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BIOTICS FOR EVERY STEP OF THE NUTRITIONAL JOURNEY

Report



Contents

The Importance of Breastmilk for a Healthy Gut Microbiota Development

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Breastfeeding is the best source of infant nutrition. Breastfeeding practices have been reported to have benefits for both mothers and infants, with functions beyond nutrition.[1,2]

For infants in the short-term the benefits include:

- protection against gastrointestinal and respiratory infections
- reduced risk of allergies
- reduced risk of necrotising enterocolitis (NEC) and sudden death.

Beneficial longer-term effects include:

- prevention to the risk of being overweight or obese, and having diabetes
- lower blood pressure and cholesterol levels
- positive behavioral and neurodevelopmental outcomes

There are also many short and long-term benefits in breastfeeding for the mother such as having a better post-partum recovery and a lower risk of breast and ovarian cancers amongst others.

The World Health Organization (WHO) recommends exclusive breastfeeding for the first 6 months of life; and the introduction of nutritionally adequate and safe complementary foods at 6 months together with continued breastfeeding up to 2 years of age or beyond.[3]

The complexity of breastmilk is amazing. Breastmilk contains a balance of nutrients for the infants as well as biotic components such as bacteria and metabolites, which support the development of a healthy gut microbiota and immune system.[4,5,6,7] Breastmilk contains human milk oligosaccharides (HMOS) which induce a prebiotic effect[8, 9, 10, 11, 12] have a direct effect on immune cells[13, 14, 15], reduces the risk of infection[16], and have a benefit to the development of the brain. It also contains bacteria and their metabolites which have probiotic and postbiotic effects for the gut and immune benefits. Breastmilk also contains antibodies and immune cells for direct protection.[17]

Breastmilk is a dynamic fluid of which the composition changes throughout the lactation period. For example, the nutritional composition of colostrum differs compared to that of mature milk. The composition can also change depending on maternal factors such as maternal health and diet, antibiotic usage, or drug exposure.

Probiotics in breastmilk

Some of the bacteria present in breastmilk exerts health benefits to the infant and are considered as probiotics. The definition of a probiotic is given in Table 1.

Breastmilk is a constant source of microbes, with a concentration of around 10^4-10^5 cfu/ml. An infant consumes around 800ml to 1L of breastmilk a day, supplying an infant with microbes that:

- assist gut and oral colonization, exerting functional properties at a structural level
- help to maintain and promote the intestinal homeostasis and metabolic level, because some of these organisms can produce short-chain fatty acids, vitamins, and other components
- protects and stimulate the immune system by producing antimicrobial components and bacteria that prevent infections.[18, 19, 20]

The most common bacteria families present in breastmilk are:

- Staphylococcus
- Streptococcus
- Bifidobacterium
- Lactobacillus

There are many factors affecting breastmilk microbiota, including, antibiotic usage, maternal health and diet, gestational age, the mode of delivery, geographical location, and the lactational stage.

Breastmilk also contains other organisms like yeasts, phages, viruses, and archaea. We are still learning about their main functions, and what the relationship between them, and the effect on the gut microbiota colonization and immune system of the infant are.

Prebiotics in breastmilk

The oligosaccharides (HMOS) present in breastmilk can be considered as a prebiotic because they are substrates that are selectively utilized by specific micro-organisms such as *Bifidobacteria* which confer a health benefit to the infant. The definition of a prebiotic can be found in Table 1. HMOS promote the growth of beneficial intestinal bacteria, generating short-chain fatty acids (SCFAs) which are critical for gut health. They also directly modulate host-epithelial immune responses and can selectively reduce binding of pathogenic bacteria and viruses to the gut epithelium preventing the emergence of a disease.[21] HMOS are also antimicrobials that act as bacteriostatic or bactericidal agents.[22] In addition, HMOS are known to act as immune modulators, directly affecting several different immune cells, and on the gut-brain axis which impacts the brain development of the infant.[22]

HMOS are a family of structurally diverse unconjugated glycans that are non-digestible and highly abundant in, and unique to, breastmilk[23]. There are more than 200 different HMO structures in breastmilk and 2-Fucosyllactose (2'-FL) is the most abundant one.

There are several factors that may affect the HMO profile in breastmilk:

- Stage of lactation: HMO composition and quantity change during lactation, with differences between colostrum, and transitional, and mature milk.[24]
- Gestational age: differences in the HMO profile are observed between preterm and term birth.[25]
- Gender of infant: there have been observed differences between the HMO profile of female and male infants.[26]
- Maternal diet: it's been recently reported that maternal diet can influence the diversity and quantity of oligosaccharides in breastmilk.[27]
- Maternal genetics: maternal genetics is a key determinant of HMO composition in human milk[26]. The secretor-HMO profile compared to no-secretor profile has been shown to have a difference in the abundance of 2'-FL within breastmilk.[21]

Postbiotics in breastmilk

The definition of a postbiotic can be found in Table 1.

As previously stated, the composition of breastmilk is highly variable, and it can be influenced by genetics, diet, lifestyle, as well as other environmental factors such geographical location. In a study by Gomez-Gallego et al.,[10] breastmilk metabolic and microbiota profiles were studied in healthy women from Finland, Spain, South Africa, and China. Their results reveal specific milk metabolomic profiles across the different geographical locations. In another study by Gay et al.,[28] breastmilk samples were collected from Australia, Japan, the USA, Norway, and South Africa and showed variation of breastmilk metabolites between the different countries.

There is a relationship between metabolites, oligosaccharides, and bacteria in breastmilk. More research is needed to understand the complex mechanisms, the interactions between them, and the potential effect on the infant.

Key take home messages

- Breastmilk is the best nutrition for newborns and has been shown to support optimal growth and development of infants.
- Breastfeeding has positive effects for both mother and infants.
- Breastmilk has a complex composition providing nutrients and other bioactive components such as HMOS, immune cells, micro-organisms, and their metabolites.
- HMOS are known to play a crucial role in the development of the gut microbiota and immune system.
- Milk microbiota and their metabolites are thought to have a role in the development of the gut microbiota and immune system, with further research needed.

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Biotics and Their Application for Different Feeding Journeys

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The ideal conditions for an infant to have a healthy microbiome are:

- Full term, vaginal delivery
- Exclusive breastfeeding for the first 6 months of life, and thereafter on demand with complementary feeding until 2 years of life
- No antibiotics during the first 2 years of life
- Healthy exposure to an environment with family, friends, and pets

However, some infants are born by cesarean section (C-section), have been exposed to early life antibiotics, and, or are not breastfed. This can result in a dysbalanced colonization, increasing the risk of gut microbiota dysbiosis. Nutritional strategies in early life can offer an opportunity to rebalance the compromised gut microbiota in these infants.

Over the years infant formulas have evolved. Whilst it is not possible for an infant formula to completely mimic breastmilk, it can move closer towards providing some of the health benefits of breastmilk.

Between 2014-2021, the International Scientific Association for Probiotics and Prebiotics (ISAPP) has proposed definitions of probiotics[29], prebiotics[30], synbiotics[31], and postbiotics,[12] as outlined in Table 1.

Table 1. Definitions as proposed by the International Scientific Association for Probiotics and Prebiotics (ISAPP)

Probiotics	Probiotics are living microorganisms that, when administered in adequate amounts, confer a health benefit to the host.
Prebiotics	Prebiotics are substrates that are selectively utilized by host microorganisms conferring a health benefit.
Synbiotics	Synbiotics are a mixture comprising living microorganisms and substrate(s) (i.e., prebiotics) that are selectively utilized by host microorganisms and that confers a health benefit on the host.
Postbiotics	The definition of postbiotics is a preparation of inanimate microorganisms and/or their components that confers a health benefit on the host.

Prebiotics were born in 1995 and since then most research has been focused on inulin, short-chain galacto-oligosaccharides (scGOS) together with long-chain fructo-oligosaccharides (lcFOS) and their health end points.[30] There is considerable research on the beneficial clinical effects of infant formulae with prebiotics which include, reducing the rate of gastrointestinal infections[32, 33], respiratory tract infections[33], allergic diseases[33, 34], and antibiotic usage[35]. Antibiotic use can be problematic, not just for the individual but as a public health concern. By 2050 antibiotic resistance is predicted to be a huge challenge worldwide and should therefore be limited. scGOS/lcFOS increase beneficial bacteria[36], which suppresses the growth of pathogens and harmful bacteria[37, 38, 35], preventing gut and respiratory tract infections[35], and therefore, reducing the need for antibiotics[39].

Non-clinical, beneficial outcomes of prebiotic supplementation in infant formula include higher stool colony counts of Bifidobacterium (the most abundant microbe in the infant gut)[40], increased SCFA concentrations, reduced fecal pH which makes the gut less susceptible to pathogens. Another favorable clinical effect is stool softening[41], which may be beneficial to some infants.

Infant formulae with prebiotics are safe to use and support the growth of the infant.[42] Not all prebiotics are the same, and therefore, each prebiotic(s) supplemented formula requires efficacy and safety assessments.

Infant formulae supplemented with probiotics also present no safety concerns with growth or adverse effects, and some favorable clinical effects are reported such as a reduction in gastrointestinal infections, infantile colic, irritability, episodes of diarrhoea, and increased defecation frequency.[43] As with prebiotics, efficacy and safety of each probiotic strain needs to be assessed in a case-to-case basis because the results are strain dependent. ESPGHAN are due to make a recommendation on the addition of specific probiotics in infant formula.

There can be a synergistic combination of pre- and probiotics. In a recent clinical study by Phavichiter et al,[44] healthy infants received either a control infant formula or a formula supplemented with infant-type *bifidobacteria*l strain *B. breve* M-16V, at a dose close to bacterial numbers found in breastmilk, combined with scGOS and lcFOS in a ratio of 9:1. Exclusively breastfed infants were included as a reference. After 6 weeks of intervention, the synbiotic formula significantly increased the *Bifidobacteria* proportions in the gut compared to a formula without synbiotics. It also decreased the prevalence of harmful bacteria to closer to the levels in the breastfed reference group. Additionally, the fecal pH was significantly lower, while L-lactate concentrations and acetate proportions were significantly higher in the synbiotic groups, creating a gut microbiota composition, and a gut environment, closer to that of breastfed infants.

Lactobacilli are aerobic bacteria which reduce the reactive potential in the gut and allows the healthy growth of *Bifidobacteria*. The vagina is abundant in *Lactobacilli*. When an infant is born via vaginal delivery, they receive *Lactobacilli* into their gut via the birth canal and vagina[45, 46]. However, if an infant is born by C-section, it will not receive the seeding of the *Lactobacilli* and the subsequent colonization of *Bifidobacteria* can be delayed by up to several weeks.

The use of synbiotic supplemented formula in infants can support the immune system through the promotion of the growth of healthy gut microbiota. In a study by Chua et al.,[47] infants were randomized to receive either a standard formula (control), a formula with prebiotics (scGOS/lcFOS), or a formula with synbiotics scGOS/lcFOS and *B. breve* M-16V from birth until week 16. The study also included 30 vaginally born subjects who were breastfed as a reference group. Synbiotic

supplementation resulted in a higher *Bifidobacteria* proportion from day 3/5 (P<0.0001) until week 8 (P=0.041) in C-section born infants compared to the non-synbiotic control. This may have the potential to reduce the development of atopic dermatitis and eczema in those infants. In another clinical study a synbiotic supplemented amino acid formula (prebiotic fructo-oligosaccharides and probiotic *Bifidobacterium breve* M-16V) was found to increase both the quantity and diversity of gut microbiota in non-IgE mediated CMA infants bringing it closer to a healthy breastfed profile.[48]

Non-viable microbes such as bacterial cells and fragments, lipids, proteins, peptides, vitamins, and other complex molecules are known under the umbrella term of "postbiotics".[12] Not all postbiotics are the same; strains and fermentation process determine which compounds are being formed. An example is the specific fermentation process Lactofidus™ which uses two bacterial strains, *Bifidobacterium breve* C50 and Streptococcus thermophilus 065 to ferment infant formulae.

Infant formula produced via the Lactofidus™ fermentation process contains specific postbiotics which modulates the gut microbiota with a higher proportion of *Bifidobacteria* and fewer-adult like species[49,50], increased poliovirus-specific intestinal antibody response[49], which in turn may lead to less severe gastrointestinal infections[51] and an increase in faecal secretory IgA immune responses.[50, 52]

In a clinical study using a formula with prebiotics scGOS/lcFOS in a ratio of 9:1, and postbiotics from the Lactofidus™ fermentation process including 3'Galactosyllactose (3'-GL), the formula was found to be safe and well-tolerated in all infants. In addition, their stool consistency and frequency were closer to those of breastfed infants, and there was a lower incidence of investigator-reported infantile colic[42]. Furthermore, the same formula, positively impacts microbiota composition and activity with higher levels of *Bifidobacteria*, lower levels of *C. difficile*, lower fecal pH, increased secretory IgA levels and increased levels of SCFAs.[53] This is reinforced by a clinical study from Beghin et al., where they showed improved gut and immune markers biomarkers such as secretory IgA levels closer to the level in breastfed infants, increased *Bifidobacterium* levels and fecal microbiota composition closer to what is observed in breastfed infants.[54]

Finally, another study with prebiotics scGOS/lcFOS (9:1), and postbiotics derived from the Lactofidus™ fermentation process with 2'-FL and milk fat in healthy term infants across 5 different European countries showed that the formula was well tolerated, supports adequate infant growth and is safe to use[55]. More research is needed to understand the clinical effects this formula, and others in the future have, on healthy term infants.

Key take home messages

- Nutritional strategies exist to modulate the gut microbiome.
- Not all biotics are the same and the individual benefits of each need to be proven.
- Clinical evidence has shown relevant immune benefits for the prebiotic mixture scGOS/lcFOS (9:1) and some specific pre-, pro-, syn- and postbiotics.

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Synbiotics to Support Gut Microbione Challenges in Early Life

BY PROF. **JAN KNOL**

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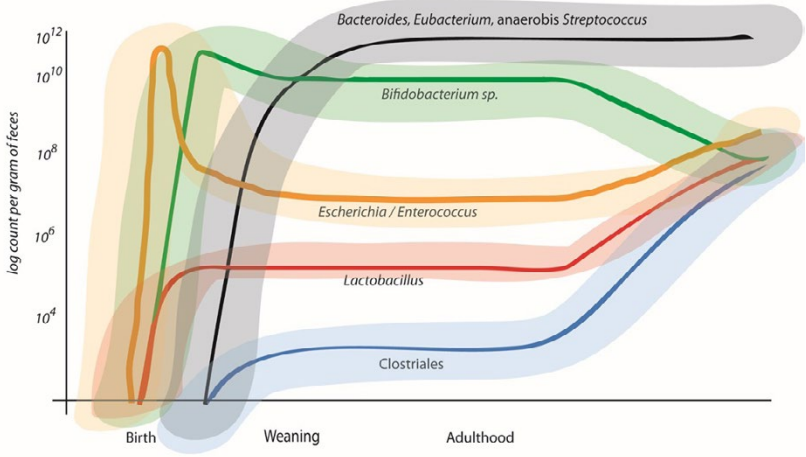
The period of early life is critically important for the development of the gut microbiome. The gut is not yet mature, the microbes need to find their place in the gut and get into sync with the infant's environment, and in addition, this happens during a period of rapid growth. Breastmilk needs to be digested and absorbed to support this growth, and at the same time, be utilized to develop the gut microbiome. The colonization of the microbiome remains to be an important topic of research.

There are some common patterns of gut microbiota dynamics and development in early life. This is shown in Figure 1[56]:

- Initially, the aerobes, *Enterococcus* and *Escherichia* quickly colonize
- This is quickly taken over by the anaerobes. In infants, the most abundant family is *Bifidobacteria* which can utilize the HMOS in breastmilk and stabilizes the microbiome to support health for the rest of life.

Figure 1



We have co-evolved with our micro-organisms. Women produce HMOS in breastmilk. In pangenome analysis of *Bifidobacteria*, the gut bacteria can both utilize and degrade HMOS, as well as produce SCFAs; simultaneously supporting microbiome and protecting it from pathogens.

Bifidobacteria colonization begins in early life. The bacteria enter the infant gut via seeding, which occurs during vaginal delivery. Once the bacteria are in the infant gut, they need nurturing within the right environment, and feeding. *Bifidobacterium* thrive in the gut by removing oxygen and producing SCFAs. Subsequently SCFAs initiate the colonization of micro-organisms, amongst others.[57]

There are many threats for healthy early life microbiome development in this sensitive period which include:

- Maternal factors
- Mode of delivery
- Antibiotic use
- Gestational age
- Environment

The incidence of C-section rate around the world has almost doubled since 2000, with recent figures reporting 3 in 10 babies are now born by C-section.[58] The mode of delivery significantly impacts the gut microbiota composition. The intestinal microbiota has been shown to be different in C-section born infants which can have an impact on the maturation of the immune system. In fact, C-section is now considered as a significant risk factor for several immune and metabolic diseases.[59] The recent MAMI trial aimed to investigate whether there are any microbiota effects seen between antibiotic administration before and after cord clamping in C-section born infants. Compared to vaginally born infants, profound differences were found in microbial diversity and composition of both C-section groups in the first month of life. However, any difference in the effect of either method is over-ruled by the negative impact of the C-section on the infant's gut microbiota.[60]

Nutritional strategies may offer the opportunity to restore the compromised gut microbiota in C-section born infants.[57] For example, a synbiotic comprising of prebiotic scGOS/lcFOS in a ratio of 9:1, plus a specific probiotic strain of *Bifidobacterium* breve M-16V feeds the proliferation of *Bifidobacteria*[44], mimics immune-modulating and bifidogenic effects of breastmilk, and increases the balance of beneficial bacteria, and may exert positive clinical effects.[61]

In a double-blind randomized controlled trial infants born by elective C-section received an infant formula supplemented with either synbiotics (as above), prebiotics, or no biotics (control) from birth until 4 months old. Vaginally born breastfed infants were included as a reference group. The objectives of the study were to measure the functional impact of delivery mode on the infant, and early life nutrition, on the development of the infant gut microbiome born via C-section. In C-section born infants, the colonization of anaerobes, such as *Bifidobacteria* are significantly delayed, however, supplementation of synbiotics via an infant formula can help to restore this. Infants receiving the infant formula with synbiotics consistently achieve similar levels of *Bifidobacteria* to that of the breastfed reference group from the first days of life.[47] In addition, supplementation with synbiotics in an infant formula supports normal gut diversity due to the cross feeding of the *Bifidobacteria*, thus contributing to favorable gut conditions.[62] In a vaginally born reference group, the infant gut becomes colonized with strict anaerobes due to the absence of oxygen. Typically, in C-section born infants, more facultative anaerobes and aerobes such as *Entreobacteria* are found, indicating there

is more oxygen present, which leads to oxidative stress in the infant gut. In the study by Lay et al.,[62] the supplementation of a synbiotic formula in C-section born infants prevents delayed colonization by strict anaerobes. In addition, in the control C-section infants, HMOS and SCFAs are present in the feces but not in the vaginally born reference, or the synbiotic supplemented group. This is due to the HMOS and SCFAs being utilized by the strict anaerobes like *Bifidobacteria*, facilitating an acidic environment and encouraging a healthy gut microbiome. In the same study, a 53% reduction in the incidence of reported skin disorders and eczema was found among C-section born infants receiving the infant formula with synbiotics compared to the control group.[47] Synbiotic supplementation in C-section born infants may offer a possible protective effect on atopic dermatitis and eczema in early life.

Key take home messages

- A compromised gut microbiome in early life can affect lifelong health
- The mode of delivery strongly determines the colonization process of the gut
- Breastmilk is the best nutrition for infants, naturally containing pre- and probiotics
- Synbiotics can support normal gut microbiome development in C-section born infants, (in terms of diversity and functionality), closer to vaginally born infants
- C section born infants receiving synbiotics have a better HMO utilization capacity
- Early life nutrition can drive normal gut colonization and thereby support healthy immune, metabolic and brain development

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