Special article

Arch Argent Pediatr. 2024;e202410489

Human milkoligosaccharides: Role in lactation and infant formulas

Mónica Del Compare[1,2], Adriana Fernández[3], Omar Tabacco[4], Gabriel Vinderola[5]

ABSTRACT

Human milk oligosaccharides (HMO) are the specific and selective growth substrate for bifidobacteria, preventing pathogen adhesion, modulating the immune system, and impacting neurodevelopment. Human milk is the best food for the neonate; infant formulas enriched with HMOs are indicated when human milk is not possible or sufficient. HMOs developed and added to available infant formulas are 2′-FL (2′-fucosyl lactose), 3-FL (3-fucosyl lactose), 3′-SL (3′-sialyl lactose), 6′-SL (6′-sialyl lactose), LNT (lacto-N-tetraose), and 3′-GL (3′-galactosyl lactose), the latter being produced in situ by microbial fermentation. These HMOs are safe and contribute to satisfactory infant development. In addition, they were shown to promote the development of the intestinal microbiota in a way that is more similar to that of infants fed human milk than that of infants fed formula without HMOs.

Keywords: *microbiota; oligosaccharides; human milk; prebiotics; infant formulas.*

doi: http://dx.doi.org/10.5546/aap.2024-10489.eng

To cite: Del Compare M, Fernández A, Tabacco O, Vinderola G. Human milk oligosaccharides: Role in lactation and infant formulas. *Arch Argent Pediatr.* 2024;e202410489. Online ahead of print 26-DEC-2024.

[1] Hospital Pediátrico Federico Falcón, Del Viso, Argentina; [2] Sanatorio Mater Dei, City of Buenos Aires, Argentina; [3] Centro de Consultas Médicas CECOMED, La Plata, Argentina; [4] Sanatorio de Niños de Rosario, Rosario, Argentina; [5] Instituto de Lactología Industrial (INLAIN, CONICET-UNL), Facultad de Ingeniería Química, Universidad Nacional del Litoral, Santa Fe, Argentina.

Correspondence to *Gabriel Vinderola: gvinde@fiq.unl.edu.ar*

Funding*: None.*

Conflict of interest: *Nutricia-Bagó facilitated the meetings to organize the work through digital platforms. The manuscript was written independently with the authors' collaboration, without the company having editorial control over the result. The authors declare that their only relationship with Nutricia-Bagó was to have participated as speakers in conferences or symposiums organized by the company. GV is a member of the board of directors of the International Scientific Association of Probiotics and Prebiotics (ISAPP).*

Received*: 7-4-2024*
Accepted*: 9-17-2024*

INTRODUCTION

Human milk oligosaccharides (HMO), formerly known as "bifidogenic factors", are a complex group of oligosaccharides (3 to 9 molecular units) and dynamic throughout lactation. HMOs are formed by combining and chaining five molecules, of which lactose is a common denominator. HMOs are the specific and selective substrate for the growth of bifidobacteria, the main microbial group in the intestine of the neonate and until the onset of complementary feeding, and also have other biological activities in the intestine (prevention of pathogen adhesion and modulators of the immune system associated with the intestine), but also in a systemic way, with a recognized impact on cognitive development.[1] In cases where breastfeeding is unfortunately not possible or sufficient, the indication is infant formulas enriched with some of these HMOs.

This narrative review aims to summarize the scientific evidence regarding the beneficial effects of HMOs naturally present in human milk and analyze the available evidence regarding those added to infant formulas.

HUMAN MILK OLIGOSACCHARIDES

Since the beginning of the 20th century, thanks to the pioneering work of Moro and Tissier, it has been known that the intestinal bacteria of infants fed with human milk (HM) are different from those of bottle-fed infants.[2] In 1930, specific carbohydrates were recognized in HM, then called "bifidogenic factors" due to their capacity to stimulate the development of certain bacteria.[1,3]

At the beginning of the 21st century, the study of these carbohydrates was deepened, and their functional effects as fundamental bioactive molecules began to be considered.

HMOs are non-digestible oligosaccharides with no direct nutritional effects, consisting of variable combinations of five monosaccharides: glucose, galactose, fucose, N-acetylglucosamine, and sialic acid. These oligosaccharides can be short or long-chain (3 to 9 units) and are present in a 9:1 ratio. Intestinal hydrolysis of lactose into its two constituent molecules (glucose and galactose) provides part of the lactose content of the substrates necessary for HMO synthesis in the mammary gland. From lactose, the addition of specific monosaccharides gives rise to different groups of HMOs: beta HMOs by the addition of galactose and N-acetylglucosamine, neutral HMOs by the addition of fucose and acidic HMOs by the addition of sialic acid.[4] More than 200 different HMOs have been described so far in HM. HMOs constitute the third solid component of HM, after lactose and lipids, and their concentration is from 100 to 300 times higher than in cow's milk. Their amount is variable throughout lactation: in colostrum, it is 20-25 grams/liter, and in mature milk, approximately 15 grams/liter. The concentration in the HM of mothers of premature infants is higher.[5] HMOs are resistant to cold and heat, pasteurization, and freezing.[6]

The variety and concentration of HMOs differ from that of other milk (bovine, caprine), standing out in HM the high concentration of compounds with fucose (70%) compared to the higher proportion of derivatives with sialic acid in cattle milk. Fucose derivatives selectively stimulate bifidobacteria.[5] Its production by the mammary gland depends on factors such as maternal nutritional status, feeding, environment, type and gestational age at birth,[7] and especially on maternal genetics due to the concentration of an enzyme, fucosyltransferase, whose production is encoded in the *FUT2* gene located on chromosome 19.[8] Because of this, the HM content can be variable; there is an approximate percentage of 5% of mothers in whom the production of HM is less than 5 grams/liter.[9]

This genetic component in the HMO content of HM is essential for the proportion of fucose conjugates. The HMO content of HM has a decisive influence on the infant's intestinal microbiota composition and function.[10]

BENEFICIAL EFFECTS OF HUMAN MILK OLIGOSACCHARIDES AND MECHANISMS OF ACTION

Prebiotics are substrates used selectively by host microorganisms that confer a health benefit.[11] In this context, HMOs can be considered prebiotics naturally present in HM. HMOs stimulate the proliferation of bacteria of the genus *Bifidobacterium*,[12] beneficial microorganisms dominant in the gut of healthy infants in the first years of life.[13] These bacteria use HMOs as an energy source and produce short-chain fatty acids (SCFA), such as butyrate, propionate, and acetate, by colonic fermentation. Butyrate promotes the thickening of the mucous layer lining the intestine, preventing potentially pathogenic microorganisms' adhesion and their entry into the bloodstream. Scientific evidence also suggests that HMO modulates the response of epithelial and immune cells, reducing the infiltration and excessive activation of leukocytes in the mucosa,

thus reducing the risk of necrotizing enterocolitis.[1]

The type of birth (vaginal or cesarean) determines the profile of microorganisms that will colonize the infant's intestine. In infants born vaginally, the microbial composition is similar to that found in the birth canal and in the maternal intestine, but in those born by cesarean section, a microbiota composition similar to the mother's skin and the hospital environment is observed.[14]

In the case of those born by cesarean section, the microbiota has a lower diversity and quantity of beneficial bacteria than those born by vaginal delivery. In the intestinal microbiota of babies born by cesarean section, the predominant bacteria are *Staphylococcus*, *Corynebacterium*, *Propionibacterium*, and a lower concentration of *Bifidobacterium*. If these infants are fed with HM, the microbiota will progressively and in direct proportion to the duration of lactation resemble that of vaginally born infants, regarding the stability and diversity of the microbiota, since bacteria from HM contribute to the colonization of the intestinal mucosa, supported by the HMOs.[15] Although different methods have been proposed to promote intestinal colonization of babies born by cesarean section, HM administration is the method that is the most effective.[14]

HMOs may benefit neurodevelopment by influencing the composition of the gut microbiota and the production of metabolites that could positively impact the brain. Although research in this area is in development, it is suggested that HMOs could have positive effects on neurodevelopment, both motor and cognitive function, especially in preterm infants who are more susceptible to neurodevelopmental deficits due to brain injury at birth and compromised brain maturation while in the neonatal intensive care unit. It has been observed that HM-fed preterm infants show better outcomes than formula-fed infants, especially those born prior to 30 weeks of gestation. In addition, MRI studies in HM-fed infants have demonstrated more mature white brain matter, fewer lesions and larger regional volumes.[16] *Figure 1* summarizes the mechanisms of action that mediate the beneficial effects of HMOs.[17]

ADDITION OF HUMAN MILK OLIGOSACCHARIDES TO INFANT FORMULAS

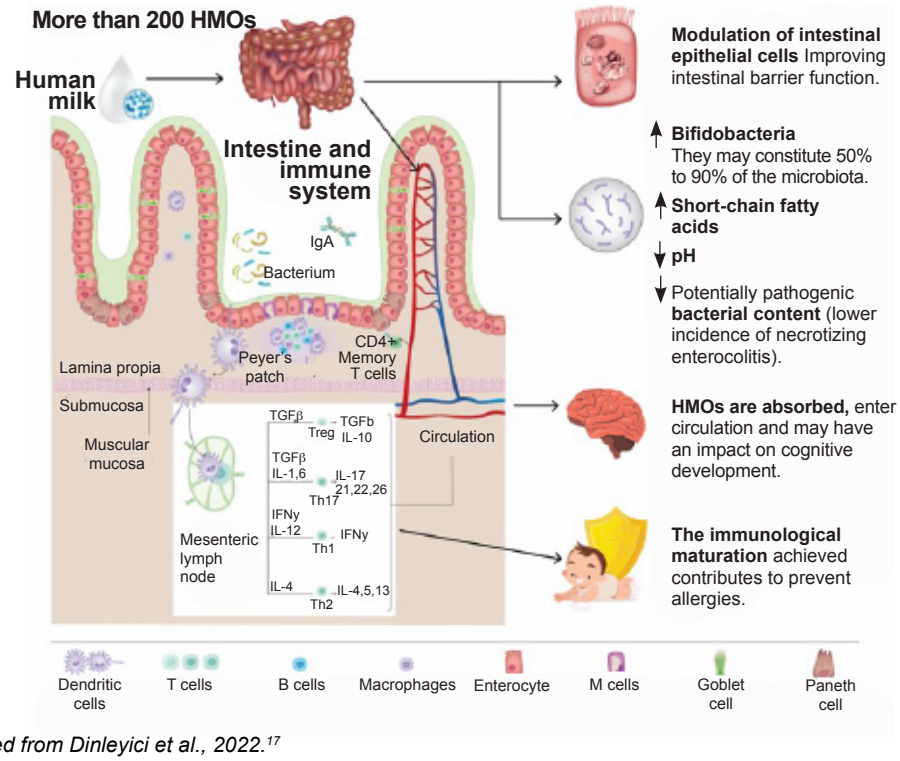
Adding HMO analogs, structurally and functionally identical to those found naturally in HM, represents infant formula technology's most important innovation of the last decade. The European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) will soon publish a position paper proposing the use of the term "HMO analogs" to refer to those obtained by chemical synthesis since those added to formulations are not isolated and purified from HM (Swajezka 2024, personal communication). 2′-fucosyl lactose (2′-FL) was the first HMO available for use in infant formulas. Currently, 2′-FL (2′-fucosyl lactose), 3-FL (3-fucosyl lactose), 3′-SL (3′-sialyl lactose), 6′-SL (6′-sialyl lactose), and LNT (lacto-N-tetraose) are available for addition to infant formulas (Hill et al.). 3′- GL (3′-galactosi lactose) is the only one produced in situ by microbial fermentation by *Bifidobacterium breve* C50 and *Streptococcus thermophilus* 065 in the same milk used as a base for formula preparation.[18]

Most infant formulas are produced from cow's milk with virtually no HMO content and little variability.[19] Advances in biotechnology now allow the synthesis of HMOs that are chemically and structurally identical to those containing HM. Organizations such as the European Food Safety Agency (EFSA) and the Food and Drug Administration (FDA) in the United States has approved its use. The first formula containing HMO was marketed in Spain and the United States in 2016. The approved use for 2'-FL is 1.2 g/L, and for LNT, 0.6 g/L, in a 2:1 ratio for infants under one year of age.[17,20,21] The number of HMOs that can be used in infant formulas has increased in recent years: 2'-FL, 3'-FL, 3′-GL, LDFT, LNnT, LNT, 3'-SL, 6'-SL.[18]

Different clinical studies have been published, which include mostly term and healthy infants.[22] In *Table 1*, some of the controlled clinical studies are summarized. These studies evaluated different HMOs, individually or in combination, at different doses. In general, most of them include aspects of safety and tolerance.[23-33] Other aspects investigated are growth, stool characteristics, microbiota composition, and biomarkers.[27,29] Some studies found that HMO supplementation is safe and well tolerated, and growth was adequate.[31]

Other results observed were that the feces where of better consistency and more frequent, similar to that of infants fed with HM.[34] Some of these studies analyzed the responses in children with cow's milk protein allergy[26,31] and observed good tolerance, improvement of symptoms, and adequate growth in the period studied. Other authors found positive effects on the composition of the microbiota.[28] In particular, it was observed

Figure 1. Proposed mechanisms of action for the local and systemic beneficial effects described for human milk oligosaccharides



Source: adapted from Dinleyici et al., 2022.[17]

that the administration of a formula with GOS/FOS and HMO 3'-GL (produced *in situ* by microbial fermentation) was able to maintain a microbiome and metabolome closer to those of human milk-fed infants than the control formula without GOS/FOS nor 3'-GL.[32] GOS/FOS are oligosaccharides of galactose and fructose (chains of between 3 and 9 units), where these sugars are chemically linked in such a way that they escape digestion and absorption in the small intestine, and reach the large intestine intact, where they are selective substrates for bifidobacterial growth. Despite these findings, further studies are needed to demonstrate health effects related to the structure and dosage of HMOs in infant formulas.

CONCLUSION

HM is the best food for all newborns, particularly because of its HMO content. HMO is a complex and dynamic group of oligosaccharides selectively utilized by bifidobacteria, which positively impacts the intestinal microbiota and the immunological, metabolic, and neurological development of newborns.

Table 1. Controlled clinical studies of infant formulas' developmental and health effects with added human milk oligosaccharides

Reference	Inclusion criterion	Intervention	Control group	Duration	Results
Marriage et al. 2015[23]	Healthy infants >2500 g > 5 days	2′-FL and GOS in 3 concentrations	Infants Exclusive HM	4 months	No significant differences in weight, length, and head circumference. Good tolerance.
Puccio et al. 2017[24]	Healthy infants 0-14 days	2′-FL (1.0 g/L) and LNnT (0.5 g/L)	Starter formula without HMO.	12 months	Safe, well-tolerated, adequate growth. Secondary outcomes: lower morbidity, lower antibiotic and antipyretic use.
Alliet et al. 2022[25]	Healthy infants <14 days	2'-FL	Formula without 2'-FL	6 months	Significantly lower content of *Costridioides difficile* and a higher content of *Bifidobacterium*
Ramírez Farías et al. 2021[26]	Infants 0-6 months, CMPA suspected	Hydrolized extensive formula with 0,2 g/L of 2'-FL	Hydrolized extensive formula	2 months	Adequate tolerance, improvement of symptoms. Adequate growth in the period studied.
Parshat et al. 2021[27]	Healthy infants <14 days	HMO: total 5.75 g/L (52% 2'-FL, 13% 3'-FL, 26% LNT, 4% 3'-SL, and 5% 6'-SL)	Starter formula without HMO HM exclusive	4 months	No difference in weight gain with both formulas. Both HMO formula group and HM group presented softer and more frequent stools than the control group.
Bosheva et al. 2022[28]	Healthy infants BW >2500 g 7-21 days	2'-FL, LNT, 3'SL, 6'-SL	Starter formula without HMO HM exclusive	15 months	The microbiota was significantly different in HMO and HM groups vs. formula without HMO: higher concentration of *Bifidobacteriem longum* subsp. *infantis* and lower concentration of *Clostridium difficile*.
Goehring et al. 2022[29]	Healthy infants 5 days of life	2'-FL, with or without GOS	Starter formula without HMO HM exclusive	4 months	29-83% lower concentration of plasma cytokines than those fed GOS-only formulas.
Vandeplas et al. 2020[30]	Infants ≥ 14 days fed with formula	Formula including 3'-GL, scGOS/lc FOS (9:1) 2'-FL	Formula with scGOS/lcFO	17 weeks	Maintained adequate growth, good tolerance, no differences in adverse events.
Vandenplas et al. 2022[31]	Infants 0-6 months diagnosed with CMPA	Hydrolized extensive formula 2'-FL, LNnt	Hydrolized extensive formula without HMO	4 months .	No significant differences in anthropometry. Significant decrease in the frequency of infections in the upper airway and otitis media.
Rodríguez Herrera 2022[32]	NB	3' -GL, 25 mg/100 mL	Infants with exclusive HM	17 weeks	Improves the composition of the microbiota and profile of the intestinal metabolome was more similar to those fed with HM.

HMO: human milk oligosaccharides, BW: birth weight, NB: newborn, HM: human milk, CMPA: cow's milk protein allergy.

REFERENCES

1. Bode L. Human Milk Oligosaccharides: every baby needs a sugar mama. *Glycobiology*. 2012;22(9):1147-52.
2. Solís G, Gueimonde M. The Gut Microbiota in Infants: Focus on Bifidobacterium. *Microorganisms*. 2023;11(2):537.
3. Kunz C. Historical aspects of human milk oligosaccharides. *Adv Nutr*. 2012;3(3):430S-9.
4. Boix-Amorós A, Collado MC, Van't Land B, Calvert A, Le Doare K, Garssen J, et al. Reviewing the evidence on breast milk composition and immunological outcomes. *Nutr Rev*. 2019;77(8):541-56.
5. Vandenplas Y, Berger B, Carnielli VP, Ksiazyk J, Lagström H, Sanchez Luna M, et al. Human Milk Oligosaccharides: 2'-Fucosyllactose (2'-FL) and Lacto-N-Neotetraose (LNnT) in Infant Formula. *Nutrients*. 2018;10(9):1161.
6. Hahn WH, Kim J, Song S, Park S, Kang NM. The human milk oligosaccharides are not affected by pasteurization and freeze-drying. *J Matern Fetal Neonatal Med*.

2019;32(6):985-91.
7. McGuire MK, Meehan CL, McGuire MA, Williams JE, Foster J, Sellen DW, et al. What s normal? Oligosaccharides concentration and profiles in milk produced by healthy women vary geographically. *Am J Clin Nutr*. 2017;105(5):1086-100.
8. Collado MC, Gueimonde M, Ruiz L, Aparicio M, Castro I, Rodríguez JM. Baby's First Microbes: The Microbiome of Human Milk. In: Azcarate-Peril MA, Arnold RR, Bruno-Bárcena JM (eds). How Fermented Foods Feed a Healthy Gut Microbiota: a nutrition continuum. Cham: Springer; 2019:3-33.
9. Cabrera-Rubio R, Kunz C, Rudloff S, García-Mantrana I, Crehuá-Gaudiza E, Martínez-Costa C, et al. Association of Maternal Secretor Status and Human Milk Oligosaccharides with Milk Microbiota: An Observational Pilot Study. *J Pediatr Gastroenterol Nutr*. 2019;68(2):256-63.
10. Lewis ZT, Totten SM, Smilowitz JT, Popovic M, Parker E, Lemay DG, et al. Maternal fructosyltransferase 2 status affects the gut bifidobacterial communities of breastfed infants. *Microbiome*. 2015;3:13.
11. Gibson GR, Hutkins R, Sanders ME, Prescott SL, Reimer RA, Salminen SJ, et al. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat Rev Gastroenterol Hepatol*. 2017;14(8):491-502.
12. Boehm G, Moro G. Structural and functional aspects of prebiotics used in infant nutrition. *J Nutr*. 2008;138(9):1818S-28.
13. Saturio S, Nogacka AM, Alvarado-Jasso GM, Salazar N, de Los Reyes-Gavilán CG, Gueimonde M, et al. Role of bifidobacteria on infant health. *Microorganisms*. 2021;9(12):2415.
14. Korpela K. Impact of delivery mode on infant gut Microbiota. *Ann Nutr Metab*. 2021;1-9.
15. Rautava S, Luoto R, Salminen S, Isolauri E. Microbial contact during pregnancy, intestinal colonization and human disease. *Nat Rev Gastroenterol Hepatol*. 2012;9(10):565-76.
16. Cohen Kadosh K, Muhardi L, Parikh P, Basso M, Jan Mohamed HJ, Prawitasari T, et al. Nutritional support of neurodevelopment and cognitive function in infants and young children-an Update and novel insights. *Nutrients*. 2021;13(1):199.
17. Dinleyici M, Barbieur J, Dinleyici EC, Vandenplas Y. Functional effects of human milk oligosaccharides (HMOs). *Gut Microbes*. 2023;15(1):2186115.
18. Salminen S, Stahl B, Vinderola G, Szajewska H. Infant formula supplemented with biotics: current knowledge and future perspectives. *Nutrients*. 2020;12(7):1952.
19. Urashima T, Taufik E, Fukuda K, Asakuma S. Recent advances in studies on milk oligosaccharides of cows and other domestic farm animals. *Biosci Biotechnol Biochem*. 2013;77(3):455-66.
20. Sprenger N, Tytgat HLP, Binia A, Austin S, Singhal A. Biology of human milk oligosaccharides: from basic science to clinical evidence. *J Hum Nutr Diet*. 2022;35(2):280-99.
21. Hegar B, Wibowo Y, Basrowi RW, Ranuh RG, Sudarmo SM, Munasir Z, et al. The role of two human milk Oligosaccharides, 2'-Fucosyllactose and Lacto-N-Neotetraose, in infant nutrition. *Pediatr Gastroenterol Hepatol Nutr*. 2019;22(4):330-40.
22. Schönknecht YB, Moreno Tovar MV, Jensen SR, Parschat K. Clinical studies on the supplementation of manufactured human milk oligosaccharides: a systematic review. *Nutrients*. 2023;15(16):3622.
23. Marriage BJ, Buck RH, Goehring KC, Oliver JS, Williams JA. Infants fed a lower calorie formula with 2'FL show growth and 2'FL uptake like breast-fed infants. *J Pediatr Gastroenterol Nutr*. 2015;61(6):649-58.
24. Puccio G, Alliet P, Cajozzo C, Janssens E, Corsello G, Sprenger N, et al. Effects of infant formula with human milk oligosaccharides on growth and morbidity: a randomized multicenter trial. *J Pediatr Gastroenterol Nutr*. 2017;64(4):624-31.
25. Alliet P, Vandenplas Y, Roggero P, Jespers SNJ, Peeters S, Stalens JP, et al. Safety and efficacy of a probiotic-containing infant formula supplemented with 2'-fucosyllactose: a double-blind randomized controlled trial. *Nutr J*. 2022;21(1):11.
26. Ramirez-Farias C, Baggs GE, Marriage BJ. Growth, tolerance, and compliance of infants fed an extensively hydrolyzed infant formula with added 2'- Fucosyllactose (2'-FL) human milk oligosaccharide. *Nutrients*. 2021;13(1):186.
27. Parschat K, Melsaether C, Jäpelt KR, Jennewein S. Clinical Evaluation of 16-Week Supplementation with 5HMO-Mix in Healthy-Term Human Infants to Determine Tolerability, Safety, and Effect on Growth. *Nutrients*. 2021;13(8):2871.
28. Bosheva M, Tokodi I, Krasnow A, Pedersen HK, Lukjancenko O, Eklund AC, et al. Infant Formula with a Specific Blend of Five Human Milk Oligosaccharides Drives the Gut Microbiota Development and Improves Gut Maturation Markers: A Randomized Controlled Trial. *Front Nutr*. 2022;9:920362.
29. Goehring KC, Marriage BJ, Oliver JS, Wilder JA, Barrett EG, Buck RH. Similar to Those Who Are Breastfed, Infants Fed a Formula Containing 2'-Fucosyllactose Have Lower Inflammatory Cytokines in a Randomized Controlled Trial. *J Nutr*. 2016;146(12):2559-66.
30. Vandenplas Y, de Halleux V, Arciszewska M, Lach P, Pokhylko V, Klymenko V, et al. A Partly Fermented Infant Formula with Postbiotics Including 3'-GL, Specific Oligosaccharides, 2'-FL, and Milk Fat Supports Adequate Growth, Is Safe and Well-Tolerated in Healthy Term Infants: A Double-Blind, Randomised, Controlled, Multi-Country Trial. *Nutrients*. 2020;12(11):3560.
31. Vandenplas Y, Żołnowska M, Berni Canani R, Ludman S, Tengelyi Z, Moreno-Álvarez A, et al. Effects of an extensively hydrolyzed formula supplemented with two human milk oligosaccharides on growth, tolerability, safety and infection risk in infants with Cow's Milk Protein Allergy: a randomized, multi-center trial. *Nutrients*. 2022;14(3):530.
32. Rodriguez-Herrera A, Tims S, Polman J, Porcel Rubio R, Muñoz Hoyos A, Agosti M, et al. Early-life fecal microbiome and metabolome dynamics in response to an intervention with infant formula containing specific prebiotics and postbiotics. *Am J Physiol Gastrointest Liver Physiol*. 2022;322(6):G571-82.
33. Wallingford JC, Neve Myers P, Barber CM. Effects of addition of 2-fucosyllactose to infant formula on growth and specific pathways of utilization by *Bifidobacterium* in healthy term infants. *Front Nutr*. 2022;9:961526.
34. Lasekan J, Choe Y, Dvoretskiy S, Devitt A, Zhang S, Mackey A, et al. Growth and Gastrointestinal Tolerance in Healthy Term Infants Fed Milk-Based Infant Formula Supplemented with Five Human Milk Oligosaccharides (HMOs): A Randomized Multicenter Trial. *Nutrients*. 2022;14(13):2625.