanism for 2'-FL inhibition of the binding of pathogenic *Campylobacter jejuni* to intestinal epithelial cells, and limited, largely indirect, evidence for a reduction of intestinal colonisation by *C. jejuni* and the incidence of diarrhoea. There are no studies which test this.

For young children:

- a) evidence that a bifidogenic effect occurs in children fed formulated supplementary foods for young children (FSFYC) supplemented with synthetic 2'-FL and/or LNnT is very limited. There is no reason to expect that the previously demonstrated effect in infants would not occur in young children.
- b) As with infants there is no direct evidence that inhibition of binding of *C. jejuni* occurs in children fed formulated supplementary foods for young children (FSFYC) supplemented with synthetic 2'-FL.

The Independent Expert Advisory Group (IEAG) for A1155 concluded that:

- the approach to the assessment taken by FSANZ is appropriate
- there is a bifidogenic effect; but that there is limited evidence in humans to estimate the size of the effect or to link the bifidogenic effect to a beneficial health outcome

- there is a dose response effect in relation to the competitive inhibition by 2'-FL of binding of *C. jejuni* to its epithelial cell receptor; but this inhibitory effect at a cellular level cannot be linked causally to a reduction in infection rates in infants or children because, for obvious reasons, *C. jejuni* challenge studies in humans are unethical.

#### *Bifidogenic effect*

There is broad scientific consensus that a *Bifidobacterium*-enriched microbiota has functional benefits in the normal growth and development of breastfed infants and plays a role in reported differences in health outcomes between breastfed and formula fed infant populations. However, it is recognised that, in most cases, the precise molecular mechanisms underlying such beneficial effects are still to be fully characterised.

The link between breastfeeding and higher levels of bifidobacteria in the infant gastrointestinal tract (GIT) is reasonably well established from numerous observational studies on the effects of formula feeding and breastfeeding on the composition of the infant gastrointestinal microbiota. Although there is variability in the results, most conclude that the proportion of bifidobacteria and lactobacilli is significantly lower for formula-fed infants. However, a direct link between levels of 2'-FL and LNnT in human milk and levels of bifidobacteria in the infant gastrointestinal tract is not well established, as there is significant variability in the results of observational studies investigating this relationship.

A credible mechanism by which human milk oligosaccharides (HMOs) influence the composition of the gastrointestinal microbiome has been established through a number of *ex vivo*, *in vitro* and *in silico* studies on the utilisation of specific HMOs by bifidobacteria isolated from infant gastrointestinal tracts. The results from a small number of human intervention and observational studies on breastfed and formula fed infants are also supportive of a bifidogenic effect of 2'-FL and LNnT, but the quality of the body of evidence limits confidence in the size of the effect. Evidence that a bifidogenic effect occurs in children fed formulated supplementary foods for young children (FSFYC) supplemented with synthetic 2'-FL and/or LNnT is very limited.

Evidence for a link between specific health outcomes and supplementation with 2'-FL and/or LNnT is limited. Secondary outcomes from one randomised controlled clinical trial support a conclusion that addition of synthetic 2'-FL and LNnT to infant formula contributes to health outcomes for formula fed infants more in line with those of healthy breastfed infants. However, certainty about the extent of any beneficial effects of 2'-FL and LNnT on infant health is low because the body of evidence is limited. The IEAG for A1155 concluded that there are many different factors which influence infant health, and that it is not possible to determine a linear effect from the presence of one substance in human milk and a specific health outcome.

#### *Pathogen-binding inhibition effect*

For obvious ethical reasons, human trials do not exist to test if 2'-FL inhibits pathogen-binding of *Campylobacter* and subsequent infection rates in infants.

Evidence from *in vitro*, *ex-vivo* and animal studies consistently demonstrate a credible mechanism for competitive inhibition by 2'-FL of the binding of pathogenic *C. jejuni* to H-2 histo-blood group antigens on intestinal epithelial cells. Results from animal studies are consistent with such inhibition reducing *C. jejuni* intestinal colonisation and the incidence of diarrhoea. However, there is no direct evidence from human clinical trials that 2'-FL undertakes this beneficial role in breastfed infants or as a component of infant formulas and/or formulated supplementary foods for young children (FSFYC).

Evidence from one human study showing a decreased incidence of *Campylobacter*-associated diarrhoea in infants of mothers with a higher proportion of 2'-FL in their milk is consistent with the proposed pathogen-binding effect of 2'-FL, but is insufficient to conclusively demonstrate the likelihood of a positive health outcome from supplementation of infant formulas and FSFYC with 2'-FL.

The capacity of 2'-FL to reduce the severity and duration of *C. jejuni* infection has been demonstrated in a mouse model of infection. Faecal shedding and infection of the intestine and other organs were significantly reduced when synthetic 2'-FL was administered prior to and/or concurrently with an inoculum of pathogenic *C. jejuni*.

*In vitro* studies demonstrate a credible mechanism of action for competitive inhibition by 2'-FL of the binding of pathogenic *C. jejuni* to intestinal epithelial cells. Cell- and antigen-binding studies show that only pathogenic strains of *C. jejuni* bind specifically to  $\alpha$ 1,2-fucosylated moieties of the H-2 antigens of intestinal epithelial cells. Synthetic 2'-FL inhibits this binding—and binding-dependent invasion of epithelial cell lines—in a dose-dependent manner. It has also been demonstrated that pathogenic *C. jejuni* bind with high avidity to immobilised 2'-FL *in vitro*.

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# 1 Introduction

In response to the review request from the Australia and New Zealand Ministerial Forum on Food Regulation, FSANZ has reassessed the bifidogenic and pathogen-binding inhibition effects of 2'-fucosyllactose (2'-FL) and lacto-*N*-neotetraose (LNnT); their possible beneficial role in the normal growth and development of infants or children; their physiological, biochemical and/or functional effects; and evidence to link those effects to specific health outcomes. Such evidence has been assessed against the effects of the compounds as normal constituents of human milk, and considering health outcomes in breastfed infants as the appropriate comparator.

## 1.1 Bifidogenic effect

In the Approval Report for Application A1155, FSANZ concluded that the two human milk oligosaccharides (HMOs)—2'-FL and LNnT—are likely to have a bifidogenic effect in infants and toddlers; that there is a biologically plausible mechanism for the effect; and that there is a substantiated beneficial role in growth and development. These conclusions were considered applicable to supplementation with synthetic 2'-FL and LNnT of all the infant formula products and formulated supplementary foods for young children (FSFYC) to which the application applies.

FSANZ identified 14 relevant studies on the bifidogenic effect not previously included in our assessments, mostly published since the A1155 Approval Report was finalised. We have included the results of these studies, along with studies previously reviewed, in this assessment. Relevant studies are summarised in Tables 1 and 2 and described in Section 2.

## 1.2 Pathogen-binding inhibition effect

In the Approval Report for Application A1155, FSANZ concluded that the evidence supported the likelihood of 2'-FL binding to invasive strains of *Campylobacter jejuni* in the intestinal lumen and inhibiting pathogen attachment to cellular receptors, thereby having an anti-infective health effect. It was concluded that this effect would be reasonably likely to apply to infants and toddlers consuming formula supplemented with 2'-FL.

FSANZ has identified no new studies relevant to the assessment. Challenge studies of *Campylobacter* infection in infants are not feasible. The studies that underpin the conclusions include one human observational study of the incidence and causes of infant diarrhoea; two studies of *C. jejuni* infection in mouse models; and a small number of *ex vivo* and *in vitro* studies of *C. jejuni* adherence to, and invasion of, human intestinal tissue and human epithelial and animal cell lines. Relevant studies are summarised in Table 3 and described in Section 3.

## 1.3 Weight of evidence approach

In order to assess the evidence of a beneficial role of 2'-FL and LNnT in the normal growth and development of infants or children, FSANZ has applied a hierarchy to the different types of study, as follows, in decreasing order of weight of evidence:

- Data from studies in humans:
  - human intervention studies: with greatest weight given to randomised, controlled, double-blinded studies
  - human observational studies: such as cohort studies, case-control studies, cross-sectional studies
  - other studies in humans
- Non-human data:
  - animal studies;
  - ex vivo studies: eg using human or animal biological samples
  - in vitro data: typically analysing mechanistic aspects of an effect.

While data from well-controlled human studies are considered of greatest value, ethical constraints limit their availability when considering health outcomes from interventions in infants and children. Therefore, the assessment for A1155 has largely considered less direct evidence for physiological, biochemical or functional effects of the two HMOs and their link to specific health outcomes. FSANZ has also considered any limitations or confounding factors in the studies which might affect the overall quality of the findings.

FSANZ notes that the Independent Expert Advisory Group (IEAG) for A1155 concluded that there are many different factors in the microbiome which influence infant health, and that it is not possible to determine a linear effect from the presence of one substance in human milk and a specific health outcome. The IEAG advised that FSANZ should focus on specific mechanisms by which health outcomes could be modified by 2'-FL and LNnT.

## 2 Bifidogenic effect

### 2.1 Role of bifidobacteria in the normal growth and development of infants or children

*Bifidobacteria are the predominant genera of the gastrointestinal tract of healthy breastfed infants*

In assessing the proposed bifidogenic effect of 2'-FL and LNnT as a beneficial role in the normal growth and development of infants or children, FSANZ defines a bifidogenic effect as: a proliferation and increase in the relative abundance of *Bifidobacterium* spp. (bifidobacteria) in the intestinal microbiome.

Bifidobacteria establish early as the predominant genus in the microbiome of healthy breastfed infants. Colonisation of the infant gastrointestinal tract (GIT) starts at birth, with the initial microorganisms being acquired from the mother during the birth, through breastfeeding and caring, and from the environment. This initial microbiota modifies the GIT environment, reducing oxygen levels and enabling the rapid proliferation of a dynamic and diverse microbial community that typically becomes dominated, in breastfed infants, by bifidobacteria and lactobacilli (around 90 percent prevalence) and a small number of other bacterial genera within the first few weeks after birth. The precise composition of that community is highly individual, being affected by numerous factors such as mode of delivery, gestational age at birth, mode of infant feeding, maternal diet, genetics and geographic location (Milani et al., 2017).

### *Bifidobacteria levels are higher in breastfed than formula-fed infants*

The proportion of bifidobacteria and lactobacilli present in the gastrointestinal tract of formula-fed infants is typically much lower—approximately 40–60 percent of the overall intestinal microbiota—with increased representation of streptococci, clostridia, staphylococci, a few genera of *Enterobacteriaceae*, and *Bacteroides* (Bian et al., 2020; Coppa et al., 2004a; Edwards & Parrett, 2002). Several studies have demonstrated the greater predominance of *Bifidobacterium* species in breastfed infants compared to formula fed infants (eg Bezirtzoglou et al., 2011; Bian et al., 2020). However, results are somewhat varied. In their comprehensive review of the topic, Davis et al. (2017) ascribe this variability to a number of factors, including the small number of infants in some studies; geographical differences; differences in analytical methods and sampling time points; changes in infant formula composition over time; and differences in oligosaccharide composition of human milk and infant formula.

### *Levels of bifidobacteria reduce from around the time of weaning*

The importance of bifidobacteria to the infant after weaning and beyond 1 year of age is less clear. Most studies report levels of bifidobacteria reducing in absolute and relative terms and the intestinal microbiome becoming gradually more diverse during and after weaning. By the second year of life, the intestinal microbiome of a child becomes increasingly similar to the stable mixed intestinal microbiome found in adults, with a greater range of bifidobacterial species present, but at significantly lower levels (Edwards and Parrett, 2002; Guarner and Malagelade, 2003; Ramakrishna, 2007; Bokulich et al., 2016).

## **2.2 Relationship between levels of bifidobacteria in the infant intestinal microbiome and health outcomes**

FSANZ has previously recognised that the presence of bifidobacteria and lactobacilli in the intestinal microbiome largely benefit the infant host<sup>1,2</sup>. Beneficial roles of this intestinal microbiota include the recovery of nutrients and energy; synthesis of vitamins; absorption of some minerals; protection against infection by pathogens; and effects on the formation and function of the host immune system (O’Hara & Shanahan, 2006). In their review of the role of bifidobacteria in the human gut, O’Callaghan & van Sinderen (2016) observed that, while it has been well established that bifidobacteria confer positive health benefits to the human host, the precise details of the molecular mechanisms underlying the effects are largely still being elucidated.

The infant intestinal microbiome undergoes a transition from birth through the first two years of life and beyond, with complex interactions between bacterial populations and with the host; and with multiple external influences on the whole system. This creates challenges for the design of experiments and the interpretation of observations and experimental data aimed at elucidating the link between individual aspects—such as levels of bifidobacteria in the infant GIT—and health outcomes for the infant.

A recent report provides some initial evidence and points to areas for further research on positive health outcomes arising from the presence of bifidobacteria as a major component of the gut microbiota of healthy infants (Berger et al., 2020a). In a randomised, double-blinded, controlled clinical trial of healthy term infants from Belgium and Italy, the authors describe three ‘faecal community types’ (FCTs)—clusters of the 26 most dominant bacterial genera in infant stool samples at 3 months of age. One FCT is characterised by high levels of

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

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

*Enterobacteriaceae* and *Lachnospiraceae*, while two show a predominance of *Bifidobacteriaceae*, being separated by the relative levels of that family (moderate vs high) and the extent to which other taxa—such as *Lachnospiraceae*, *Enterobacteriaceae* and *Peptostreptococcaceae*—are present. In an analysis of health outcomes for the formula fed infants in the study, the authors report that infants at 3 months of age with gut microbiota in the FCT characterised by the highest relative and absolute levels of bifidobacteria and lowest diversity of bacterial genera were less likely to require antibiotics up to 12 months (odds ratio [OR], 0.4; 95% confidence interval [CI], 0.17 to 0.93;  $P < 0.033$ ) than infants with gut microbiota in the other two FCTs. However, these results were secondary measures from a clinical trial assessing the safety of synthetic 2'-FL and LNnT supplementation of infant formula and their effects on the composition of the intestinal microbiota, and relied up on parent-reported morbidity. The authors conclude that they represent an opportunity to formulate hypotheses for future research and to inform the design of future clinical trials.

### **2.3 Relationship between levels of 2'-FL and LNnT in human milk and levels of bifidobacteria**

Most investigations on the link between 2'-FL and infant bifidobacteria levels rely on observational studies of the faecal microbiota of infants of mothers with different capacities to express  $\alpha$ 1,2-fucosyltransferase, which catalyses the addition of fucose to oligosaccharides at the 2'-O position. Mothers with the secretor phenotype contain a functional gene for the enzyme and produce milk containing  $\alpha$ 1,2-fucosylated human milk oligosaccharides—including 2'-FL—which are absent (or present in only minimal amounts) in the milk of non-secretor mothers. However, there is significant variability in the results of such studies, since the effect of secretor status is not solely on levels of 2'-FL in human milk, and there are many other factors affecting the composition of both human milk and the infant microbiota.

Lewis et al. (2015) examined milk samples and infant stool samples from mothers and their predominantly breastfed infants ( $n=44$ ) on days 6, 21, 71 and/or 120 postpartum. Mothers were secretor ( $n=32$ ) and non-secretor ( $n=12$ ) phenotype, as determined by analysis of levels of 2'-fucosylated HMOs in their milk. Levels of 2'-FL, for example, were two orders of magnitude higher, on average in the milk of secretor-phenotype mothers than in milk of non-secretors. Secretor-fed infants were shown to have significantly higher absolute levels of bifidobacteria in their faeces ( $10^9/\text{g}$  cf  $10^{7.7}/\text{g}$ ;  $p < 0.001$ ). High levels of bifidobacteria were established earlier and more often in infants fed by secretor mothers than in infants fed by non-secretor mothers. The findings demonstrate that maternal secretor status was a significant, but not the sole, determinant of levels of infant faecal bifidobacteria levels. The extent to which the effect is directly attributable to the different levels of 2'-FL in the mothers' milk was not determined, although the authors found that bifidobacteria isolates from secretor-fed infants were three times more likely to grow readily *in vitro* using 2'-FL as sole carbon source.

Smith-Brown et al. (2016) investigated the effect of mothers' secretor status and breast-feeding on the infant microbiota composition at 2 to 3 years of age ( $n=37$  children and  $n=17$  of their mothers). They showed that higher levels of bifidobacteria were sustained at 2-3 years of age in children exclusively breastfed to four months of age with secretor-phenotype mothers compared to those of non-secretor mothers. However, only 11 children were assessed at that age ( $n=8/3$  secretor/non-secretor mothers). The authors did not confirm that secretor status, determined by haemagglutination inhibition assays of maternal blood and saliva samples, correlated with levels of 2'-FL in the mothers' milk.

In their analysis of paired milk and stool samples from Chinese mothers ( $n=56$ ; comprising  $n=43/13$  secretors/non-secretors) and their breast-fed children up to 6 months of age (at which stage,  $n=21/6$  secretor/non) Bai et al. (2018) describe a correlation between levels of

fucosylated HMOs in mothers' milk and infant faecal levels of *Bifidobacterium* spp. at days 6 and 42, but not later, suggesting an early promoting effect of fucosylated HMOs on gut bifidobacteria.

Korpela et al. (2018) examined the effect of birth mode and maternal secretor status on the gut microbiota of fully or partially breastfed 3 month old Finnish infants (secretor n=76; non-secretor n=15). They demonstrated significant differences in levels of 2'-FL in milk collected 3 days postpartum from secretor mothers (mean  $\pm$  sd: 5664  $\pm$  2048 mg/L (vaginal birth); 3810  $\pm$  1183 mg/L (Caesarean birth)) compared to that from non-secretor mothers (mean < 50 mg/L). In vaginally born infants at 3 months, maternal secretor status was not associated with microbiota composition. However, bifidobacteria levels were lower for the caesarean-born infants, and significantly so for those of non-secretor mothers. The authors conclude that the combination of caesarean birth and lack of milk 2'-FL profoundly alters the infants' microbiota.

Conversely, Borewicz et al. (2019), in a study of 121 healthy, breastfed 1 month old infants from the KOALA Birth Cohort (Netherlands), found no correlation between a mother's secretor status and their infant's microbiota profile. Only gender and mode of delivery were significantly correlated with the composition of the infants microbiota. In contrast to most studies, the authors found that levels of 2'-FL were associated with a microbiota cluster type relatively low in abundance of *Bacteroides* and *Bifidobacterium*. However, comparison of levels of HMOs in breast milk and genetic markers of specific bacterial phyla in infant faecal samples showed correlations between the abundance of specific bifidobacteria phylotypes and the breakdown of 2'-FL, LNnT and other HMOs in transit through the GIT. Noting the changing nature of the intestinal microbiome in the first 12 month of life, the strong influence of birth mode, and the relatively low relative abundance of bifidobacteria reported in this study (mean 32%: range 0–91.5%), it is possible that samples from these 1 month old infants are indicative of a relatively immature gastrointestinal microbiome which had not yet reached their peak bifidobacterial levels.

Further conflicting evidence can be found in the work of Wang et al. (2015). They report that 2'-FL in mothers' milk was negatively associated with *Bifidobacterium* levels in 3 month old infants. This study was of limited size (n=16), and they did not control for, or report on, maternal secretor status.

Two *ex vivo* experiments investigating effects of HMOs on infant faecal samples inoculated into a semi-continuous colon simulator provide support for the bifidogenic effect of 2'-FL. Salli et al. (2019) incubated faecal samples from eight breastfed and formula fed Finnish infants between 0.5 and 8 months old with synthetic 2'-FL, comparing results to those obtained with galactooligosaccharides (GOS) and lactose as reference compounds and a control without an additional carbon source. They observed promotion of growth of *Firmicutes* and *Actinobacteria* (including bifidobacteria) by 2'-FL relative to GOS, lactose and control. Van den Abbeele et al. (2019) conducted similar experiments on faecal samples from three 6 month old Belgian infants that had been exclusively formula-fed prior to starting solid food at 4 months old. Samples were incubated with a sugar-depleted medium designed to mimic digested infant formula, with or without the addition of synthetic 2'-FL (2 g/L). The predominant phyla upregulated by 2'-FL included *Firmicutes* and *Actinobacteria*, with levels of *B. adolescentis*, in particular, increasing and *B. bifidum* declining.

In two studies, Azagra-Boronat et al. (2019a; 2019b) investigated the effects of synthetic 2'-FL supplementation of the diets of suckling rats. In the first study, rats were given a daily dose of 0.2 g 2'-FL per 100 g bodyweight by oral gavage from day 2 to day 16, with mineral water carrier as control, and effects were assessed on day 8 and day 16 (Azagra-Boronat et al., 2019a). Rats fed 2'-FL displayed a higher proportion of caecal lactobacilli by day 8, but no effect on bifidobacteria levels were seen. However, bifidobacteria represented only a very

minor component of the microbiota of the 8-day-old rats in this study—with *Rothia* spp. being the main *Actinobacteria* genus observed. In a second rat feeding study, effects of 2'-FL and other oligosaccharides on the diarrhoeagenic effect of rotavirus were assessed in neonatal Lewis rats (Azagra-Boronat et al., 2019b). Dietary interventions were administered daily by oral gavage from days 2–8 of life, with rotavirus challenge from day 5. It was observed that 2'-FL induced a significant increase in *Bifidobacterium animalis* compared to other treatments. However, the effect was off a very low base, with *Bifidobacterium* species representing only  $0.014 \pm 0.011\%$  in control animals subjected to no treatments.

Using juvenile animal studies as proxies for studies on human infants is subject to a number of confounding factors, as developmental time points are difficult to compare (Kim et al., 2017). The 8-day point applied by Azagra-Boronat and colleagues in these studies corresponds to sometime late in the first month of a human infant's life, which is early relative to the time of assessment in most of the human studies considered above. Noting that, and given the extremely low representation of bifidobacteria observed in the neonatal rat gastrointestinal tract, the results of these studies are considered to be of limited applicability to human infants.

Many studies have demonstrated preferential utilisation of certain HMOs by bacteria in the infant GIT—particularly certain species of *Bifidobacterium* and *Bacteroides* (Garrido et al., 2015; Ruiz-Moyano et al., 2013; Yu et al., 2013a, 2013b). The phenomenon is well-established and increasingly well understood at a microbial genetic level.

Most recently, Lawson et al. (2020) characterised carbohydrate metabolism genes in 19 strains of bifidobacteria isolated from breastfed infants, with a particular focus on genes and gene clusters involved in utilisation of HMOs. They demonstrate significant inter- and intra-species variability in the presence of genes for metabolising 2'-FL and LNnT and the ability to grow on these HMOs in vitro: ie HMO utilisation is dependent on the type of HMO and the strain (rather than the species) tested. They further demonstrated the ability of strains lacking the ability to directly metabolise HMOs to grow on the by-products of HMO metabolism—fucose, galactose, acetate, and *N*-acetylglucosamine—by HMO-degrading strains (ie cross-feeding), strengthening the concept of an interdependent gastrointestinal microbial community.

In a review of HMO usage by bifidobacteria, Sakanaka et al. (2020) undertook a metagenomics data mining analysis of 34 breastfed and 27 formula fed infants (<1 year old) residing in the USA ( $n = 37$ ), Malawi ( $n = 14$ ), and Venezuela ( $n = 10$ ). They show that the abundance of bifidobacteria in the Malawian and Venezuelan breast-fed infants positively correlated to the abundance of genes responsible for an intracellular digestion strategy for HMOs while in the infants from the USA the abundance of bifidobacteria was positively associated with genes responsible for extracellular digestion. While the practical effects of such differences have not been fully elucidated, the results demonstrate significant geographic and/or population differences in breastfed infant gut bifidobacteria utilisation strategies for HMOs, and imply that some study results might not be applicable outside the country/population on which they are derived.

**Table 1: Summary of findings of human studies on the bifidogenic effects of 2'-FL and LNnT**

Reference Country	Study design/methods	Summary of Findings (relevance to beneficial role; cause & effect; mechanism of action; dose-response)	Significance of finding
Human intervention studies			
*Berger et al. (2020a)  Belgium & Italy	<p>Randomised, double-blinded, controlled multicentric clinical trial of healthy, full-term (gestational age 37–42 weeks) infants. Recruited at &lt; 14 days old. Followed until 1 year old.</p> <p>Number of participants at 3mo/1yo: breast fed (BF: n=35/30); control infant formula (IF: n=63/49); test IF supplemented with synthetic 2'-FL (1.0-1.2 g/L) &amp; LNnT (0.5-0.6 g/L) (n=58/47). Feeding supplementation ended at 6 months of age.</p> <p>Exclusion criteria: congenital illness or malformation that may affect growth; significant prenatal and/or serious postnatal disease before enrolment; minor parent(s); parents/caregivers not expected to comply with study procedures; concurrent or prior participation in another clinical trial since birth (except for BF group, where vaccine studies were allowed.)</p>	<p>Primarily a safety study.</p> <p>Secondary findings include: test group had more similar microbiota composition to BF (<i>cf</i> control IF) group in stool samples at 3 months. Supplementation correlated with higher levels of bifidobacteria in stool samples at 3 months. Higher levels of bifidobacteria in stools at 3 months correlated with significantly less likelihood to require antibiotics during the first year. Supplementation significantly correlated with lower risk of bronchitis up to 3 or 12 months compared to control group (as reported in Puccio et al., 2017).</p>	<p>Evidence for:</p> <ul style="list-style-type: none"> <li>• a beneficial role for bifidobacteria in the normal growth and development of infants</li> <li>• more bifidobacteria being more beneficial than less</li> <li>• synthetic 2'-FL and/or LNnT promoting slightly higher levels of bifidobacteria in IF-fed infants.</li> </ul>
Puccio et al. (2017)  Belgium & Italy	<p>Randomised, double-blinded, controlled multicentric clinical trial of healthy, full-term (gestational age 37–42 weeks) infants. Recruited at &lt; 14 days old. Followed until 1 year old.</p> <p>Number of participants at enrolment/4mo: BF (n=35/30); control IF (n=87/67); test IF supplemented with synthetic 2'-FL (1.0-1.2 g/L) &amp; LNnT (0.5-0.6 g/L) (n=88/64). Feeding supplementation ended at 6 months of age.</p> <p>Exclusion criteria: congenital illness or malformation that could affect growth; significant prenatal and/or serious postnatal disease before enrolment; minor parent(s); parents not expected to comply with study procedures; current or previous participation in another clinical trial.</p>	<p>Primarily a safety study.</p> <p>Secondary findings include correlation of supplementation with lower risk of parent-reported morbidities, including bronchitis, lower respiratory tract infection and lower antibiotic use compared to control group. Further data on parent-reported morbidity and a study of microbiota effects is reported in Berger et al. (2020a).</p>	<p>Evidence for beneficial health outcomes for infants fed infant formula supplemented with synthetic 2'-FL and LNnT.</p>

Reference	Study design/methods	Summary of Findings (relevance to beneficial role; cause & effect; mechanism of action; dose-response)	Significance of finding
<p>Elison et al. (2016)</p> <p>Denmark</p>	<p>Randomised, double-blinded, placebo-controlled feeding study of adults, aged between 18 and 60 years.</p> <p>Exclusion criteria: participation in a clinical study 1 month before and during the study; abnormal results of laboratory and clinical screening tests; compliance with Gastrointestinal Symptom Rating Scale criteria; any GI and/or other severe diseases; highly dosed probiotic supplement and/or antibiotic use 3 months before and during the study; regular consumption of medication that might interfere with symptom evaluation; pregnancy or seeking pregnancy; and nursing.</p> <p>Fourteen days of dietary supplementation with synthetic 2'-FL and LNnT separately and together at 5g, 10g and 20g/day, with glucose (2g/day) as placebo control (n=10 per group). Diet not otherwise controlled. Faecal microbiota composition analysed before intervention and during the intervention.</p>	<p>Increasing intakes of HMOs correlated with increasing levels of bifidobacteria and a reduction in relative abundance of <i>Firmicutes</i> and <i>Proteobacteria</i> in faecal samples.</p>	<p>Evidence that synthetic 2'-FL and LNnT promote higher levels of bifidobacteria in (adult) gut microbiota in a dose-dependent manner (ie dose-response).</p>
Human observational studies			
<p>*Bian et al. (2020)</p> <p>Sub-Saharan Africa (The Gambia, Kenya, Mali, and Mozambique) and South Asia (Bangladesh, India, and Pakistan)</p>	<p>Observational study of the impact of breastfeeding on symptomatic (n=128) and case-matched asymptomatic (n=105) <i>Campylobacter</i> infected infants.</p> <p>At time of faecal sample collection, infants were stratified by age: 0–5 months old (n=110); 6–11 months old (n=123).</p> <p>Infants were either exclusively breastfed (n=142) or not breastfed (n=91) at the time of sample collection.</p>	<p>Levels of bifidobacteria were more than fourfold higher in exclusively breastfed than in non-breastfed <i>Campylobacter</i>-infected infants.</p>	<p>Evidence for bifidobacteria levels being higher in breastfed infants</p>
Reference	Study design/methods	Summary of Findings (relevance to beneficial role; cause & effect; mechanism of action; dose-response)	Significance of finding
Country			

<p>*Borewicz et al. (2019)</p> <p>The Netherlands</p>	<p>Observational study of healthy full-term (gestational age <math>\geq</math> 37 weeks), exclusively breastfed infants (n=121) and their mothers from the KOALA Birth Cohort. Mothers recruited at between 14 and 18 weeks of gestation.</p> <p>Infant faecal samples and mothers' milk samples collected at 1 month of age.</p> <p>Exclusion criteria: prematurity, twins, congenital abnormalities; use of antimicrobial agents before faeces collection.</p>	<p>Human milk 2'-FL levels ranged from 0.0–852.8 mg/L, (median 460.0; mean 372.7 SD 242.3).</p> <p>Infant faecal microbiota is influenced by gender, delivery mode and 2'-FL (<math>p &lt; 0.06</math>). 2'-FL was associated with a microbiota cluster type relatively low in abundance of <i>Bacteroides</i> and <i>Bifidobacterium</i>. Correlations were observed between the abundance of specific bifidobacteria phylotypes and the breakdown of 2'-FL.</p>	<p>Evidence against 2'-FL in breast milk leading to higher bifidobacteria levels.</p> <p>Evidence for breakdown of 2'-FL in the infant GIT by certain species and/or strains of <i>Bifidobacterium</i>.</p>
<p>*Korpela et al. (2018)</p> <p>Finland</p>	<p>Observational study of mothers and their fully or partially breastfed infants recruited from the placebo cohort of a probiotic intervention trial (Kuitunen et al., 2012).</p> <p>Breast milk samples collected on postpartum day 3 and infant faecal samples at age 3 months.</p> <p>Exclusion criteria: probiotic intake by mother or baby; antibiotics use in infant prior to faecal sample collection.</p> <p>Mothers' secretor<sup>3</sup> status determined by quantification of human milk 2'-FL levels: secretor (n=76) and non-secretor (n=15).</p>	<p>2'-FL values (mean <math>\pm</math> sd) in the milk of secretor mothers was 5664 <math>\pm</math> 2048 mg/L (vaginal birth) and 3810 <math>\pm</math> 1183 mg/L (Caesarean birth). The mean amount in the milk of non-secretor mothers was below 50 mg/L. In vaginally born infants at 3 months, maternal secretor status was not associated with microbiota composition. Among the caesarean-born infants of non-secretor mothers, bifidobacteria were strongly depleted. For caesarean born infants of secretor mothers, microbiota was more similar to that of vaginal born infants, including higher levels of bifidobacteria.</p>	<p>Evidence, in caesarean born infants only, for 2'-FL promoting higher levels of bifidobacteria in gut microbiota in a dose-dependent manner (ie dose-response).</p>

<sup>3</sup> Secretor mothers produce milk containing  $\alpha$ 1-2-fucosylated human milk oligosaccharides (including 2'-fucosyllactose), which are absent (or present in only minimal amounts) in the milk of non-secretor mothers.

Reference Country	Study design/methods	Summary of Findings (relevance to beneficial role; cause & effect; mechanism of action; dose-response)	Significance of finding
*Bai et al. (2018) China	<p>Longitudinal observational study of mothers and their exclusively breastfed infants. Mothers recruited at 34 weeks of gestation. Infants followed to 6 months of age.</p> <p>Exclusion criteria: caesarean-born infants; antibiotic, probiotic or formula powder intake by infants.</p> <p>Number of participants: 56 mothers (n=43 secretor phenotype; n=13 non-secretor) and infants at enrolment. At 6 months, n=21/6 secretor/non-secretor.</p>	<p>Paired milk and stool samples were analysed from mothers and their breast-fed children out to 6 months. Significant correlation was observed between levels of fucosylated HMOs and <i>Bifidobacterium</i> spp. at days 6 and 42, but not later, suggesting an early promoting effect of fucosylated HMOs on gut bifidobacteria.</p>	<p>Evidence for fucosylated HMOs promoting higher levels of bifidobacteria in gut microbiota early in an infant's life.</p>
Smith-Brown et al. (2016) Australia	<p>Observational study of mothers and their infants from the Feeding Queensland Babies Study (FQBS) cohort. Infants followed to 2-3 years of age.</p> <p>Number of participants: 37 children and 17 of their mothers at enrolment.</p> <p>Analysis of the effects of mothers' secretor status on child microbiota at 4 months and 2-3 years of age.</p>	<p>Found higher levels of <i>Bifidobacterium</i> and <i>Bacteroides</i> species at 4 months in breastfed infants of secretor mothers compared to those of non-secretor mothers. Higher relative levels of <i>Bifidobacterium</i> species (but not <i>Bacteroides</i>) was sustained at 2-3 years in breastfed (to at least 4 months) children of secretor mothers.</p> <p>Note: small numbers of children who had been exclusively breastfed remained at the 2-3 year time point (8 with secretor mothers, 3 with non-secretor mothers), and did not assay for levels of 2'-FL in breast milk.</p>	<p>Evidence for 2'-FL promoting higher levels of bifidobacteria in gut microbiota in a dose-dependent manner (ie dose-response).</p>
Lewis et al. (2015) USA	<p>Observational study of a subset of 44 infants and their mothers from the UC Davis Foods For Health Institute Lactation Study. Subjects were enrolled at approximately 34 weeks of gestation. Followed until day 120 of the infants' lives.</p> <p>Breast milk samples and infant stool samples obtained from mothers (n=44) and their predominantly breastfed infants (n=44) on days 6, 21, 71 and/or 120 postpartum. Mothers were secretor (n=32) and non-secretor (n=12) phenotype.</p>	<p>High levels of bifidobacteria were established earlier and more often in infants fed by secretor mothers than in infants fed by non-secretor mothers. A higher percentage of bifidobacteria isolated from secretor-fed infants were able to metabolise 2'-FL.</p>	<p>Evidence that 2'-fucosylated HMOs, including 2'-FL, promote higher levels of bifidobacteria in gut microbiota.</p>

Reference Country	Study design/methods	Summary of Findings (relevance to beneficial role; cause & effect; mechanism of action; dose-response)	Significance of finding
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*Wang et al. (2015) USA	<p>Observational study of healthy, full term, vaginally-delivered, exclusively breastfed or formula fed infants. Mothers were recruited between the third trimester of pregnancy and 1 month postpartum. Infants were followed until 3 months of age.</p> <p>Number of participants: Breastfed (n=16); formula fed (n=6).</p> <p>Exclusion criteria: infants diagnosed with intolerance to cow's milk; partially breastfed infants; infants who became clinically ill with fever, contagious diseases or active diarrhoea; antibiotic treatment within 2 weeks prior to sample collection.</p>	<p><i>Bifidobacterium</i> predominated in both breastfed and formula fed infants at 3 months of age, with no difference in abundance or in overall faecal microbiota phylogenetic diversity between the two groups. Found 2'-FL associated with <i>Bacteroides</i> and negatively associated with <i>Bifidobacterium</i>. Note: participant numbers are small, and did not control for secretor status of mothers.</p>	<p>Evidence against 2'-FL in breast milk leading to higher bifidobacteria levels.</p>
Bezirtzoglou et al. (2011) Greece	<p>Observational study of healthy, vaginally-delivered, exclusively breastfed or formula fed infants. Followed between days 11–22 (breastfed) or 14–36 (formula fed).</p> <p>Number of participants: Breastfed (n=6); formula fed (n=6).</p> <p>Faecal bacterial composition analysed by fluorescence <i>in situ</i> hybridisation.</p>	<p>Found breastfed infants had higher levels of bifidobacteria in faeces than formula fed infants. Bifidobacteria were still dominant, but at lower and more variable relative levels, in the formula fed infants, who also had higher <i>Bacteroides</i> and <i>Prevotella</i> levels and a more diverse microbiota. Note: participant numbers are small; different ages between cohorts; and did not control for secretor status of mothers. Did not assess 2'-FL composition of breast milk.</p>	<p>Evidence for breastfeeding promoting higher levels of bifidobacteria in gut microbiota</p>
Other human studies			
*Cabrera-Rubio et al. (2019)	<p>Observational study of healthy Spanish mothers with exclusive breast-feeding practices, carried out within the MAMI birth cohort (NCT03552939). Number of participants: n=25. Breast milk (BM) samples were collected longitudinally during the first month of lactation (<math>\leq 5</math> days: colostrum, <math>&lt; 15</math> days: transitional milk, and 1 month: mature milk).</p>	<p>Microbiota composition and quantity in breast milk depends on secretor/non-secretor status. Bifidobacteria more prevalent in secretor breast milk samples during the first 4 weeks post-partum. Future studies to determine effect on neonate GIT colonization.</p>	<p>Suggests one mechanism by which bifidobacteria come to be established and dominate the microbiota of breastfed infants.</p>

**Table 2: Summary of findings of animal, *ex-vivo* and *in vitro* studies on the bifidogenic effects of 2'-FL and LNnT**

Reference	Summary of Findings	Significance of finding
<b>Animal data</b>		
*Azagra-Boronat et al. (2019a)	Rats fed 2'-FL produced by microbial fermentation (> 90% purity) displayed higher Lactobacillus proportion. No effect observed on bifidobacteria – they represented only a very minor component of the microbiota of the 8-day-old rats in this study.	Not supportive of synthetic 2'-FL promoting higher levels of bifidobacteria in gut microbiota.
*Azagra-Boronat et al. (2019b)	Rat feeding study with rotavirus challenge. Synthetic 2'-FL induced an increase in <i>Bifidobacterium animalis</i> population off a very low base, and this did not correlate to the diarrhoea index.	Slightly supportive of synthetic 2'-FL promoting higher levels of bifidobacteria.
<b><i>ex vivo</i> data</b>		
*Salli et al. (2019)	A semi-continuous colon simulator inoculated with faecal samples from BF and FF Finnish infants between 0.5 and 8 mo and incubated with synthetic 2'-FL with GOS and lactose as reference compounds and control without additional carbon source. 2'-FL promoted the growth of bifidobacteria. The predominant phyla upregulated by 2'-FL included <i>Firmicutes</i> and <i>Actinobacteria</i> (which includes the genus <i>Bifidobacterium</i> ).	Support for 2'-FL in breast milk or synthetic 2'-FL in infant formula leading to higher bifidobacteria levels.
*Van den Abbeele et al. (2019)	A semi-continuous colon simulator inoculated with faecal samples from three 6 mo Belgian infants, exclusively formula-fed prior to beginning consuming solids food at 4 mo. Incubated with 2 g/L synthetic 2'-FL in a sugar-depleted carrier medium of composition to mimic digested IF. The predominant phyla upregulated by 2'-FL included <i>Firmicutes</i> and <i>Actinobacteria</i> (particularly <i>Bifidobacterium adolescentis</i> , at the expense of <i>B. bifidum</i> ). Claimed to support supplementation of follow-on formula with 2'-FL.	Support for 2'-FL in breast milk or synthetic 2'-FL in infant formula leading to higher bifidobacteria levels.
<b>Studies on HMO utilisation by bifidobacteria <i>in vitro</i></b>		
*Sakanaka et al. (2020)	Review of HMO usage by bifidobacteria, including meta-analysis showing geographic and/or population differences in breast-fed infant gut bifidobacteria utilisation strategies for HMOs. Implies some study results might not be applicable outside the country/population on which they are derived.	Evidence on the mechanism of utilisation of 2'-FL and LNnT by <i>Bifidobacterium</i> species
<b>Other relevant evidence (eg reviews)</b>		
*Lawson et al. (2020)	Investigation of HMO usage—including synthetic 2'-FL and LNnT—by 19 strains of bifidobacteria derived from BF infants. Includes analysis of genes / gene clusters.	Evidence on the mechanism of use of 2'-FL and LNnT by <i>Bifidobacterium</i> species
*Berger et al. (2020b)	Human milk oligosaccharide 2'-fucosyllactose links feedings at 1 month to cognitive development at 24 months in infants of normal and overweight mothers. No measure of bifidobacteria. Cite Carlson et al. (2018) in support of microbiome/cognitive development link.	Support for a positive health outcome from synthetic 2'-FL addition to infant formula.

Reference	Summary of Findings	Significance of finding
Sprenger et al. (2017)	Single centre, longitudinal cohort study of n=50 mothers and their 50 children (equal male and female). Breast milk collected at 30, 60, and 120 days postpartum. Infants assessed up to 4 mo. Mother secretor status based on milk 2'-FL concentrations at 30 days: low (n=16: 95% CI of mean 12±42 mg/L) and high concentrations (n=34: 95% CI of mean 1880±2460 mg/L). No correlation between infant growth up to 4 months and 2'-FL levels. Observe correlation between level of LNnT and 2'-FL in breast milk samples.	Supports combined addition of synthetic 2'-FL and LNnT to infant formula.

\* Study not previously included in our assessments of A1155.

## 2.4 Effect of supplementation of infant diets with 2'-FL and LNnT on levels of bifidobacteria and health outcomes

Until relatively recently it has not been possible to conduct large-scale interventional clinical trials of HMOs, as the cost to manufacture them at sufficient levels was prohibitive. However, some synthetic HMOs—including 2'-FL and LNnT—have been available at industrial scale since 2016, and the results of a small number of preclinical studies, observational clinical trials and interventional clinical trials have been published.

Puccio et al. (2017) and Berger et al. (2020a) report the results of a randomised double-blinded controlled multicentre clinical trial of healthy term infants from Belgium and Italy from birth through to 1 year old. The study was primarily aimed at assessing the safety of synthetic 2'-FL and how well it was tolerated in the infant diet. Three groups of infants were enrolled (at age 0-14 days), with numbers of participants at enrolment and at 4 months as follows: exclusively breastfed (n=35; 30); control cow's milk-based infant formula fed (n=87; 67); and test group with infant formula supplemented with synthetic 2'-FL (1.0-1.2 g/L) and LNnT (0.5-0.6 g/L) (n=88; 64). From 6 to 12 months of age all infants received standard follow-up formula without HMOs.

While the primary endpoint of the study was weight gain through 4 months, Puccio et al. (2017) report on a number of secondary endpoints, including parent-reported morbidity. Information provided by parents was reviewed and confirmed by a physician at each study visit, at 1, 2, 3, 4, 6, and 12 months of age. Findings from these secondary outcomes included significant correlation ( $p \leq 0.05$ ) of supplementation of infant formula with lower risk of:

- bronchitis to 4, 6 and 12 months: OR(95%CI) = 0.16(0.02–0.78); 0.26(0.08–0.74); and 0.30(0.11–0.73), respectively.
- lower respiratory tract infection to 12 months: OR = 0.45; 95%CI 0.21–0.95.
- antibiotic use to 12 months: OR = 0.47; 95%CI 0.24–0.89.

Berger et al. (2020a) undertook further analysis of the results of this trial, limiting their analysis to the infants who completed the 6-month treatment and for whom they had good quality stool samples at 3 months of age, termed the per-protocol (PP) groups (control n = 63; test n = 58; breastfed n = 35). In this well-controlled trial subpopulation, only the correlations between supplementation of infant formula with lower risk of bronchitis at 6 (OR = 0.20; 95%CI 0.06–0.64) and 12 months (OR = 0.26; 95%CI 0.10–0.66) were significant (ie  $p \leq 0.05$ ). Both reports conclude that additional studies are needed to confirm whether HMO-supplemented infant formulas confer protection from illness.

In results from the same trial, Berger et al. (2020a) analysed the taxonomic composition of the stool microbiota of the PP groups of infants at 3 and 12 months of age by 16S rRNA gene sequencing. The phylogenetic diversity of the test group was significantly lower than the control group ( $P < 0.05$ ), and more similar to the breastfed group, which was the least diverse. The microbiota compositions of the three groups were significantly different at the genus level ( $p < 0.001$ ), with the test group closer to the breastfed group than to the control. Supplementation correlated with significantly higher levels of *Bifidobacterium* and lower levels of three taxa with potentially pathogenic members: *Escherichia*, *Streptococcus* and unclassified *Peptostreptococcaceae* (all  $p < 0.05$ ).

Further evidence that synthetic 2'-FL and LNnT promote higher levels of bifidobacteria in gut microbiota in a dose-dependent manner can be found in the double blind, randomised, placebo-controlled adult feeding study of Elison et al. (2016). The study involved ten groups of Danish adults fed either 2'-FL, LNnT or 2'-FL + LNnT (2:1 mass ratio) at 5, 10 or 20 g per day or 2 g of glucose as placebo control over 14 days. The daily doses were chosen to be within the range of the average daily intake per kg body weight in breastfed infants.

Diet was not otherwise controlled, but subjects were asked not to change their diet over the course of the study. Increasing intakes of the oligosaccharides correlated with increasing levels of

bifidobacteria—from around 6 percent to up to 25 percent abundance in some individuals—and a reduction in relative abundance of *Firmicutes* and *Proteobacteria* in faecal samples compared to the control. The increase in bifidobacteria was dose dependent, but unrelated to the initial bifidobacteria abundance, and was observed to be more pronounced for the 2'-FL + LNnT mix.

## 2.5 Conclusions

FSANZ concludes that the addition of synthetic 2'-FL and LNnT to infant formula leads to a *Bifidobacterium*-enriched microbiota that is more similar to that observed in breastfed infants than in those fed unsupplemented formula. However, evidence for a link between the presence of 2'-FL and/or LNnT in human milk or formula and any specific health outcome (and the size of such an outcome) is limited and largely indirect. Evidence that the effect is applicable to formulated supplementary foods for young children (FSFYC) supplemented with synthetic 2'-FL and/or LNnT is very limited.

There is broad scientific consensus that a *Bifidobacterium*-enriched microbiota has functional benefits in the normal growth and development of breastfed infants and plays a role in reported differences in health outcomes between breastfed and formula fed infant populations. However, it is recognised that, in most cases, the precise molecular mechanisms underlying such beneficial effects are still to be fully characterised.

The link between breastfeeding and higher levels of bifidobacteria in the infant gastrointestinal tract (GIT) is reasonably well established from numerous observational studies on the effects of formula feeding and breastfeeding on the composition of the infant gastrointestinal microbiota. Although there is variability in the results, most conclude that the proportion of bifidobacteria and lactobacilli is significantly lower for formula-fed infants. However, a direct link between levels of 2'-FL and LNnT in human milk and levels of bifidobacteria in the infant gastrointestinal tract is not well established, as there is significant variability in the results of observational studies investigating this relationship.

A credible mechanism by which HMOs influence the composition of the gastrointestinal microbiome has been established through a small number of human intervention and observational studies on breastfed and formula fed infants, and is supported by *ex vivo*, *in vitro* and *in silico* studies on the utilisation of specific HMOs by bifidobacteria isolated from infant gastrointestinal tracts. There is a very limited amount of evidence that the effect is applicable to formulated supplementary foods for young children (FSFYC) supplemented with synthetic 2'-FL and LNnT.

Secondary outcomes from one randomised controlled clinical trial support a conclusion that addition of synthetic 2'-FL and LNnT to infant formula contributes to health outcomes for formula fed infants more in line with those of healthy breastfed infants. However, results are considered to be preliminary.

The IEAG for A1155 concluded that there are many different factors which influence infant health, and that it is not possible to determine a linear effect from the presence of one substance in human milk and a specific health outcome.

## 3 Pathogen-binding inhibition effect

### 3.1 Protective effect of breastfeeding against campylobacteriosis

Gastrointestinal illness is a leading cause of infant and toddler morbidity and mortality, with an estimated 300 000 episodes of diarrhoea leading to the death of infants globally in 2011 (Walker et al., 2013). *Campylobacter jejuni* is recognised as one of the major bacterial causes of infant diarrhoea in developing and developed countries (Fullerton et al., 2007; Kotloff et al., 2013).

In Australia, the rate of notifications of *Campylobacter* infection is highest in children aged up to 4 years olds (NNDSS Annual Report Writing Working Group, 2019). In 2018, the notification rate for the age group was 210.1 cases per 100 000 resident population, compared to 135.5 cases per 100 000 resident population for all age groups (NNDSS, 2020). In a review of the epidemiology of *Campylobacter* infection in Queensland from 1991 to 1995, Stafford et al. (1996) found that the notification rate was highest in children aged 12–23 months—more than double the rate for the 0–11 months age group. The reason for the higher notification rate in the 12–23 month age group was not established in the study. A similar trend is observed in New Zealand. For example, in 2017, children aged 1–4 years (257.9 per 100,000) and infants less than 1 year (241.0 per 100,000) had the highest *Campylobacter* infection notification rates (Institute of Environmental Science and Research Ltd., 2019).

It has been well established that breastfeeding has clear short-term benefit in reducing morbidity and mortality due to infectious diseases in childhood, including diarrhoeal disease. This has been observed in low, middle and high-income countries. In a systematic review and meta-analysis of studies on the effect of breastfeeding on respiratory infections and diarrheal disease in childhood, Horta and Victora (2013) found that the intensity of breastfeeding was inversely correlated with risk of diarrhoeal illness at any age up to 5 years. More intense breastfeeding was strongly protective compared to less intensive in infants up to 6 months of age, with a pooled relative risk of 0.37 (95% CI: 0.27–0.50). Breastfeeding also decreased the risk of hospitalisation and death from diarrhoea at any age up to 5 years: pooled relative risk 0.28 (95% CI: 0.16–0.50) and 0.23 (95% CI: 0.13–0.42), respectively.

Several studies report a similar strong protective effect of exclusive and partial breastfeeding specifically for *Campylobacter* infection and diarrhoea (Ruiz-Palacios et al., 1990; Nachamkin et al., 1994; Fullerton et al., 2007; Bilenko et al., 2008). Many of the studies that aim to assess the protection afforded by breastfeeding fail to adequately control for the possibility that breastfeeding simply shields infants from exposure to possibly contaminated alternative food and water sources (Quigley et al., 2007). However, several human observational studies provide evidence to indicate that it is due to the functional properties of human milk itself (Ruiz-Palacios et al., 1990; Nachamkin et al., 1994; Victora et al., 1989).

### 3.2 Inverse correlation between 2'-FL in mothers' milk and *Campylobacter*-associated diarrhoea

Morrow et al. (2004) investigated the protective effects of naturally-occurring human milk oligosaccharides (HMOs) against diarrhoeal illness in breastfed infants in San Pedro Martir, Mexico City. Ninety three breastfeeding mother-infant pairs were prospectively studied from birth to 2 years of age 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 pathogenic microorganisms, including *Campylobacter*. A single milk sample from each mother, collected between 1 and 5 weeks postpartum, was analysed by HPLC for levels of 2'-linked fucosylated oligosaccharides, including 2'-FL, in the HMO fraction. Longitudinal data from 11 Mexican secretor mothers confirmed the representativeness of this sample for the course of lactation. Infant stool samples were collected weekly and whenever diarrhoea occurred. Diarrhoea samples were routinely tested for *Campylobacter 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 HMOs) was approximately 8.7 cases per 100 child months, compared to an incidence of approximately 1.5 and 1.6 cases per 100 child months for mothers with intermediate (29-36% of total HMOs) and high ( $\geq$ 37% of total HMOs) levels of 2'-FL, respectively. No protective effect against other pathogens was observed for 2'-FL in human milk. The concentrations of 2'-FL and HMOs in human milk was not reported by Morrow et al. (2004), so it is not possible to infer a minimal protective concentration of 2'-FL in milk. The results are consistent with findings from animal, cell culture and *in vitro* studies demonstrating the binding of *C. jejuni* to intestinal epithelial cells through 2'-linked fucosylated epitopes and inhibition of binding by 2'-FL, HMO fractions and H-2 antigen binding competitors.

### **3.3 Protective effect of 2'-FL against *C. jejuni* infection in mice**

In a challenge study with pathogenic *C. jejuni* strain 81-176 in an experimental murine model, Yu et al. (2016) demonstrated a strong protective effect of 2'-FL produced by microbial fermentation (>99% purity) when added with the bacterial challenge and/or to the diet of C57BL/6 mice at around twice its average level in human milk. Mice were treated for 7 days with antibiotic to disrupt the intestinal microbiota, followed by inoculations of  $10^8$  cfu/mouse in 100  $\mu$ L saline gavage on three consecutive days. Addition of 5 g/L 2'-FL concurrently with *C. jejuni* challenge reduced faecal shedding and infection of the intestine, spleen and mesenteric lymph nodes by 90%, 80%, 96% and 93%, respectively. Addition of 5 g/L 2'-FL in drinking water *ad libitum* for 3 days before the bacterial challenge, along with its inclusion at 5 g/L with the challenge dose, reduced faecal shedding and infection of the intestine, spleen and mesenteric lymph nodes by 99%, 97%, 97% and 98%, respectively.

Ruiz-Palacios et al. (2003) investigated intestinal colonisation by pathogenic *C. jejuni* of pups of BG/SJL mice transfected with a plasmid containing the human FUT1 gene in order to express  $\alpha$ 1,2-fucosyltransferase in mammary gland tissue and milk. Pups and their dams were confirmed to be free of *Campylobacter* infection prior to experimental inoculation. Prieto et al. (1995) had previously shown that transgenic mice constructed in the same manner produce H-2 blood group antigen and 2'-FL (mean concentration 2 g/L) in their milk, while non-transgenic mice did not have any significant amount of 2'-FL in their milk. Ruiz-Palacios et al. (2003) observed that intestinal clearance of pathogenic *C. jejuni* strain 287ip was significantly quicker in pups nursing transgenic versus non-transgenic dams when the pups were challenged with identical inocula ( $10^6$  CFU). Wild-type mice inoculated with  $10^6$  CFU generally remained infected throughout the 15 days of the study, while none of the infected pups nursing transgenic dams remained infected after 9 days of nursing.

The results of these animal studies support the conclusion that invasive *C. jejuni* strains require binding to  $\alpha$ 1,2-fucosylated receptors on the surface of intestinal epithelial cells to initiate infection, and that  $\alpha$ 1,2-fucosylated moieties in milk—including 2'-FL (synthetic or endogenous) at levels similar to its normal concentration in human milk—inhibit infection by invasive strains of *C. jejuni*.

### **3.4 Competitive inhibition of binding of *Campylobacter jejuni* to $\alpha$ 1,2-fucosylated histo-blood group epitopes by 2'-FL**

A role for fucose in the binding of *C. jejuni* to intestinal mucosa and epithelium was implied in the experiments of Cinco et al. (1984) and Moser et al. (1992), but the cellular and antigen binding studies of Ruiz-Palacios et al. (2003) were the first to clearly demonstrate the role of the  $\alpha$ 1,2-fucosylated epitopes of H-2 blood group antigens on intestinal epithelial cells. Ruiz-Palacios et al. (2003) transfected Chinese hamster ovary (CHO) cells with genes for  $\alpha$ 1,2-fucosyltransferase (CHO-FUT1),  $\alpha$ 1,3/4-fucosyltransferase (CHO-FUT3), and  $\alpha$ 1,3-fucosyltransferase (CHO-FUT4). CHO cells are an epithelial cell line that do not express  $\alpha$ 1,2-linked fucosylated epitopes on their surface and have low *Campylobacter* binding affinity relative to HEp-2 cells. Transfection with the FUT1 gene confers on CHO cells the ability to complete the synthesis of all  $\alpha$ 1,2-fucosylated antigens. Expression of the antigens on the surface of transfected CHO cells was confirmed by immunofluorescence assay using specific monoclonal antibodies. Binding of *C. jejuni* was

assessed by light microscopy of cells stained with Warthin-Starry and by scanning electron microscopy.

Binding of four adherent pathogenic *C. jejuni* strains isolated from children and two non-adherent, non-pathogenic strains isolated from healthy children was tested. Cells transfected with FUT1 gene expressed the  $\alpha$ 1,2-fucosylated H-2 antigen on their surface and 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. Binding of *C. jejuni* to FUT1-transfected CHO cells was inhibited by agents that bind specifically to H-2 antigen (*L. tetragonolobus* or UEA I lectins, or anti-H-2 mAbs), but not by lectins and mAbs which bind other epitopes, such as the H-1 antigen.

Ruiz-Palacios et al. (2003) also demonstrated specific binding of *C. jejuni* to the H-2 antigen prepared as a neoglycoprotein—a glycoconjugate of bovine serum albumin—and immobilized on nitrocellulose membranes. Ten of 12 pathogenic *C. jejuni* strains tested bound to the immobilised H-2 antigen, while none of four non-pathogenic strains bound. In a competitive binding assay, pre-incubation of the membranes with monoclonal antibodies against the H-2 antigen strongly inhibited binding of pathogenic *C. jejuni*, strain 287ip, implying a specific binding mechanism between bacterial cell and antigen.

Inhibition of the interaction between *C. jejuni* and H-2 antigen by 2'-FL has been directly demonstrated in a number of ways. In the experiments described above, Ruiz-Palacios et al. (2003) showed that binding of *Campylobacter* to FUT1-transfected CHO cells was competitively inhibited by soluble mimetics of the H-2 epitope, such as the H-2 neoglycoprotein, HMO extracts and synthetic 2'-FL. The half maximal inhibitory concentration for 2'-FL was 2.5 g/L, which is similar to its average level in human milk. Further, strong inhibition of binding of pathogenic *C. jejuni* strain 287ip to the immobilised H-2 neoglycoprotein was observed with 2'-FL concentrations as low as 10 mg/L, two orders of magnitude lower than average 2'-FL levels in human milk.

Cell binding assays using biosensors demonstrate a strong binding affinity of 2'-FL with invasive *C. jejuni* but not with other pathogens tested, including *Pseudomonas aeruginosa*, *Cronobacter sakazakii*, *Salmonella* Typhimurium, *Staphylococcus aureus*, *Listeria monocytogenes*, *Streptococcus dysgalactiae*, and *Streptococcus mutans* (Lane et al., 2011). The interaction was 60% inhibited by free synthetic 2'-FL at 50 mg/L, one fiftieth its average level in human milk. This provides supportive evidence for a specific mechanism for the competitive inhibition by 2'-FL of the binding of *C. jejuni* to H-2 antigen.

### **3.5 Inhibition of binding of *C. jejuni* to human intestinal tissue and epithelial cell lines by 2'-FL**

An *ex vivo* study on human mucosal tissue and *in vitro* studies in various human epithelial cell lines—including HEp-2, HT-29 and Caco-2—have demonstrated 2'-FL inhibition of *C. jejuni* adhesion and invasion (Lane et al., 2012; Ruiz-Palacios et al., 2003; Weichert et al., 2013; Yu et al., 2016) in an apparent dose dependent manner (Yu et al., 2016).

Ruiz-Palacios et al. (2003) investigated *ex vivo* the binding of *C. jejuni* strains 287ip (pathogenic) and 57sp (non-pathogenic) to human ileum specimens obtained from patients who required intestinal resection. Ileum samples were incubated with a bacterial suspension that had been pre-incubated with saline (as control) or synthetic 2'-FL at 2 g/L—a level below its average level in human milk. It was observed that strain 57sp colonized samples at half the rate of the pathogenic strain. 2'-FL reduced colonization by strain 287ip by 69%, but did not inhibit colonisation by strain 57sp.

Results are consistent with strain 287ip binding to a 2'-fucosylated epitope on the mucosal surface, while the non-pathogenic strain bound with lower avidity to other epitopes for which 2'-FL is not a competitive inhibitor.

Lane et al. (2012) demonstrated 2'-FL inhibition of binding of pathogenic *C. jejuni* strain 81-176 to HT-29 cells, a human colorectal adenocarcinoma cell line with epithelial morphology. Synthetic

2'-FL at 1 g/L—a level below its average level in human milk—reduced the adhesion of *C. jejuni* to HT-29 cells by 55% but did not reduce cellular invasion, suggesting that factors other than the H-2 antigen are involved in the mechanism of *C. jejuni* pathogenesis post-adhesion to the epithelium.

Weichert et al. (2013) undertook a cell culture assay of the effect of 2'-FL and 3-fucosyllactose (3-FL) on the adhesion of pathogenic *C. jejuni* strain 81-176 and other pathogenic bacteria to differentiated Caco-2 cells—a continuous line of heterogeneous human epithelial colorectal adenocarcinoma cells used as a cell model of small intestinal enterocytes. Infection of Caco-2 cells in the presence of 10 g/L 2'-FL produced by microbial fermentation (>95% purity) inhibited adhesion of *C. jejuni* by 26% and three other pathogens by up to 18%. However, the concentration of 2'-FL used in these inhibition assays was higher than levels normally found in human milk, and fourfold higher than the permissible level proposed by FSANZ for 2'-FL in infant formulas and FSFYC in this application. The suitability of the Caco-2 cell line for this work is questionable. It has been reported that only a subset of differentiated Caco-2 cells express the type H-2 histo-blood group antigen—the putative cellular receptor for *C. jejuni* (Murakami et al., 2013). It is possible that a significant proportion of the binding of *C. jejuni* to the Caco-2 cells in this study is occurring non-specifically or through other epitopes which are not susceptible to inhibition by 2'-FL.

In a cell culture assay, Yu et al. (2016) investigated the effect of 2'-FL produced by microbial fermentation (>99% purity)—at concentrations up to 5 g/L—on the invasion of HEp-2 and HT-29 cells by *C. jejuni* strain 81-176. HEp-2 is a human epithelial type 2 cell line from a human carcinoma. Infection, measured as the number of *C. jejuni* per cell, was similar for both cell lines. 2'-FL inhibited *C. jejuni* infection in a dose-dependent manner in both cell lines, with a half maximum inhibitory concentration of 1 g/L—a level below its average level in human milk.

### 3.6 Conclusions

FSANZ concludes that there is a consistent body of indirect evidence to demonstrate a credible mechanism for 2'-FL inhibition of the binding of pathogenic *Campylobacter jejuni* to intestinal epithelial cells, and limited and largely indirect evidence for a reduction of intestinal colonisation by *C. jejuni* and the incidence of diarrhoea.

There is no direct evidence that inhibition of binding of *C. jejuni* occurs in children fed formulated supplementary foods for young children (FSFYC) supplemented with synthetic 2'-FL.

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 is consistent with the proposed anti-infective effect of 2'-FL, but is insufficient to conclusively demonstrate the likelihood of a beneficial health outcome from supplementation of infant formulas and FSFYC with 2'-FL.

The capacity of 2'-FL to reduce the severity and duration of *C. jejuni* infection has been demonstrated in a mouse model of infection. Reductions in faecal shedding and infection of the intestine, spleen and mesenteric lymph nodes of up to 99 percent were observed when 2'-FL produced by microbial fermentation was administered at 5 g/L prior to and/or concurrently with the inoculum of *C. jejuni*. This level of 2'-FL is twice the level proposed for use in infant formula in this application, but well within the normal range observed in human milk.

*In vitro* studies provide a credible mechanism of action for the anti-infective effect of 2'-FL through competitive inhibition of the binding of pathogenic *C. jejuni* to H 2 histo-blood group antigens on intestinal epithelial cells. It has been satisfactorily established through cell- and antigen-binding studies that pathogenic strains of *C. jejuni* bind specifically to  $\alpha$ 1,2-fucosylated moieties of the H-2 antigens of intestinal epithelial cells. While it is not known whether this is the sole mechanism for binding to the intestinal epithelium, such binding is not observed for non-pathogenic, non-adherent strains of *C. jejuni*. Synthetic 2'-FL at concentrations in the range normally found in breast milk inhibits this binding—and binding-dependent invasion of epithelial cell lines—in a dose-dependent manner. Biosensor experiments have demonstrated that pathogenic *C. jejuni* also bind with high avidity to immobilised 2'-FL moieties *in vitro*. Disruption of that binding by free, soluble synthetic

2'-FL demonstrates its specificity.

The IEAG) for A1155 concluded that there is a dose response effect in relation to the competitive inhibition by 2'-FL of binding of *C. jejuni* to its epithelial cell receptor; but that this cannot be extrapolated to a dose response effect on reducing infection in infants or children, because those types of studies cannot be done in humans.

**Table 3: Summary of the findings on anti-infective effects of 2'-FL and LNnT**

Reference	Summary of Findings (relevance to beneficial role; cause and effect; mechanism of action; dose-response)	Significance of finding
Human observational studies		
Morrow et al. (2004)	Prospective study of breastfeeding mother-infant pairs (n=93) in Mexico. Infants were enrolled from birth and monitored to 2 years postpartum. The incidence of <i>Campylobacter jejuni</i> -associated diarrhoea in infants was significantly inversely correlated with levels of naturally-occurring 2'-FL in their mother's milk. No other pathogen-protective effect correlating with levels of 2'-FL was observed.	Observation in infants correlating levels of naturally-occurring 2'-FL with protection against <i>Campylobacter</i> -associated diarrhoea.
Animal studies		
Ruiz-Palacios et al. (2003)	Study of intestinal colonisation—by pathogenic <i>C. jejuni</i> strain 287ip—of pups of BG/SJL mice transfected with a plasmid containing the human FUT1 gene (and non-transgenic controls). Intestinal clearance of <i>Campylobacter</i> was significantly quicker in pups nursing transgenic versus non-transgenic dams when the pups were challenged with identical inocula ( $10^6$ CFU). Wild-type mice inoculated with $10^6$ CFU generally remained infected throughout the 15 days of the study, while none of the infected pups nursing transgenic dams remained infected after 9 days of nursing.	Results support a conclusion that $\alpha$ 1,2-fucosylated moieties in milk inhibit infection by invasive strains of <i>C. jejuni</i> .
Yu et al. (2016)	Challenge study of the effect of 2'-FL produced by microbial fermentation (>99% purity) on infection with <i>C. jejuni</i> strain 81-176 in an experimental murine model. C57BL/6 mice were inoculated with $10^8$ cfu/mouse in 100 $\mu$ L saline gavage on three consecutive days. Addition of 5 g/L of 2'-FL concurrently with <i>C. jejuni</i> challenge reduced faecal shedding and infection of the intestine, spleen and mesenteric lymph nodes by 90%, 80%, 96% and 93%, respectively. Addition of 5 g/L 2'-FL in drinking water <i>ad libitum</i> for 3 days before the bacterial challenge, along with its inclusion at 5 g/L with the <i>C. jejuni</i> challenge, reduced faecal shedding and infection of the intestine, spleen and mesenteric lymph nodes by 99%, 97%, 97% and 98%, respectively.	Synthetic 2'-FL added with bacterial challenge and/or to the diet of mice at around twice its mean concentration in human milk is protective against invasive <i>C. jejuni</i> strains that require binding to fucosylated receptors on the surface of intestinal epithelial cells to initiate infection.
<i>Ex vivo</i> studies		
Ruiz-Palacios et al. (2003)	Study of the effect of synthetic 2'-FL on the adherence of <i>C. jejuni</i> to human intestinal mucosa <i>ex vivo</i> . 2'-FL (2 g/L) reduced colonisation of fresh human ileal samples by pathogenic strain 287ip by 69%, but did not inhibit colonisation by non-pathogenic strain 57sp. Results are consistent with strain 287ip binding to a 2'-fucosylated epitope on the mucosal surface, while the non-pathogenic strain bound with lower avidity to other epitopes for which 2'-FL is not a competitive inhibitor.	Binding of pathogenic <i>C. jejuni</i> to a putative 2'-fucosylated mucosal epitope is shown to be specifically inhibited by synthetic 2'-FL at a level below its mean concentration in human milk.

<i>In vitro</i> studies		
Ruiz-Palacios et al. (2003)	<p>Study of the binding of <i>C. jejuni</i> to histo-blood group antigens prepared as glycoconjugates of bovine serum albumin (termed neoglycoproteins) immobilized on nitrocellulose membranes. Assayed by Western blot after exposure to digoxigenin-labeled <i>C. jejuni</i> strains.</p> <p>Ten of 12 pathogenic <i>C. jejuni</i> strains bound to the fucosylated antigens, while none of four non-pathogenic strains bound.</p> <p>In a competitive binding assay, pre-incubation of the membranes with monoclonal antibodies against the antigens strongly inhibited binding of pathogenic <i>C. jejuni</i> strain 287ip to H-2 antigen while only weak or no inhibition of binding to other antigens was observed, implying a non-specific binding mechanism in those cases. The specificity of binding to H-1 and H-2 antigens was further assessed in a competitive binding assay with synthetic 2'-FL. Strong inhibition of binding of strain 287ip to H-2 was observed with 2'-FL concentrations as low as 10 mg/L. 250 mg/L 2'-FL was required to inhibit the binding to H-1, again suggesting a non-specific interaction between H-1 and <i>C. jejuni</i>.</p>	<p>Results suggest a specific high avidity binding between pathogenic strains of <i>C. jejuni</i> and the <math>\alpha</math>1,2-fucosylated H-2 histo-blood group antigen.</p> <p>Binding is shown to be specifically inhibited by synthetic 2'-FL at a concentration of 10 mg/L, approximately 1/250<sup>th</sup> of its mean concentration in human milk.</p>
Ruiz-Palacios et al. (2003)	<p>Study of the binding of two pathogenic strains of <i>C. jejuni</i> to Chinese hamster ovary (CHO) cells transfected with human genes for <math>\alpha</math>1,2-fucosyltransferase (FUT1 gene), <math>\alpha</math>1,3/4-fucosyltransferase (FUT3), and <math>\alpha</math>1,3-fucosyltransferase (FUT4).</p> <p>Substantially more invasive <i>C. jejuni</i> bound to the FUT1 transfected cells than to other cell types, demonstrating specific binding affinity for the <math>\alpha</math>1,2-fucosyl moiety. An agglutination assay confirmed the ability of only the pathogenic strains of <i>C. jejuni</i> to bind FUT1-transfected CHO cells.</p> <p><i>Campylobacter</i> binding to FUT1-transfected CHO cells was inhibited by agents that bind specifically to H-2 antigen (<i>L. tetragonolobus</i> or UEA I lectins, or anti-H-2 mAbs), but not by lectins and mAbs which bind other epitopes, such as the H-1 antigen.</p> <p>Binding was also inhibited by soluble mimetics of the H-2 ligand, such as neoglycoprotein containing <math>\alpha</math>1,2-fucosylated ligands, human milk oligosaccharides, and synthetic 2'-FL. The half maximal inhibitory concentration for 2'-FL was 2.5 g/L.</p>	<p>Results support a specific high avidity binding between pathogenic strains of <i>C. jejuni</i> and the <math>\alpha</math>1,2-fucosylated H-2 histo-blood group antigen.</p> <p>Binding is shown to be specifically inhibited by synthetic 2'-FL at a concentration similar to its mean concentration in human milk.</p>
Lane et al. (2011)	<p>Three different experiments demonstrated a specific high-avidity binding interaction between a well-characterized invasive strain of <i>C. jejuni</i> (strain 81-176) and 2'-FL immobilised to a gold biosensor chip surface. The interaction was 60% inhibited by free synthetic 2'-FL at 0.05 g/L. No binding was observed between immobilised 2'-FL and strains of <i>Staphylococcus aureus</i>, <i>Cronobacter sakazakii</i>, <i>Pseudomonas aeruginosa</i>, <i>Salmonella</i> Typhimurium, <i>Streptococcus mutans</i>, <i>Streptococcus dysgalactiae</i> or <i>Listeria monocytogenes</i>.</p>	<p>Specific binding of <i>C. jejuni</i> to immobilised 2'-FL was shown to be strongly inhibited by free synthetic 2'-FL at a concentration of 0.05 g/L, approximately 1/50<sup>th</sup> of its mean concentration in human milk.</p>

Lane et al. (2012)	Cell culture assay of the effect of bovine colostrum oligosaccharides and individual bovine and human milk oligosaccharides on adhesion to, invasion of and translocation of HT-29 cells by a well-characterized invasive strain of <i>C. jejuni</i> (strain 81-176). Synthetic 2'-FL at 1 g/L reduced the adhesion of <i>C. jejuni</i> , to HT-29 cells by 55%, but did not reduce cellular invasion. Its effect on translocation was not reported.	Demonstration of inhibition of binding of a pathogenic strain of <i>C. jejuni</i> to a cell line model of human gut epithelium by synthetic 2'-FL
Weichert et al. (2013)	Cell culture assay of the effect of 2'-FL and 3-fucosyllactose (3-FL) produced by microbial fermentation (>95% purity) on the adhesion of <i>C. jejuni</i> and other pathogenic bacteria to differentiated Caco-2 cells. Infection of Caco-2 cell line in the presence of 10 g/L 2'-FL inhibited adhesion of <i>C. jejuni</i> by 26%, and three other pathogens by up to 18%.	Demonstration of moderate inhibition by high levels of synthetic 2'-FL of binding of a pathogenic strain of <i>C. jejuni</i> to a cell line model of human small intestinal enterocytes.
Yu et al. (2016)	Cell culture assay of the effect of 2'-FL produced by microbial fermentation (>99% purity) on the invasion of HEP-2 or HT-29 cells by <i>C. jejuni</i> strain 81-176. Infection, measured as the number of <i>C. jejuni</i> per cell, was similar for both cell lines. <i>C. jejuni</i> infection was inhibited by 2'-FL in a dose-dependent manner in both cell lines, with a half maximum inhibitory concentration of 1 g/L.	Demonstration of strong inhibition of <i>C. jejuni</i> invasion of two human epithelial cell lines by synthetic 2'-FL at levels similar to its mean concentration in human milk.

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