dsm-firmenich

Synergistic Synbiotics:

Innovating in Early Life Nutrition with HMOs and Probiotics

Whitepaper • December 2024

Gut microbiome

Optimal gut microbiota acquisition and establishment in early life is one of the essential processes that determines the life-long health of an individual.[1,2,3]

Importantly, the individual's signature microbiome is driven by factors that have already begun during pregnancy but are most profoundly impacted by early life events, such as mode of delivery (Cesarean section vs vaginal), gestational age, antibiotic exposure in early life, genetics and especially feeding mode (breastfeeding vs infant formula feeding).[4]

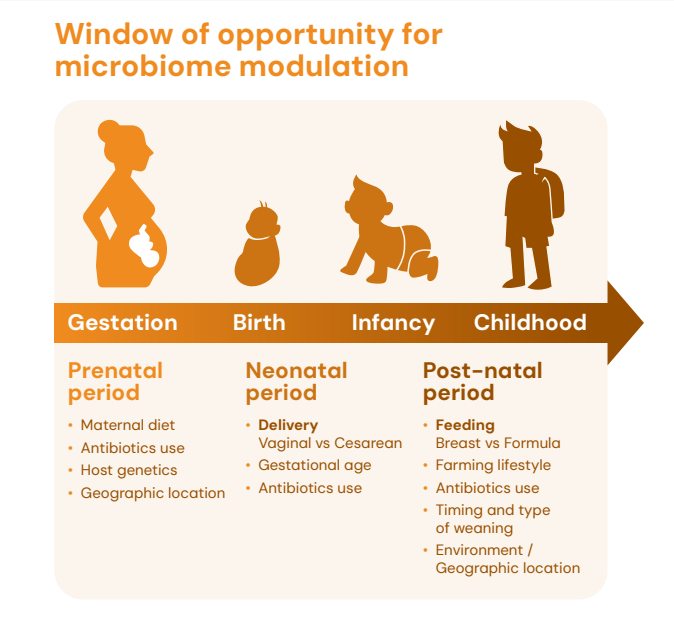


Figure 1. Adapted from Moriki D, et al. *Nutrients*. 2022[5]

The microbes that newborns are exposed to in early life, and that are established soon after birth, are essential to the development of this complex microbial signature.

Several studies have shown that there is a mother-to-infant transfer of bacterial strains; for example, newborns delivered vaginally have a richness of ***Lactobacillus*** which is the principal species of the maternal vaginal microbiota.[2,7] Infants delivered by Cesarean section (C-section) bypass exposure of the mother's vaginal and gut microbiota, and instead acquire species from the mother's skin as well as her surrounding environment (commonly the hospital).[4] By around the 4th to 7th day after (vaginal) birth ***Bifidobacterium species*** appear to become most dominant.[2,8] The infant gut microbiota however remains limited in terms of diversity (dominated by ***Bifidobacterium*** [up to 90%][9] and expands during different stages of development, including complementary feeding.[2]

It has been proposed that the gut microbiome develops in three distinct phases:

1 Developmental phase

from 3–14 months where the gut microbiota is being established (e.g., pregnancy, birth and feeding mode)

2 Transitional phase

from 15–30 months where the variety and abundance of species are changing (diversifying e.g., initiation of complementary feeding)

3 Stable phase

from 31–46 months with the establishment of the adult-type gut microbiome (with higher diversity).[10]

> The first months of life are therefore often referred as the critical window or window of opportunity when establishing the optimal gut microbiota is key.

It is now well established that breastfeeding is a dominant factor influencing the gut microbiota development during infancy.[9,10]

It is no coincidence that exclusive human milk feeding for the first six months of life is considered the gold standard of infant nutrition, providing infants with the best start in life. And numerous studies have demonstrated a difference in the gut microbiota composition between formula-fed compared to breastfed infants.[11,12,13,14]

Breastfeeding provides the infant gut with several important species including ***Bifidobacterium species (sp.)***, and ***Lactobacillus sp.*** all naturally present in human milk.[9] These microorganisms are transmitted from mother-to-infant and act as probiotics in the infant gut, exerting specific health benefits such as reducing gut inflammation[15,17] and immunomodulatory effects.[1,16]

Bifidobacterium sp. is especially dominant in the gut of breastfed infants[18] and is represented by numerous sub-species.[1] Several of these sub-species have been shown to support the growth of other beneficial microorganisms facilitated by cross-feeding, which helps sustain a balanced gut microbiome.[1]

Exposure of the breastfed infant to this diversity of bacterial phylotypes may also exert benefits against several diseases such as respiratory[6,19,20], gastrointestinal and non-communicable diseases[6,21,22], laying the foundations for long-term health.

Consequently, infants born vaginally who receive breastmilk develop a markedly different (bifido-dominant) gut microbiota compared to those born by C-section and who are non-breastfed. The differences are primarily due to the distinct microbial exposures that occur during (vaginal) birth and early (breast) feeding.

These gut-implanted bifidobacteria from mother-to-infant are in turn fueled through utilization of human milk oligosaccharides (HMOs) which serve as their primary energy source.[9,23] A key focus of research, aims to uncover ways to nudge the gut profile of formula-fed infants towards that of the breastfed infant.

Human milk oligosaccharides (HMOs)

HMOs are complex carbohydrates that can reach the infant's large gut intact, where they act as substrate seeding the growth of beneficial bacterial species.[24]

HMOs are the third largest solid component of human milk after lactose and fat[27,25,26] and are structurally, as well as functionally, different to oligosaccharides present in bovine milk, the common basis for many infant nutrition. Until recently, the lack of HMOs in infant nutrition was the biggest compositional difference between human milk and infant formula.

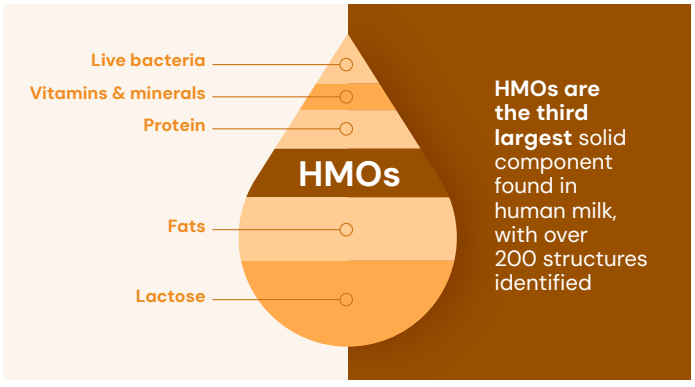


Figure 2: Human milk composition[33]

Interest, especially in specific HMOs, has mostly expanded in the last two decades with more than 200 different HMOs identified to date.[23]

These are categorized into three distinct groups – fucosylated, non-fucosylated (also called neutral HMOs) and sialylated (also called acidic HMOs). It's been suggested that the neutral HMOs account for >75% of HMOs in human milk.[29]

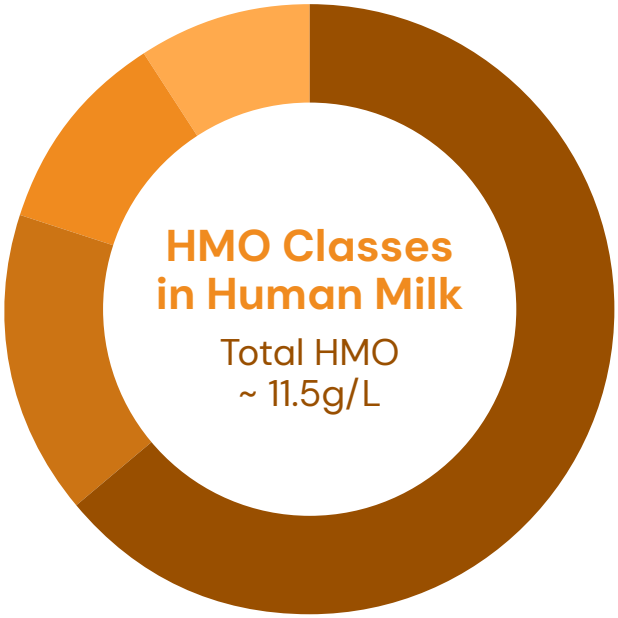
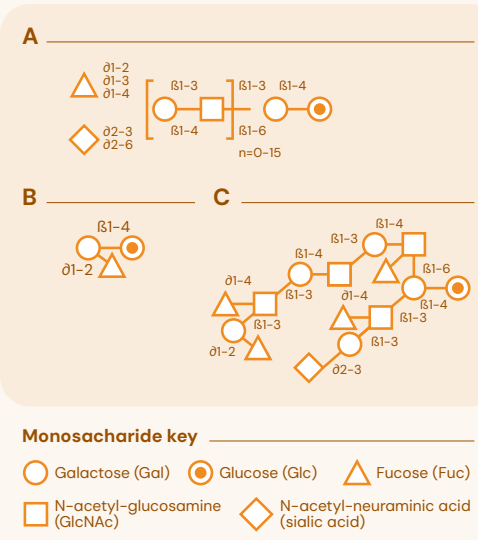


Figure 3. HMO classes in human milk[31]



In recent years, human identical HMOs have become available at commercial scale for addition in infant nutrition. Two HMOs, 2'-FL (Fucosyllactose) and LNnT (Lacto-*N*-neotetraose), were the first to be commercialized and are the most clinically studied. Infant nutrition solutions are now available with between 1-6 HMOs added.

In addition, the exploration of the sialyated HMOs such as 3'-SL (Sialyllactose) and 6'-SL have recently come to the forefront of research, given their role in supporting brain development and cognitive function.

Figure 4. Adapted from Bode & Jantscher-Krenn 2012

HMO structures: A. All HMOs have a lactose background that can either be fucosylated or sialylated. B. 2'FL is an example of a fucosylated HMO C. Complex HMOs such as iso-lacto-N-decaose shown here, can also be formed which can be branched and modified with fucose, syalic acid, and/or N-acetyl-glucosamine.

Human milk contains significant quantities of HMOs; from 9–22g/ liter in colostrum to 6–15g/ liter found in mature milk after one month lactation.[31]

By avoiding the digestive enzymes, the majority of these oligosaccharides (>98%) reach the large intestine where they act as substrate for health-enhancing bacterial species, offering numerous health benefits to the infant.[32]

It's been suggested that these health benefits arise through several distinct mechanisms, both directly and indirectly in the infant gut.

Firstly, they act as substrate selectively promoting the **growth of beneficial bacterial species** in the gut (**e.g. bifidobacteria**); secondly, **HMOs increase the production of metabolites such as sialic acid** which play an important role in critical phases of neurodevelopment; thirdly they strengthen **the gut barrier function**; fourthly, they **prevent pathogen adhesion in the gut**; and finally, HMOs **directly modulate the infant immune system**.[33,34]

1 HMOs promote the growth of beneficial bacteria

HMOs act as metabolic substrates promoting the growth of bifidobacteria as well as other health-enhancing species. The Bifidobacterium sp. are dominant in the gut of breastfed infants, representing ~90% in the first days of life in breastfed infants.[9] These bacteria in turn induce several additional benefits. For example, certain strains of bifidobacteria are able to ferment HMOs to short-chain fatty acids (SCFA), such as lactate, butyrate, acetate, pyruvate and others, which encourage the growth of other beneficial species in a process called cross-feeding.[33,35]

2 HMOs increase the production of metabolites such as sialic acid

SCFAs cross the blood brain barrier and may play a role in maintaining the barrier integrity. Although the precise mechanisms involved in the action of SCFAs on the central nervous system remain largely unknown, a multitude of animal studies have shown that they exert widespread influence on key neurological and behavioral processes and may be involved in critical phases of neurodevelopment.[36] Additionally, sialylated HMOs are a source of sialic acid, a nutrient that is vital for brain development and cognitive processes. It's been reported that enhanced levels of sialic acid may contribute to improved neurological outcomes in infants.[33,37]

3 HMOs strengthen the gut barrier function

The intestinal barrier is the first line of defence in innate immunity.[37] HMOs strengthen it by modulating the intestinal epithelial cells as well as immune cell responses, which positively promotes gut immune development.[34,37]

4 HMOs prevent pathogen adhesion in the gut

Many pathogens need to attach to glycans on the gut epithelial surface as a first step to initiate infection and disease.[28] Fucosylated HMOs, for example (see Figure 2), resemble the host epithelial cell surface glycans to which pathogens adhere.[28] These HMOs therefore serve as "decoy receptors" which in turn blocks the pathogen from attaching to the gut wall, reducing the subsequent initiation of infection and disease.[28] HMOs also possess direct bacteriostatic activity with antimicrobial effects[33] which potentially protect infants against the invasion of certain pathogenic species.

5 HMOs can directly modulate the infant immune system

HMOs not only act on the infant's gut immune system indirectly but results from in vitro studies suggest that HMOs also directly modulate the infant's immune system through, for example, the reduction of pro-inflammatory cytokine secretion.[29]

Thus, establishing an optimal gut microbiota early in life can have a profound impact on the development and functioning of the host's immune system. This foundational microbiome plays a crucial role in shaping the long-term well-being of the host and may provide protection against several diseases later in life.[39]

However, there are circumstances when the gut microbiota of the child is disrupted.

Or when the supply of substrate needed to drive the growth of these health promoting bacterial species is lacking. Importantly, studies in infants who were formula fed have shown that they had an altered microbiota development when compared to that of breastfed infants.[40]

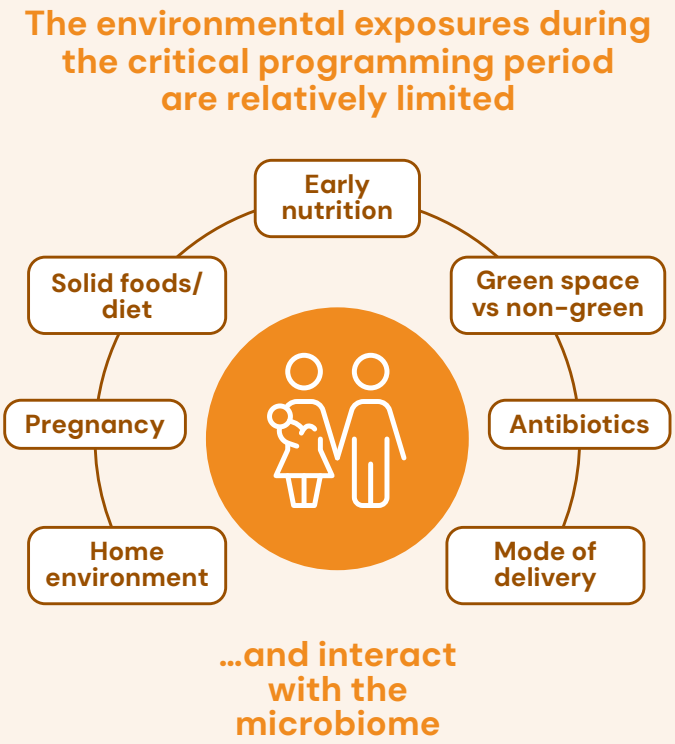


Figure 5. Environmental exposures of the child.

Under these conditions there might be an increased risk of disease development, both acute and chronic, including infections, inflammation, and other complex disorders. In such circumstances a rebalancing of the gut environment is essential.

The latest advancement has been the establishment of synergistic synbiotics, where the substrate HMO is paired with a co-administered probiotic (a live microorganism). Luckily the human gut microbiome is receptive to external exploitations making it ideal for these therapeutic interventions.[41] In the subsequent section we will discuss some of these approaches.

Probiotics

The International Scientific Association for Probiotics and Prebiotics (ISAPP) defined probiotics as *"live microorganisms that when administered in adequate amounts, confer a health benefit on the host"*.[9,42]

It is known that certain probiotic strains promote gut and immune health by balancing gut bacteria, producing metabolites such as SCFA, sialic acid and others, increasing secretory immunoglobulin A (sIgA) as well as cross-feeding and communicating with different host cells.[43]

The ***Lactobacillus*** species along with ***Bifidobacterium*** are among the most studied probiotics;[43] particularly their use during infancy, as these are the dominant species found in human milk[9] and in the gut of breastfed infants. Infants fed formula supplemented with a combination of these species e.g., ***Bifidobacterium infantis R0033***, ***Bifidobacterium bifidum R0071***, ***Lactobacillus helveticus R0052,*** all had higher levels of fecal sIgA after 4 weeks of feeding, compared to un-supplemented infants.[40] Furthermore, formula supplementation with ***Bifidobacterium animalis subspecies lactis (Bb12)*** augmented sIgA concentrations which were more pronounced in C-section delivered infants.[44]

The authors concluded that the immune-related effects of not breastfeeding and C-section delivery might be mitigated by including ***Bifidobacterium animalis subspecies lactis (Bb12)*** to infant formula, providing these infants with a safe, immune-modulating bacterial species.[44] In another study, supplementation with a mixture of ***Lactobacillus*** and ***Bifidobacterium*** immediately after C-section delivery enriched the numbers of these species in the infant gut (measured in feces).[45] Meanwhile, others have reported that certain health-promoting bacteria, again commonly those of ***Bifidobacterium*** and ***Lactobacillus*** origin or blends of multiple strains, were successful in improving symptoms such as depression, stress, anxiety, and learning in humans.[41]

Therefore, certain probiotic strains appear to benefit early life health and development through their actions on gut health and immune maturation, reducing inflammation and infections, promotion of natural defenses and protection against some metabolic conditions; and importantly, their ability to potentially improve behavior and cognition, and reduce anxiety.

Prebiotics

ISAPP defines a prebiotic as a *"substrate that is selectively utilized by host microorganisms conferring a health benefit"*.[9,46] This definition suggests that a prebiotic is selectively metabolized by beneficial microorganisms, rather than being broadly consumed by the entire gut microbiota.[47] Prebiotics must resist digestion, be fermented by gut microbes, and selectively promote the growth or activity of health-enhancing intestinal bacteria.[47]

Consequently, prebiotic oligosaccharides such as fructo-oligosaccharides (FOS) (of plant origin) and galacto-oligosaccharides (GOS) (of milk origin) have been developed to mimic the actions of natural oligosaccharides that are found in human milk.[47] The main bacterial metabolites coming from the fermentation of these prebiotic fibers are SCFAs (mostly acetate, butyrate, and propionate), which are potent immunomodulators.[9,46] Despite the proposed benefits of this blend, some suggest the effects of GOS and/or FOS are not comparable to the effects seen with using HMOs, for example, on the immune system.[30]

Synbiotics

ISAPP defines a synbiotic as *"a mixture comprising live microorganisms and substrate(s) selectively utilized by host microorganisms that confers a health benefit on the host"*.[9,48] These biotics were intended to replicate some of the functional effects of human milk feeding on infant health when human-milk feeding was not possible; by combining the live microorganism with the active substrate, a synergistic effect would be achieved, amplifying the benefits beyond what would occur when probiotics or prebiotics were provided individually.

Recently, synbiotics have been further classified into two distinct categories: complementary and synergistic (see Figure 6). *Complementary* synbiotics consist of a combination of a probiotic and a prebiotic that function independently of each other. The addition of the prebiotic being aimed at enhancing the growth of the host's existing beneficial microorganisms, while the probiotic introduces new beneficial microorganisms. Both components must meet the minimum criteria set out for probiotics and prebiotics individually.[48] While *synergistic* synbiotics combine a substrate and a live microorganism, with the substrate specifically chosen to drive the growth of the co-administered live microorganism.[43] In this case, neither the live microorganism nor the substrate needs to meet the criteria of probiotic or prebiotic individually; instead, they work together to enhance the health benefit; [48,43] *syn* in the word synbiotic thus means together.[43] Guarner and colleagues proposed that a key requirement to be named a *synergistic* synbiotic is that "there is at least one appropriately designed study of the synbiotic in the target host that demonstrates both selective utilization of the substrate and a health benefit".[43]

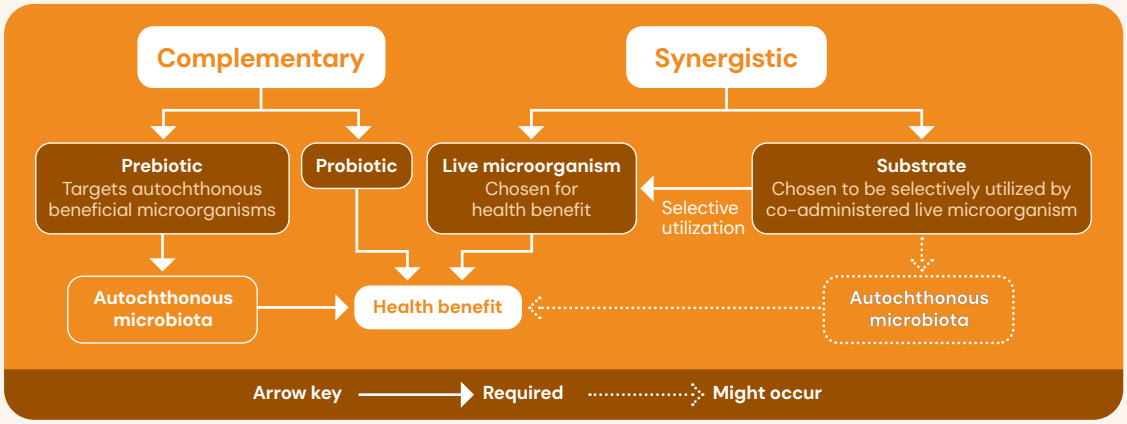


Figure 6. Complementary vs Synergistic Synbiotics adapted from Guarner et al., 2023.[4]

To date most studies conducted have focused on ***complementary*** synbiotics, demonstrating a range of associated health benefits. For example, feeding infants with cow's milk allergy a hypoallergenic formula with a blend of ***Bifidobacterium breve M-16V*** and galacto-/fructo-oligosaccharides (GOS/FOS) significantly reduced regurgitation frequency and atopic dermatitis.[49] While healthy infants fed a standard formula with added ***Lactobacillus fermentum CECT5716*** and galacto-oligosaccharides (GOS) had significantly fewer gastrointestinal infections (P=0.02) during the study period.[50]

Meanwhile there are limited studies exploring ***synergistic*** synbiotics in human subjects. The relatively recent introduction of ***human-identical*** HMOs as synbiotic substrates, replacing traditional prebiotic substrates such as GOS and FOS, creates the opportunity to generate synbiotic combinations closer to the composition of human breastmilk; while unlocking true synergies between selected combinations of HMOs and probiotic strains.[33] Preclinical research shows that HMOs selectively enhance the growth of the co-selected probiotic, boosting the production of key metabolites associated with health benefits in infants.
In a recent (ex-vivo) study where six HMOs were combined with ***B. infantis LMG 11588***, they found a greater increase in SCFA production when compared to the SCFA production from the individual ingredients.[17] De Bruyn and colleagues (2024) reported that specific bacterial taxa were associated with a differential response pattern to the HMOs, suggesting that adding HMOs to infant formula with a co-administered ***synergistic*** probiotic may yield above and beyond that of adding each individual ingredient.[17]

Postbiotics

ISAPP defines a postbiotic as a *"preparation of inanimate (non-viable) microorganisms and/or their components that confers a health benefit on the host".*[9,51]

Although the knowledge about the benefits of postbiotics is not recent, an agreement on the definition has only been established since 2021. Postbiotics provide several immune benefits, including modulation of systemic and local immune responses, enhancement of epithelial barrier function, modulation of resident microbiota with possible anti-inflammatory effects.[51]

Lactic acid bacteria for example, plays a key role in many fermentation processes, producing various cellular structures and metabolites linked to human health, such as lactic acid, SCFAs, and bioactive peptides.[51] The advantage of using postbiotics is that they pose no risk of bacterial translocation, making them safer for some vulnerable populations of infants and children, for example those with a weakened immune system or gut barrier dysfunction.[52]

dsm-firmenich synbiotic approach

Parents are focused on giving their baby the best start in life and are becoming increasingly aware of the critical role that the microbiome plays in early life nutrition.

For many years now, prebiotics and probiotics (and more recently synbiotics) have been added to infant nutrition with the aim of bringing it closer to some of the compositional and functional benefits of human breastmilk. However, synergistic combinations have not yet been sufficiently studied and described.

Three years ago, dsm-firmenich & Lallemand Health Solutions embarked on a research journey to explore the potential ***synergistic*** synbiotic benefits between the GlyCare® Human Milk Oligosaccharides (HMO) portfolio and Lallemand Health Solutions infant probiotic strain portfolio. Lallemand Health Solutions infant probiotics are produced under strict quality control; while their use has been clinically documented in infants, and they are already approved for use in infant formula in some key markets such as European, US and Chinese.

A two-phased study was designed to identify ***synergistic*** synbiotic combinations that could beneficially impact infant health. The first phase was to test various strains from the ***Lactobacillus*** and ***Bifidobacterium*** families along with co-administered HMO substrates, to establish their ability to metabolize different oligosaccharides, including fucosylated, neutral and sialylated classes. In the second phase, combinations were tested in an ex-vivo fermentation model using infant fecal samples, taken from different donors (e.g., from various ethnicities, breastfed vs formula fed, vaginal delivered vs Cesarean-section babies).

These fecal samples were monitored for the production of short-chain fatty acids (SCFA) as well as other beneficial gut-derived metabolites, using metabolomics to identify synergies. Intriguingly, the results indicated strong synergies between the selected combinations of ***Bifidobacterium*** and the co-administered HMOs that appeared to indicate potential improvements to immunity, gut health and cognition in infants.

In this model, ***Bifidobacterium infantis Rosell®-33*** had a very strong preference for the fucosylated HMOs including 2'-fucosyllactose (2'-FL, 2'-fucosyllactose/Di-fucosyllactose (2'-FL/DFL) and 3-fucosyllactose (3-FL). Furthermore, the specific combination of 2'-FL with ***Bifidobacterium infantis Rosell®-33*** boosted the production in our ex-vivo model of certain SCFA including lactic acid and acetic acid, indicating potential gut health benefits; and putrescine, indicating immunity benefits in infants.

Bifidobacterium bifidum Rosell®-71 appears to be capable of metabolizing all classes of HMO with similar levels of execution.

When this probiotic was combined with 2'-FL and 3'-SL or was included with a blend consisting of 2'-FL, 3'-SL and Lacto-N-neotetraose (LNnT), it appeared to enhance gut health and promoted the establishment of the gut-brain axis. Interestingly, both Gamma-aminobutyric acid (GABA), a neurotransmitter involved in neuronal proliferation and differentiation, and dopamine, a neurotransmitter involved in key functions such as locomotion, attention and cognition, were significantly increased in our ex-vivo model when 2'-FL, 3'-SL, or a combination of these with LNnT were tested with this specific probiotic strain.

This work is still in its infancy but is paving the way for exciting new developments of tailored combinations of ***synergistic*** synbiotic solutions. This advancement aims to further optimize infant nutrition by bringing it a step closer to human milk, both in composition and functionality, imparting some of the key benefits to non-breastfed infants to support a healthy start in life.

To strengthen the science already generated in this space, dsm-firmenich and Lallemand Health Solutions are exploring more complex combinations of HMOs with specific probiotic strains.

In parallel, we are aiming to study these synergistic solutions in a randomized clinical trial in healthy infants. For example, we are already engaged with our partner Lallemand Health Solutions in a clinical trial where newborn infants, whose mothers received antibiotics during delivery, will be supplemented with ***synergistic*** synbiotics while monitoring the impact of supplementation on the child's growth, microbiota as well as immune related outcomes. This exciting clinical study is the first of its kind which illustrates our ambition to bring science-backed innovative synergistic synbiotics to our Early Life Nutrition solutions.

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