# Human milk oligosaccharides:

## *2'-fucosyllactose as a nutritional supplement*

➢ Jennewein Biotechnologie

The World Health Organization recommends breastfeeding for infants up to the age of 6 months, as breast milk is considered the best form of nutrition for babies because it contains multifunctional proteins, lipids and oligosaccharides. One litre of human breast milk contains 5–10 g of complex oligosaccharides, also known as human milk oligosaccharides (HMOs). More than 200 different HMOs have been identified and together comprise the third most abundant component of human breast milk.

HMOs are chains of monosaccharide building blocks such as glucose, galactose, L fucose, N-acetylneuraminic acid and N-acetylglucosamine. The quantity and specific composition of HMOs in human breast milk varies among mothers and changes during lactation. HMOs include both neutral and sialylated (acidic) oligosaccharides. About 80% of European and American women are 'secretors', which means they express the *FUT2* gene and thus produce the enzyme α-1,2-fucosyltransferase, which adds L-fucose to oligosaccharide chains via α(1,2) linkages. Because of the activity of this enzyme, the most abundant HMO in 'secretor' mothers is 2'-fucosyllactose, with levels of up to 4.6 g per litre of breast milk.

The role of HMOs in infant health has been recognized only since the early twentieth century. The German–Austrian paediatrician and bacteriologist Theodor Escherich and the German paediatrician Ernst Moro observed lower mortality rates among breastfed infants compared to their bottle-fed peers, and found that the infants raised on breast milk were generally healthier and more disease resistant. In the 1950s, the positive effect of breast milk was attributed to specific carbohydrates, namely the HMOs. These complex molecules have a prebiotic effect and promote the development of a healthy intestinal microbiome in breastfed infants. International scientific studies have also demonstrated the protective effect of HMOs against bacterial and viral infections, particularly those responsible for bacterial diarrhoea. Furthermore, specific HMOs can reduce the risk of Norovirus infection, stimulate brain development and protect the immune system in babies. They also reduce the risk of necrotizing enterocolitis, the most common and fatal disorder in preterm neonates.

Jennewein Biotechnologie GmbH has conducted extensive research on the production of HMOs and has developed a new and innovative method that allows HMOs to be produced on an industrial scale for use in the food industry. The HMOs produced by Jennewein Biotechnologie are currently used as food ingredients in infant formula, bringing the health benefits of HMOs to growing families.

## Composition and technical specifications

The main product manufactured by Jennewein Biotechnologie is the most abundant HMO, namely 2'-fucosyllactose, marketed under the brand-name Mum's Sweet Secret. This is produced by fermentation in bacteria, which guarantees high quality and consistency. The technical specifications are provided in Table 1.

They are also approved for kosher and halal food products.

## Efficacy

### *Preclinical studies*

Many scientific studies describe the effects of HMOs in vitro and in vivo. HMOs are significant modulators of neonatal gut colonization. They are not digested by humans and enter the colon largely intact, where they act as prebiotics to selectively promote the growth of specific intestinal bacteria, particularly bifidobacteria and bacteroides. Certain species of these bacteria produce enzymes that degrade or even fully metabolize HMOs. The predominance of these commensal bacteria in the gut of breastfed infants prevents the growth of potentially harmful bacteria by outcompeting them for nutrients and by producing metabolites, such as acids and short-chain fatty acids, that make the environment unfavourable for pathogens (reviewed in Bode [1] and Lewis and Mills [2]). Colonialization of the gut by pathogenic bacteria is also prevented by the anti-adhesive effect of HMOs. Many gastrointestinal pathogens bind to glycan structures on the surface of the mucosal epithelium to initiate an infection. Soluble HMOs with similar structures act as decoys to prevent this critical adhesion step. The HMO 2'-fucosyllactose prevents the binding of several pathogens to human

| ***Physical and chemical properties*** | |
|---|---|
| Appearance | Spray-dried powder |
| Colour | White to ivory-coloured |
| Solubility in water | Min. 500 g/l (ambient temperature) |
| Appearance in solution | Clear, colourless to slightly yellow |
| GMO detection | Negative |
| 2′-Fucosyllactose (%DM) | ≥90% |
| Lactose (%) | ≤5 |
| 3-Fucosyllactose (%) | ≤5 |
| Difucosyllactose (%) | ≤5 |
| Fucosyl-galactose (%) | ≤3 |
| Glucose (%) | ≤3 |
| Galactose (%) | ≤3 |
| Fucose (%) | ≤3 |
| Water content (%) | ≤9.0 |
| Protein content (µg/g) | ≤100 |
| Total ash (%) | ≤0.5 |
| ***Heavy metals and contaminants*** | |
| Arsenic (mg/kg) | ≤0.2 |
| Cadmium (mg/kg) | ≤0.1 |
| Lead (mg/kg) | ≤0.02 |
| Mercury (mg/kg) | ≤0.5 |
| Aflatoxin M1 (µg/kg) | ≤0.025 |
| ***Microbiological properties*** | |
| Standard plate count (CFU/g) | ≤10000 |
| Yeast and mould (CFU/g) | ≤100 |
| Coliform (CFU/g) | ≤10 |
| *Enterobacteriaceae* (CFU/g) | ≤10 |
| *Escherichia coli* | Absent in 11 g |
| *Salmonella* | Absent in 100 g |
| *Cronobacter sakazakii* | Absent in 100 g |
| *Staphylococcus aureus* (CFU/g) | ≤10 |
| Endotoxins (EU/g) | ≤300 |
| ***Shelf life*** | 2 years from production date |

**Table 1** - Technical specifications of 2′-fucosyllactose powder

cells in vitro, including *Campylobacter jejuni* (one of the most harmful pathogens responsible for bacterial diarrhoea), enteropathogenic *Escherichia coli*, *Salmonella enterica* serovar fyris and *Pseudomonas aeruginosa*. Other neutral HMOs have been shown to block *Vibrio cholerae* [3], while acidic HMOs similarly block *Helicobacter pylori*, which promotes the development of gastritis and gastric ulcers [4]. Certain neutral HMOs were recently shown to have a direct antimicrobial effect against Streptococcus agalactiae, which in new-borns can increase the risk of pneumonia, septicaemia and meningitis [5]. HMOs, and specifically fucosylated HMOs such as 2′-fucosyllactose, 3-fucosyllactose and more complex HMOs containing an α[1,2]-fucose epitope, also prevent Norovirus infection [4], which is the most common cause of diarrhoea-related death in children up to 5 years of age. Norovirus infection also requires the initial adhesion of the pathogen to mucosal cell surface glycans. Free fucosylated HMOs resemble these sugars, and therefore act as decoy receptors to reduce the risk of infection. In a study using new-born piglets, HMOs were shown to influence the gastrointestinal immune cell population, reducing susceptibility to *Rotavirus infections* [3]. The direct influence of HMOs on the immune system may reflect the absorption of small quantities of the sugars into the systemic circulation. Several studies have shown that HMOs have an immunomodulatory effect [1], and 2′-fucosyllactose also shows anti-inflammatory activity, quenching inflammation signals caused by enterotoxigenic *E. coli* [4]. In preterm infants, necrotizing enterocolitis is one of the most fatal intestinal disorders (having mortality rates of up to 40%), and is characterized by ischaemia and necrosis of the small intestine and the colon. Breastfeeding in the first 14 days of life is associated with a reduced risk of this disease. Studies in rodents demonstrated that 2′-fucosyllactose, and also the more complex HMO disialyllacto-N-tetraose, significantly reduce the incidence and severity of necrotizing enterocolitis [6].

## *Clinical studies*

A clinical study has been carried out to evaluate growth and tolerance in infants fed on synthetic formulas containing 2′-fucosyllactose [7], and to investigate the effects of formulas containing 2′-fucosyllactose on the developing immune system [8]. In the first part of the study (NCT01808105), three synthetic formulas with the same caloric density as human milk were compared to breast milk. The control formula contained only galacto-oligosaccharides (GOS), which are the predominant complex oligosaccharides found in formulas based on bovine milk. In contrast, the two test formulas contained different concentrations of 2′-fucosyllactose (0.2 g/l and 1.0 g/l) in addition to GOS. No significant differences in weight, length or head circumference were observed among infants fed on breast milk or formula from birth to 4 months of age. The formulas containing 2′-fucosyllactose were well tolerated, and HMO absorption was similar in the formula-fed group and the breastfed infants. The second part of the study also comprised one breastfed group and three randomized formula-fed groups given the same compositions as above. The objective was to examine how 2′-fucosyllactose affected immune system biomarkers, particularly cell-signalling molecules known as cytokines that regulate both the innate and adaptive immune responses and guide the differentiation and development of immune cells. The cytokine profiles differed between breastfed infants and those fed on the GOS control formula, whereas

the cytokine profiles in the infants provided with formula containing 2′-fucosyllactose were similar to those of the breastfed infants. The concentration of plasma inflammatory cytokines was significantly lower in the breastfed group and the two groups supplied with formula containing 2′-fucosyllactose compared to the GOS control formula group. This study therefore indicates that formulas containing 2′-fucosyllactose support immune development and regulation similarly to breast milk.

## Safety and tolerance

The 2′-fucosyllactose produced by Jennewein Biotechnologie received GRAS status (generally recognized as safe) from the US Food and Drug Administration in 2015 for general nutrition, medical nutrition and for use in dietary supplements.

Various international scientific studies have demonstrated that 2′-fucosyllactose has positive effects on the health of both infants and adults and is well tolerated.

## Applications and dosage

The 2′-fucosyllactose produced by Jennewein Biotechnologie can be used for general, medical and infant nutrition. For infant nutrition, the recommended dose is 2 g/l.

## REFERENCES


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**Jennewein Biotechnologie GmbH in a nutshell**

Jennewein Biotechnologie was founded in 2005, and is a leading company specialized in the production of rare functional monosaccharides and oligosaccharides for nutritional and medical applications. The HMO production process was developed by Jennewein following extensive in-house research and is based on bacterial fermentation.


For information
Bettina Gutiérrez, Press and Public Relations
Tel: +49 2224 9197346
Bettina.gutierrez@jennewein-biotech.de
Katja Parschat, Deputy Head of R&D
Tel: +49 2224 9197344
Katja.parschat@jennewein-biotech.de
www.jennewein-biotech.de