

# Establishing a healthy microbiome with human milk oligosaccharides

*Supporting immune system development in infancy and beyond*

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ABSTRACT

Human milk oligosaccharides (HMOs) are unique oligosaccharides found in mother's milk. Collectively, HMOs constitute the third largest solute in human milk, after lactose and fat. The types and levels of HMO vary considerably among women, geographical regions and the stages of lactation. Over 130 different oligosaccharides have been identified in human milk, with 2'-fucosyllactose (2'-FL) being the most abundant (~2 g/l). Recent innovations and regulatory approvals have enabled infant formula companies to incorporate 2'-FL into their products, helping infant formulas to become more like mother's milk. This article explores the latest research on HMOs and their role in supporting digestive, immune and cognitive development in infants.

## Keywords

Human milk oligosaccharides  
2'-Fucosyllactose  
Infant formula  
Mother's milk  
Microbiome

## Human milk oligosaccharides: the missing link

Breast milk is the gold standard for infant nutrition, and with good reason: its composition changes to meet a baby's growing nutritional needs, and it contains numerous bioactive agents that play a key role in brain growth and in the development of a healthy gastrointestinal tract and immune system.

While the composition of breast milk is dynamic (Fig. 1), in general it is made up of 87% water, 7% lactose (the primary carbohydrate), 3.8% lipids (fat) and 1% protein [1]. The third most abundant solid components in breast milk, after lactose and lipids, are non-nutrient complex carbohydrates called human milk oligosaccharides (HMOs) [2].

HMOs are important bioactives that help support and maintain health from birth through early life, including

- promoting the predominance of a *Bifidobacterium*-rich microbiota;
- strengthening the gut-barrier function and acting as receptor decoys for pathogens; and
- appropriately potentiating the immune system for long-term health [3].

More than 130 different HMOs have been identified in human milk [4]. The types and levels of HMOs vary considerably among women, depending on their geographical region and stage of lactation. Of the 20 predominant HMOs, the most abundant is 2'-fucosyllactose (2'-FL), averaging 2.4±1.9 g/l [5].

Recent scientific advances have enabled the commercial production of HMOs. DuPont Nutrition & Health in collaboration with Inbiose, a Ghent University spin-off company, has developed a proprietary, patented process that ferments glucose and lactose to form HMOs and other carbohydrates. This process allows the large-scale production of 2'-FL that is identical to the 2'-FL HMO found in human milk.

## Regulatory approval of 2'-fucosyllactose

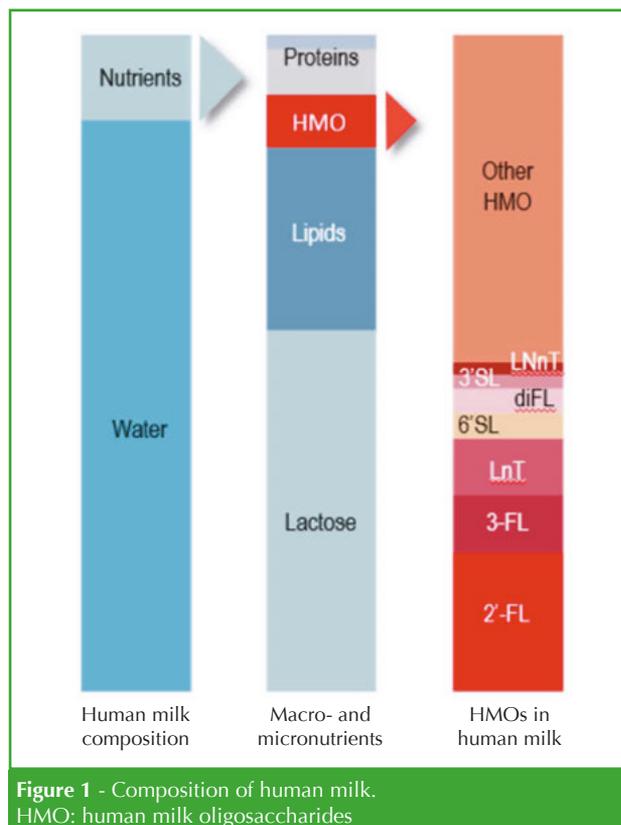
In 2016, Inbiose and DuPont Nutrition & Health announced a joint development and licensing agreement for the exclusive rights to produce and commercialize 2'-fucosyllactose (2'-FL) and other selected fucosylated HMOs for food and dietary supplement applications.

DuPont 2'-FL is branded as CARE4U™, and with more than 98% 2'-FL, CARE4U™ is the highest-purity 2'-FL product currently on the market.

CARE4U™ 2'-FL received EU Novel Food approval in December 2017 for use in infant and follow-on formula, foods for young children, medical foods and other foods. In the USA, CARE4U™ 2'-FL was considered as GRAS (Generally Recognized as Safe) for use in infant formula and toddler foods in late 2017. CARE4U™ 2'-FL may also be used as a dietary ingredient for supplements in both the EU and the USA. Additional regulatory submissions are being filed in other relevant countries.

CARE4U™ 2'-FL received the NutraIngredients-USA "Ingredient of the Year Award 2018".

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Research indicates that commercially produced 2'-FL confers the same health-promoting benefits of HMO in human milk, including supporting digestive, immune and cognitive development.

### Safety and tolerance of commercially produced HMOs in infant formula

To evaluate whether infant formula with 2'-FL HMO was well-tolerated and impacted growth among infants, a four months prospective study was conducted among 254 infants between 0 and 5 days of life [6]. Infants in the reference group were exclusively fed human milk collected from breastfeeding mothers, while the remaining infants were exclusively fed infant formula. Three infant formulas, calorically similar to human breast milk (approximately 19 kcal/fl oz or 0.63 kcal/ml), were prepared; two of the formulas were fortified with either 0.2 or 1.0 g/l of 2'-FL, while the third, with no 2'-FL, served as the control formula.

Tolerance to the formulas was evaluated by stool consistency, number of stools per day and percentage of feedings associated with spitting up or vomit. Growth measurements included weight, length and head circumference. At the end of the four months trial, no significant differences were observed between the infants fed the 2'-FL-supplemented formulas versus human-milk-fed infants with respect to tolerance or

growth. Relative to the concentrations fed, there was also no significant difference in 2'-FL absorption between infants fed the 2'-FL-fortified formulas and those fed human milk.

This leading study showed that 2'-FL-supplemented formulas with caloric densities similar to breast milk are well-tolerated by formula-fed infants, supporting growth patterns and 2'-FL absorption profiles similar to breastfed infants.

### HMOs: shaping a healthy microbiota

Among the many functions of breast milk is its ability to help establish a healthy and diverse microbiome for the baby. Establishing a healthy microbiome in infancy has a lifelong impact on human health, supporting digestive and immune health as well as cognitive development [3].

HMOs act like a prebiotic fibre, with more than 90% reaching an infant's large intestine undigested [7]. There, the bioactive components in HMOs provide nourishment for beneficial bacteria, particularly *Bifidobacteria*. 2'-FL, when added to infant formula, can help establish beneficial bacteria linked to immunity, such as *Bifidobacteria* (Fig. 2).

### Colonizing the gut microbiome with pre- and probiotics

Introduction of beneficial bacteria, such as *Bifidobacteria* and *Lactobacilli*, in the form of probiotics can also help establish a healthy and diverse microbiome. However, innate resistance from the indigenous gut microbiota may hinder or prevent their survival and colonization. By selectively combining certain prebiotics and probiotics, researchers were able to improve the survival, implantation and growth of probiotic bacteria in the gut. The term *synbiotic* was coined to describe the synergies observed in combination products with prebiotic compounds that selectively favour the probiotic organisms [8].

The effect of feeding a formula supplemented with a synbiotic mixture of bovine-milk-derived oligosaccharides (BMOs), some of which are structurally identical or similar to those found in human milk, and the probiotic *Bifidobacterium animalis* subsp. *lactis* (*B. lactis*) strain CNCM I-3446, was examined in a controlled, randomized, double-blinded clinical trial [9].

A total of 115 healthy, full-term infants with a mean age of 5 days were enrolled in the study and randomized into one of three groups: exclusively breastfed, fed a control formula, or fed a formula supplemented with a prebiotic (BMO) and a probiotic (*B. lactis* strain CNCM I-3446) for three months.

There were no significant differences in anthropometric measurements among the three groups at baseline or over the 12-week feeding period.

Researchers found that compared with the control formula, the synbiotic intervention had a marked impact on the development of the gut microbiota, stimulating a shift to a *Bifidobacterium*-dominated faecal microbiota through an increase in endogenous *Bifidobacteria* (*B. longum*, *B. breve*, *B. bifidum*, *B. pseudocatenulatum*), along with a 100-fold increase in the probiotic *B. lactis* in the stool.

This study demonstrated that the gut microbiota of formula-fed infants can be modulated by a synbiotic nutritional intervention where the diversity and composition of the microbiota becomes more like that of breastfed infants. Developing synbiotic infant formulas that contain both pre- and probiotics is an area for future study.

### Immune-supporting benefits of HMOs

Emerging research is revealing how HMOs act as important bioactives, helping to colonize the microbiome, bind pathogens and other toxins, and support the development of a healthy immune system.

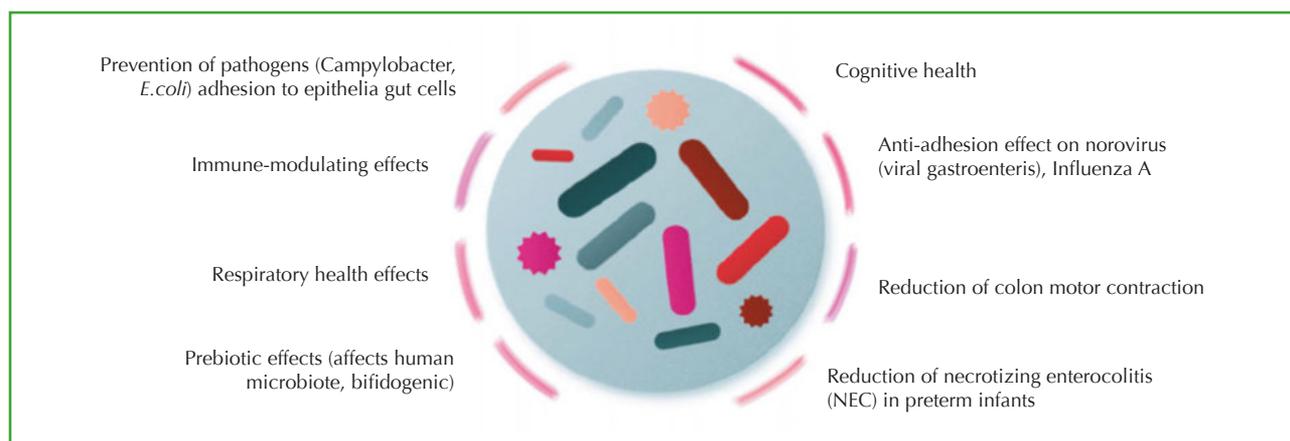
A study published in the *Journal of Nutrition* looked at the impact of 2'-FL-supplemented formulas on immune system biomarkers in healthy infants [10]. The infants, exclusively either fed formula or breastfed since birth, were enrolled in the study by 5 days old and exclusively formula-fed or breastfed from enrolment to 4 months of age. Formula-fed infants were randomly assigned to be fed one of three formulas: formulas with 0.2 or 1 g/l 2'-FL, or a control formula without 2'-FL.

Infants fed 2'-FL-supplemented formula had modified innate and adaptive immune profiles more like those of breastfed

infants in the study. These findings indicate that 2'-FL fortification supports aspects of immune development and regulation in formula-fed infants similar to that of breastfed infants. It is well-established that breastfed infants have a lower risk of diarrhoea than formula-fed infants. However, not all mothers produce 2'-FL in their breast milk. Only women with the enzyme FUT2 (approximately 80%) can make and secrete 2'-FL in their breast milk; 2'-FL concentration levels can also vary depending on region and stage of lactation. A study investigating the contribution of fucosylated oligosaccharides in breast milk to the innate immune system of breastfed infants found that the relative content of 2'-FL in human milk is significantly associated with the incidence of diarrhoea in breastfed infants [11]. A higher and more severe incidence of diarrhoea was reported in infants consuming milk with significantly lower ratios of 2'-FL compared with that consumed by infants without diarrhoeal symptoms while breastfeeding, suggesting a major role for these oligosaccharides in immunity.

Studies have also demonstrated that 2'-FL serves as an anti-adhesive antimicrobial that can reduce the risk of microbial infections by inhibiting certain pathogens from binding to cells in vitro, such as those that cause bacterial diarrhoea, like *Campylobacter jejuni* and *Escherichia coli* [12, 13].

According to the Centers for Disease Control and Prevention, approximately one out of every five cases of gastroenteritis (acute diarrhoea and vomiting) is caused by norovirus, a highly contagious virus spread by contaminated food or water. Norovirus is a leading cause of death in children younger than 5, responsible for an estimated 50,000 deaths, mostly in developing countries. Histo-blood group antigens (HBGAs) are important binding factors for norovirus infections. Researchers in Germany found that the HMOs 2'-FL and 3'-fucosyllactose (3'-FL) mimic HBGA structurally, and



**Figure 2** - Human milk oligosaccharides (HMOs) help shape a healthy microbiota

can act as receptor decoys, limiting the ability of pathogens like norovirus from binding in the gut, and thereby reducing the risk of norovirus infections [14].

There is also growing evidence linking the anti-adhesive and immunomodulatory effects of HMOs with a lower incidence of respiratory-tract-related infections in breastfed infants compared with infants fed formula.

A recent clinical trial looked at how the addition of two HMOs to infant formula—2'-FL and lacto-N-neotetraose (LNnT), which together represent about 37% of the total oligosaccharides in breast milk—might impact growth, gastrointestinal tolerance and morbidity in the first year of life [15]. The study included 175 healthy newborn infants monitored from birth to 2 weeks of age and randomly assigned to two groups. The infants initially received either an intact protein, cow's-milk-based standard formula (control group), or the same formula with added HMOs (1 g 2'-FL/0.5 g LNnT) (test group) from enrolment to 6 months of age, and then all infants were given standard follow-up formula without HMOs from 6 to 12 months, with appropriate foods added to the diet at 4 months.

Regular examinations were conducted throughout the study, and both groups had similar weight gain and growth, confirming that the HMO-supplemented formula was well-tolerated. Infants in the test group had significantly softer stools and fewer incidents of waking during the night during the first two months. Among those born via caesarean section, infants consuming the HMO-supplemented formula reported a lower incidence of colic at 4 months and had significantly lower rates of bronchitis and lower respiratory tract infections, and required less medications. This finding is notable, because gut colonization of caesarean-born infants is compromised compared to those born by vaginal delivery, making this group of particular interest for nutritional interventions, such as HMOs, that can support the development of a healthy, diverse microbiome.

Some of the immunomodulation effects attributed to HMOs are not only related to their impact in the gastrointestinal tract but may also be mediated by systemic effects. While the majority of ingested HMOs arrive in the colon intact, some HMOs are absorbed in the infant's intestine and reach the systemic circulation. In one study, researchers found that levels of 2'-FL, 3'-FL and LNnT in both plasma and urine were significantly correlated with concentrations in the corresponding breast milk, but not so in formula-fed infants [16]. This finding is significant, as in order for HMOs to exert systemic effects on the immune system, they must be transported in the blood system to be able to interact with

specific cells. This may explain some of the hypothesized benefits of breast milk, since even small levels of absorbed HMOs have been shown to have biological effects in vitro. Further research is needed to understand the mechanisms supporting the observed benefits of HMOs.

## HMOs and brain development

Studies have demonstrated that breastfeeding is correlated with having higher intelligent quotient (IQ) scores compared with formula feeding [17]. There is growing interest in investigating the impact of 2'-FL and HMO supplementation on infant brain development.

In an early study to determine if HMOs, particularly 2'-FL, can impact brain development and whether this effect persists beyond weaning, researchers fed rat pups an oral supplementation of 2'-FL or water (control group) during the lactation period [18]. Using standard behavioural tests, the animals were evaluated at 4–6 weeks of age, and again at 1 year. While differences between the groups were less noticeable after weaning, at 1 year of age the group fed the 2'-FL supplement performed significantly better in tests involving recognition and memory. Based on this study, the researchers hypothesized that exposure to 2'-FL in infancy could potentially impact age-related cognitive decline, although this outcome must be confirmed by further studies.

In addition to learning and memory, Vazquez and colleagues found that 2'-FL stimulates the development of central nervous system functions in rats. Their studies found that orally administered 2'-FL enhances brain function and cognition, including hippocampal long-term potentiation, through the gut brain axis via the vagus nerve [19].

The findings in these studies are encouraging; they warrant future studies to investigate the benefits of 2'-FL and HMO supplementation on human brain development.

## Benefits of HMOs for adults

Just as HMOs can modulate immune function and strengthen the gut-barrier function in infants, they also show promise in helping to restore microbiota balance in adults. When the microbiome is out of balance, meaning there is an overgrowth of harmful bacteria in the gut compared with beneficial bacteria, it can lead to conditions such as inflammatory bowel syndrome and other metabolic diseases, such as obesity and diabetes [20].

In a prospective study conducted in 100 healthy adults (49 females and 51 males) aged 19–57 years, the diet was sup-

plemented for two weeks with 2'-FL as a fucosylated HMO and LNnT as a non-fucosylated neutral HMO, to determine how HMO supplementation in adults affects the gut microbiota, as well as to measure overall safety and tolerance [21]. Each group was randomly assigned a supplement of 2'-FL, LNnT, or a 2:1 mix of 2'-FL+LNnT at 5, 10, or 20 g, or 2 g of glucose as a placebo, daily for two weeks. Analysis of the microbiota showed that HMO supplementation substantially increased the relative abundance of *Actinobacteria* and *Bifidobacteria* (greater than 25% in some individuals) along with a reduction in the abundance of firmicutes and proteobacteria. Blood samples were analysed before and after the study intervention but revealed no irregularities, and a gastrointestinal symptoms rating scale revealed that supplementation at doses of up to 20 g was safe and well-tolerated.

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