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Chapter

Probiotics and Prebiotics in Infant Formulae

José Maldonado

Abstract

Human breast milk provides all necessary nutrients for the development of term infants. In addition to its universally recognized nutrients, human breast milk contains a number of non-nutritive components that play a potential role in supporting infant growth. Human breast milk also contains bioactive compounds exerting a wide range of beneficial effects, such as promoting immune system maturation and exerting protection against infections. Supplementation of infant formulae with oligosaccharides and bacteria with proven beneficial health effects seems to be well-founded. The purpose of supplementation is to mimic the functional effects of oligosaccharides and bacteria found in human breast milk. Oligosaccharides with prebiotic functions and bacteria strains with probiotic functions have recently been added to infant formulae in the European Union and other countries. However, a systematic review conducted by the Committee on Nutrition of the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition revealed that there is no conclusive evidence supporting the routine use of probiotic- and/or prebiotic-fortified infant formulae. The aim of this review is to analyze the scientific basis for supplementation of infant formula with these compounds.

Keywords: intestinal microbiota, infant formulae, probiotic, prebiotic, symbiotic

1. Introduction

Human breast milk (HBM) is a complex physiological fluid uniquely suited to nourish infants. Its composition is specifically adapted to the digestive system and nutritional and growth needs of infants. HBM does not only contain essential nutrients, but also a vast array of non-nutritional bioactive components and microbes (microbiota) that confer benefits to the health of infants in the short and long terms. The microbiota mediates bacterial colonization of the newborn gut and supports immune system maturation and metabolic and cognitive development. Protective constituents such as cytokines, oligosaccharides, and bacteria facilitate newborn's adaptation to the extrauterine environment [1, 2]. HBM has been long known to protect neonates and infants from infections. It has been suggested that this protective role could be regulated by the action of a group of components that might inactivate bacteria and viruses individually, additively, and synergistically [3].

Gut microbiota has effects on health, and HBM contributes decisively to its composition via its bacteria and oligosaccharides supply. In order to achieve the same health effects, infant formulae are supplemented with live bacteria (probiotics), which favor bifidobacteria and lactobacilli growth (prebiotics) or a combination of these components (symbiotics).

2. Gut microbiota

Our body hosts a vast, diverse community of stable and varying microorganisms that are referred to as microbiota. The gut is the niche with the highest number and diversity of micro-organisms, containing over 10^{14} microbial cells, 10 times the amount of somatic and germinal cells in our body. The microbes that inhabit our gut are known as gut microbiota [4].

Gut microbiota is an open ecosystem that contains a broad diversity of metabolically active microbes that coexist in space and time and play a relevant role in the health of their host. The gut microbiota is considered a metabolic organ that is adaptable and rapidly renewable. There is a mutually beneficial interplay between the host and gut microbiota [4, 5].

2.1 Gut microbiota and immunity

The relationship between the lymphatic system and gut microbiota in early stages of life is crucial to the appropriate development of interactions between mucosal cell communities and systemic immunomodulation [5]. Animals with a sterile gut have been proven to be highly vulnerable to infections, which demonstrate the important role that gut microbiota plays in the immune system [4].

Bacterial colonization of the newborn's gut is influenced by a variety of factors such as gestation and delivery and breast-feeding mode [6]. HBM is an excellent continuous source of commensal bacteria for the infant gut. Evidence has been provided of a vertical transfer of bacteria from mother to child via breast milk [7, 8]. The fact that facultative anaerobic bacteria in newborn's gut are the predominant bacterial community in HBM microbiota is not a chance. These bacteria play a key role in the prevention of infections in the newborn [9]. Gut microbiota disorders (dysbiosis) in the first stages of life reportedly precede the development of atopy [10].

During the first week of life, the total bacterial count and, more specifically, anaerobic bacteria count progressively increase. The feeding mode of the newborn has a decisive impact on bacterial gut colonization. Bifidobacteria, lactobacilli, and Gram-positive cocci predominate in the feces of breastfed infants, whereas the bifidobacteria count is lower in the feces of formula-fed infants, with the predominance of bacteroides, clostridia, and coliforms [11–13]. Differences in the composition of newborn's gut microbiota based on the type of feeding could be the clue to identifying the bacteria that exert protective effects to breastfed infants [4].

3. Probiotics

Bacterial concentrations in HBM range between 10^2 and 10^4 ufc/mL. This means that an infant ingesting over 800 ml of milk a day would receive 10^5 to 10^7 ufc [14]. Therefore, HBM is a primary source of commensal and probiotic bacteria to the infant and plays a key role in the initial colonization of the gut. Some bacteria isolated from HBM have proven to have immunomodulatory and anti-infective effects. Therefore, the protective effects of HBM may be conferred by these bacteria. Supplementation of infant formulae with probiotic bacteria isolated from HBM could help improve gut microbial balance in formula-fed infants, thereby mimicking the beneficial effects of HBM.

Evidence has been published that probiotics modulate mucosal and systemic immune function, improve intestinal barrier function, and exert metabolic effects on the host [4]. Some of the lactobacillus strains isolated from HBM [15] have been reported to compete with enteropathogenic bacteria for nutrients and epithelium

adhesion and improve gut barrier functions. The ability of lactobacillus and bifidobacteria strains to stabilize the integrity of gut barrier has been demonstrated [16]. These types of bacteria potentially reduce antigen systemic load and influence immune function via enterocytes, antigen-presenting cells (monocytes and dendritic cells), regulatory T cells, and effector T and B cells [17, 18].

3.1 Infant formula supplemented with probiotics

European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) Committee on Nutrition [19] published a systematic review of studies assessing the safety and health effects of probiotic-supplemented infant formulae. No conclusive data were obtained from ESPGHAN's analysis of infant and follow-on formulae due to considerable variability in the type and dose of probiotics used and supplementation periods.

3.1.1 Safety

Formulae supplemented with probiotics do not raise safety concerns with regard to growth and adverse effects [19]. There are sufficient data supporting the safety of probiotics for infants older than 6 months. However, data on the use of probiotic supplementation in infants younger than 4 months are more limited. Studies in breastfed infants younger than 6 months who received a formula supplemented with either *Lactobacillus fermentum* CECT5716 or *Lactobacillus rhamnosus* GG revealed that formulae were well tolerated and had no adverse effects on growth either during the study period or at 3–5 years of age [20–23]. A recent study revealed that growth and food tolerance improved in premature infants >30 weeks of gestational age fed with a formula supplemented with *Saccharomyces boulardii*, and no adverse effects were detected [24].

3.1.2 Prevention and treatment of infant disorders

Conflicting results have been obtained regarding the effects of probiotics on the composition of fecal microbiota. A decrease in bifidobacteria and enterobacteria concentrations has been reported with respect to controls [25, 26]. Also, no differences have been observed in lactobacillus and bacteroides. By contrast, Maldonado et al. [27] reported an increase in fecal bifidobacteria and lactobacilli concentrations in infants fed with a formula supplemented with *Lactobacillus fermentum* CECT 5716. Also, no differences were found in other bacteria strains. Evidence has been provided that a formula containing *Bifidobacterium lactis* can influence the composition, stability, and function of gut microbiota in low-weight newborns [28].

The literature reports that probiotic supplementation of formula beyond early infancy can produce a decrease in the use of antibiotics and incidence of diarrhea, colic, and/or irritability. Yet, the variety of methods, type and dose of probiotics, and duration of interventions hinders that conclusive data can be obtained on clear clinical effects of probiotic-supplemented formulae in infants younger than 4 months [19].

In general, there is no consistent evidence supporting that supplementation of follow-on formula with probiotics has protective effects against infectious diarrhea [19]. Yet, a reduction has been reported in the duration and number of episodes of diarrhea associated with the use of probiotic-supplemented formulae [27, 29–31].

A systematic review conducted by Mugambi et al. [32] of controlled, randomized trials did not reveal that supplementation had any effects on infectious

diarrhea, colic, crying/irritability, regurgitation, or vomiting. No beneficial effects were documented on either crying or irritability in the review by ESPGHAN Committee on Nutrition. A study that was not included in ESPGHAN study showed that colic symptoms substantially improved with the administration of *Lactobacillus reuteri* DSM 17938 in breastfed infants [33]. There is no sufficient evidence, however, supporting routine supplementation with probiotics for the treatment or prevention of colic, especially in formula-fed infants [34].

In a review on the effects of a variety of immunonutrients in the prevention of necrotizing enterocolitis [35], the authors gathered sufficient data supporting supplementation of infant formulae with probiotics. Several meta-analyses combined these randomized controlled trials and observational studies demonstrated that the use of probiotics was beneficial for the prevention of severe necrotizing enterocolitis, late-onset sepsis, and all-cause mortality in very-low-birth-weight infants, as well as the time to achieve full enteral feeding in preterm infants [36–38]. By contrast, no differences were observed in a multicenter study involving 1315 preterm newborns fed with a hydrolyzed formula supplemented and non-supplemented with the probiotic *Bifidobacterium breve* BBG-001 [39]; the results of this trial provide no evidence of benefit of this probiotic intervention in reducing late-onset sepsis and necrotizing enterocolitis or death.

In relation to respiratory infections, limited available evidence from randomized controlled trials showed that formula supplementation with the probiotics studied is not associated with a reduction in the duration or risk of respiratory infections [19, 32]. A number of studies on formulae supplemented with different probiotic bacteria [30, 27, 40, 41] have shown a significant reduction in the number of upper airway tract infections in infants fed with these formulae. A study on *Lactobacillus fermentum* CECT 5716 [27] reports a significant 30% reduction in the total number of infections.

Significant reductions have been documented in the incidence of influenza and respiratory symptoms in several studies, where *Lactobacillus fermentum* CECT 5716 was administered in combination with anti-influenza vaccine [42]. This effect is explained by increased levels of NK cells and T-helper and T-cytotoxic lymphocytes.

Sufficient evidence has not been published supporting the beneficial effects of supplementation of infant formulae with probiotics on allergies. Several meta-analyses, however, have shown that the use of probiotics reduces the incidence of atopic dermatitis in infants but not of other types of allergies [43–45].

Evidence has been published that dietary treatment with a extensively hydrolyzed formula containing *Lactobacillus rhamnosus* GG is associated with a higher rate of acquisition of tolerance in infants allergic to cow's milk proteins, as compared to infants treated with a non-supplemented hydrolyzed formula [46, 47]. A relationship has been documented between dysbiosis in gut microbiota composition and the pathogenesis of cow's milk allergy [48, 49]. In addition, the administration of a hydrolyzed formula supplemented with probiotics reduces the incidence of other allergies and favors tolerance, as it changes the composition of infant's gut microbiota [23, 50].

Some studies suggest that gut microbiota alterations precede the development of the allergic phenotype. Therefore, probiotics could exert preventive and therapeutical effects [51]. The potential of some strains to favor Th1 and Th3 immune response against Th2 activity in patients with atopy can create the optimal conditions to redirect immune memory and reduce the risk of atopic disease. The Work Allergy Organization (WAO) [52] determined that probiotics confer health benefits in the prevention of eczema. Thus, WAO recommends the use of probiotics in pregnant or breastfeeding women whose infants have a high risk of developing allergies and in infants with a high risk of allergy.

3.1.3 Conclusion

ESPGHAN Committee on Nutrition does not recommend the routine use of infant formulae supplemented with probiotics. However, the evidence obtained in recent studies suggests that infant formulae containing some specific bacteria strains can confer beneficial health effects. A large number of infant formulae currently available on the market contain probiotics, and several panels support their use provided that their safety and benefits for the health and development of the infant have been demonstrated [19, 53, 54]. The European Food Safety Authority (EFSA) supports the safety of formula supplementation with probiotic bacteria. Yet, EFSA recommends that further studies are conducted to obtain the highest quality evidence on their efficacy [55].

4. Prebiotics

Prebiotics are defined as oligosaccharides refractory to the human digestive process with ability to stimulate and promote the growth and/or metabolism of bifidobacteria and lactobacilli in human gut [56]. More than 200 oligosaccharide complexes (neutral and cyclical oligosaccharides) have been identified in human breast milk [57]. Neutral oligosaccharides account for 70% of the total count and include the isomers lacto-N-tetraose, lacto-N-neotetraose, lacto-N-hexaose, monofucosyl-lacto-N-hexaose, and difucosyl-lacto-N-hexaose. Low levels of acidic oligosaccharides containing sialic acid or sulfate groups are present in HBM, and they primarily contain 5-N-acetyl-neuraminic acid [58].

Colostrum is composed of higher oligosaccharide concentrations (15–23 g/L), whereas mature HBM contents range from 1 to 10 g/L [59]. Oligosaccharides account for 8% of the total nutrient contents of HBM and are the third prevalent component following lactose and lipids.

Most of these oligosaccharides are non-absorbable and reach the colon, where they have different functions. Thus, they compete for membrane receptors with pathogenic bacteria and viruses in intestinal epithelium; they contribute to acidification via fermentation by colon bacteria; inhibit the growth of bacteroides, clostridia, and coliforms; promote lactobacilli and bifidobacteria growth; and stimulate the development of infant's immune system. A direct relationship has been documented between oligosaccharides and selectins, integrins, and other receptors, and they mediate leukocyte-endothelial cell interactions [59]. Fermentation of prebiotics by gut bacteria produces short-chain fatty acids, which exert a direct anti-inflammatory effect and promote intestinal barrier integrity by stimulating the proliferation and differentiation of gut mucosal cells.

Cow milk oligosaccharide content is substantially lower than that of HBM, and infant formula supplementation with prebiotics with the purpose of obtaining their health benefits is well founded. At present, GOS and FOS combinations are used, and other HBM oligosaccharides have been recently incorporated to infant formulae.

4.1 Infant formulae supplemented with prebiotics

The European Scientific Committee on Food approved prebiotic supplementation in infant and follow-on formulate up to a maximum of 0.8 g/100 ml to a GOS:FOS ratio of 9:1. By contrast, a systematic review on the safety and health effects of prebiotic-supplemented infant formulae conducted by ESPGHAN Committee on Nutrition [19] did not provide conclusive evidence due to variability in the type and dose of the prebiotic used and period of intervention.

2′-flucosyllactose, a HBM oligosaccharide, was recently synthesized and has been incorporated to some infant formula [60].

4.1.1 Safety

Formulae fortified with prebiotics do not raise safety concerns with regard to growth and adverse effects. [19]. Infant formulae containing HBM oligosaccharides have proven to be safe and well tolerated, and synthetic oligosaccharides have demonstrated to have similar effects to those of HBM oligosaccharides [60].

4.1.2 Prevention and treatment of infant disorders

There is solid evidence that infant formula containing some prebiotics is associated with less-consistent feces and a higher frequency of defecation [61]. However, inconsistent evidence has been obtained on the association between prebiotics and the frequency of defecations [32, 62].

The use of prebiotic-fortified formulae has been associated with a lower risk for intestinal and respiratory infections [63, 64] and an increase in fecal secretory IgA levels [65]. By contrast, they have not been proven to exert any effects on humoral and cellular immunity [66]. In general terms, there is no conclusive evidence supporting that supplementation of infant formulae with prebiotics exerts any protective effects against infections, colic, crying/irritability, regurgitation, or vomiting [19, 32]. Fortification with 2′-flucosyllactose does seem to improve infant immunity, as it has been reported to be related to a lower incidence of infections, especially respiratory infections [60].

GOS:FOS mixtures favor the growth of bifidobacteria and lactobacilli in the feces of infants receiving fortified formulae. However, they have a limited effect on the reduction of pathogenic bacteria [19]. Yet, some studies suggest that prebiotics reduce pathogenic micro-organism concentrations, while the infant is receiving a formula supplemented with oligosaccharides [67]. A number of studies [25, 32, 62] have failed to demonstrate that bifidobacteria, lactobacilli, or pathogen count decreases with prebiotics.

Other studies have shown similarities between the bifidogenic effect of prebiotic-fortified formulae and HBM, as compared to non-fortified formulae [59, 68, 69]. Indeed, prebiotics have been reported to have special effects on some bifidobacteria species such as *Bifidobacterium breve*. Thus, fecal *Bifidobacterium breve* concentrations in infants fed with a fortified formula have been documented to be similar to those found in breastfed infants.

Although prebiotic-supplemented formulae are thought to prevent eczema in infants at high risk of developing allergies [43, 63, 70], there is no sufficient evidence on the role that prebiotics play in the prevention of eczema, atopic dermatitis, or food hypersensitivity [71, 72]. A partially hydrolyzed formula containing specific prebiotics has been reported to generate a gut microbiota similar to that of breastfed infants. A potential link between microbial activity and eczema onset was identified, which could suggest a suboptimal implementation of gut microbiota in specific developmental stages of infants at high risk of developing allergy [73].

4.1.3 Conclusion

ESPGHAN Committee on Nutrition does not recommend routine use of infant formulae supplemented with prebiotic. In agreement with the American Academy of Pediatrics, they recommend that further studies are conducted to assess the safety and efficacy of prebiotic supplementation.

5. Symbiotics

Symbiotics are mixtures of probiotics and prebiotics that beneficially affect the host by improving the survival and implantation of the probiotic bacteria and stimulate the activity of the host's endogenous bacteria [56]. Symbiotics are believed to act synergistically to increase the overall gut health by offering more benefits than the use of either a probiotic or prebiotic agent alone. Considering a huge number of possible combinations, the application of symbiotics for the modulation of intestinal microbiota in humans seems promising [74]. A disadvantage to using symbiotics is that it is difficult to predict the selectivity and specificity of each of the components and what the resulting mechanisms of action will be.

Limited data have been provided on concomitant prebiotic and probiotic supplementation of infant formulae. The few studies carried out with symbiotics [19, 32, 75] revealed that symbiotics: (a) do not exert effects on growth; (b) do not reduce the incidence of digestive disorders (colic, regurgitation, crying, vomiting, to name a few) or infections; (c) increase the frequency of daily defecations but do not influence fecal consistency; and (d) no data are available on their effects on the composition of gut microbiota or on immune response.

There is no conclusive evidence on the effects of supplementation of infant formula with symbiotics. Therefore, ESPGHAN Committee on Nutrition does not recommend routine use of infant formula fortified with symbiotics.

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References

[1] Ojo-Okunola A, Nicol M, du Toit E. Human breast milk bacteriome in health and disease. Nutrients. 2018;**10**:1643. DOI: 10.3390/nu10111643

[2] Aakko J, Kumar H, Rautava S, Wise A, Austran C, Bode L, et al. Human milk oligosaccharide categories define the microbiota composition in human calostrum. Beneficial Microbes. 2017;**8**:563-567. DOI: 10.3920/BM2016.0185

[3] Isaacs CE. Human milk inactivates pathogen individually, additively, and synergistically. The Journal of Nutrition. 2005;**51**:1286-1288. DOI: 10.1093/jn/135.5.1286

[4] Wallace TC, Guarner F, Madsen K, Cobano MD, Gibson G, Hentges E, et al. Human gut microbiota and its relationship to health and disease. Nutrition Reviews. 2011;**69**:392-403. DOI: 10.1111/j.1753-4887.2011.00402

[5] Guarner F, Melagelada JR. Gut flora in health and disease. Lancet. 2003;**361**:512-519. DOI: 10.1016/S0140-6736(03)12489-0

[6] López Moriana C, Mach N. Influencia de la gestación, el parto y el tipo de lactancia sobre la microbiota intestinal del neonato. Acta Pediátrica Española. 2014;**72**:37-44

[7] Martín R, Heilig GH, Zoetendal EG, Smidt H, Rodríguez JM. Diversity of the Lactobacillus group in breast milk and vagina of healthy women and potential role in colonization of the infant gut. Journal of Applied Microbiology. 2007;**103**:2638-2644. DOI: 10.1111/j.1365-2672.2007.03497.x

[8] Milani C, Mancabelli L, Lugli GA, Duranti S, Turroni F, Ferrario C, et al. Exploring vertical transmission of bifidobacteria from mother to child. Applied and Environmental Microbiology. 2015;**81**:7078-7087. DOI: 10.1128/AEM.02037-15

[9] Lara-Villoslada F, Olivares M, Sierra S, Rodríguez JM, Boza J, Xaus J. Beneficial effects of probiotic bacteria isolated from breast milk. The British Journal of Nutrition. 2007;**98**(Suppl. 1):S96-S100. DOI: 10.1017/S0007114507832910

[10] Rachid R, Chatila TA. The role of the gut microbiota in food allergy. Current Opinion in Pediatrics. 2016;**28**:748-753. DOI: 10.1097/MOP.0000000000000427

[11] Fallani M, Young D, Scott J, Norin E, Amarri S, Adam R, et al. Intestinal microbiota of 6-week-old infants across Europe: Geographic influence beyond delivery mode, breast-feeding, and antibiotics. Journal of Pediatric Gastroenterology and Nutrition. 2010;**51**:77-84. DOI: 10.1097/MPG.obo13e3181d1b11e

[12] Bezirtzoglou E, Tsiotsias A, Welling GW. Microbiota profile in feces of breast- and formula-fed newborns by using fluorescence in situ hybridization (FISH). Anaerobe. 2011;**17**:478-482. DOI: 10.1016/j.anaerobe.2011.03.009

[13] O'Sullivan A, Farver M, Smilowitz JT. The influence of early infant-feeding practices on the intestinal microbiome and body composition in infants. Nutrition and Metabolic Insights. 2015;**8**:1-9. DOI: 10.4137/NMI.S29530

[14] Rodríguez JM, Jiménez E, Merino V, Maldonado A, Marín ML, Fernández L, et al. Microbiota de la leche humana en condiciones fisiológicas. Acta Pediátrica Española. 2008;**66**:77-82

[15] Olivares M, Díaz-Ropero MP, Martin R, Rodríguez JM, Xaus J. Antimicrobial potential of

four lactobacillus strains isolated from breast milk. Journal of Applied Microbiology. 2006;**101**:72-79. DOI: 10.1111/j.1365-2672.2006.02981.x

[16] Rosenfeldt V, Benfeldt E, Valerius NH, Paerregaard A, Michaelsen KF. Effects of probiotics on gastrointestinal symptoms and small intestinal permeability in children with atopic dermatitis. The Journal of Pediatrics. 2004;**145**:612-616. DOI: 10.1016/j.jpeds.2004.06.068

[17] Prescott SL, Björkstén B. Probiotics for the prevention or treatment of allergy diseases. The Journal of Allergy and Clinical Immunology. 2007;**120**:255-262. DOI: 10.1016/j.jaci.2007.04.027

[18] Bermúdez-Brito M, PlaZA-Díaz J, Muñoz-Quezada S, Gómez-Llorente C, Gil A. Probiotics mechanism of action. Annals of Nutrition & Metabolism. 2012;**61**:160-174. DOI: 10.1159/000342079

[19] Braegger C, Chmielewika A, Decsi T, Kolacek SMihatsch W, Morfeno L, ESPGHAN Committee on Nutrition, et al. Supplementation of infant formula with probiotics and/or prebiotics: A systematic review and comment by the ESPGHAN Committee on Nutrition. Journal of Pediatric Gastroenterology and Nutrition. 2011;**52**:238-250. DOI: 10.1097/MPG.0b013e31817b9e80

[20] Gil-Campos M, López MA, Rodríguez-Benítez MV, Romero J, Roncero I, Linares MD, et al. *Lactobacillus fermentum* CECT 5716 is safe and well tolered in infants of 1-6 months of age: A randomized controlled trial. Pharmacological Research. 2012;**65**:231-238. DOI: 10.1016/j.phrs.2011.11.016

[21] Maldonado-Lobón JA, Gil-Campos M, Maldonado J, López-Huertas E, Flores-Rojas K, Valero AD, et al. Long-term safety of early consumption of *Lactobacillus fermentum* CECT5716: A 3-year follow-up of a randomized controlled trial. Pharmacological Research. 2015;**95-96**:12-19. DOI: 10.1016/j.phrs.2015.01.006

[22] Scalabrin DMF, Harris C, Johnston WH, Bersth CL. Long-term safety assessment in children who received hydrolyzed protein formulas with *Lactobacillus rhamnosus* GG: A 5-year follow-up. European Journal of Pediatrics. 2017;**176**:217-224. DOI: 10.1007/soo431-016-2825-4

[23] Berni Canani R, Di Costanzo M, Bedogni G, Amoroso A, Cosenza L, Di Scala C, et al. Extensively hydrolyzed casein formula containing *Lactobacillus rhamnosus* GG reduces the occurrence of other allergic manifestations in children with cow's milk allergy: 3-year randomized controlled trial. The Journal of Allergy and Clinical Immunology. 2017;**139**:1906-1913. DOI: 10.1111/pai.12687

[24] Xu L, Wang Y, Wang Y, Fu J, Sun M, Mao Z, et al. A double-blinded randomized trial on growth and feeding tolerance with *Saccharomyces boulardii* CNCMI-745 in formula-fed preterm infants. Jornal de Pediatria. 2016;**92**:296-301. DOI: 10.1016/j.jped.2015.08.013

[25] Brunser O, Figueroa G, Gatteland M, Haschke-Becher F, Magliola C, Rochat F, et al. Effects of probiotic or prebiotic supplemented milk formulas on fecal microbiota composition in infants. Asia Pacific Journal of Clinical Nutrition. 2006;**15**:368-376

[26] Langhendries JP, Detry J, van Hees J, Lambray JM, Darimont J, Mozin M, et al. Effect of a fermented infant formula containing viable bifidobacteria on the faecal flora composition and pH of healthy full-term infants. Journal of Pediatric Gastroenterology and Nutrition. 1995;**21**:177-181

[27] Maldonado J, Cañabate F, Sempere L, Vela F, Sánchez AR, Narbona E, et al. Human milk probiotic *Lactobacillus fermentum* CECT 5716 reduces the incidence of gastrointestinal and upper respiratory tract infections in infants. Journal of Pediatric Gastroenterology and Nutrition. 2012;**54**:55-61. DOI: 10.1097/MPG.pbo13e318233f18

[28] Chi C, Xue Y, Liu R, Wang Y, Lv N, Zeng H, et al. Effects of a formula with a probiotic *Bifidobacterium lactis* supplement on the gut microbiota of low birth weight infants. European Journal of Nutrition. 13 Jun 2019. DOI: 10.1007/s00394-019-02006-4

[29] Weizman Z, Asli G, Alsheikh A. Effects of a probiotic infant formula on infections in child care centers; comparison of two probiotic agents. Pediatrics. 2005;**115**:5-9. DOI: 10.1542/peds.2004-1815

[30] Maldonado J, Lara-Villoslada F, Sierra S, Sempere L, Gómez M, Rodríguez JM, et al. Safety and tolerance of the human milk probiotic strain *Lactobacillus salivarius* CECT 5713 in 6-month-old children. Nutrition. 2010;**26**:1082-1087. DOI: 10.1016/j.nut.2009.08.023

[31] Corrêa NB, Péret Filho LA, Penna FJ, Lima FM, Nicoli JR. A randomized formula controlled trial of *Bifidobacterium lactis* and *Streptococcus thermophilus* for prevention of antibiotic-associated diarrhea in infants. Journal of Clinical Gastroenterology. 2005;**39**:385-389. DOI: 10.1097/01.mcg.0000159217.47419.56

[32] Mugambi MN, Musekiwa A, Lombard M, Young T, Blaauw R. Symbiotics, probiotics or prebiotics in infant formula for full term infants: A systematic review. Nutrition Journal. 2012;**11**:81. DOI: 10.1186/1475-2891-11-81

[33] Savino F, Cordisco L, Tarasco V, Palumeri E, Calabrese R, Oggero R, et al. *Lactobacillus reuteri* DSM 17938 in infantile colic: A randomized, double-blind, placebo-controlled trial. Pediatrics. 2010;**126**:e526-e533. DOI: 10.1542/peds.2010-0433

[34] Sung V, Collet S, de Gooyer T, Hiscock H, Tang M, Wake M. Probiotics to prevent or treat excessive infant crying. JAMA Pediatrics. 2013;**167**:1150-1157. DOI: 10.1001/jamapediatrics.2013.2572

[35] Zhou P, Li Y, Ma LY, Lin HC. The role of immunonutrients in the prevention of necrotizing enterocolitis in preterm very low birth weight infants. Nutrients. 2015;**7**:7256-7270. DOI: 10.3390/nu7095334

[36] Dermyshi E, Wang Y, Yan C, Hong W, Qiu G, Gong X, et al. The "golden age" of probiotics: A systematic review and meta-analysis of randomized and observational studies in preterm infants. Neonatology. 2017;**112**:9-23. DOI: 10.1159/000454668

[37] Sun J, Marwah G, Westgarth M, Buys N, Ellwood D, Gray PH. Effects of probiotics on necrotizing enterocolitis, sepsis, intraventricular hemorrhage, mortality, length of hospital stay, and weight gain in very preterm infants: A meta-analysis. Advances in Nutrition. 2017;**8**:749-763. DOI: 10.3945/an.116.014605

[38] Aceti A, Gori D, Barone G, Callegari ML, Fantini MP, Indrio F, et al. Probiotics and time to achieve full enteral feeding in human milk-fed and formula-fed preterm infants: Systematic review and meta-analysis. Nutrients. 2016;**8**:471. DOI: 10.3390/nu8080471

[39] Costeloe K, Bowler U, Brocklehurst P, Hardy P, Heal P, Juszczak E, et al. A randomised controlled trial of the probiotic *Bifidobacterium breve*

BBG-001 in preterm babies to prevent sepsis, necrotising enterocolitis and death: The Probiotics in Preterm infantS (PiPS) trial. Health Technology Assessment. 2016;**20**:1-194. DOI: 10.3310/hta20660

[40] Taipale T, Pienihäkkinen K, Isolauri E, Larsen C, Brockmann E, Alanen P, et al. *Bifidobacterium animalis* subsp. *lactis* BB-12 in reducing the risk of infections in infancy. The British Journal of Nutrition. 2011;**105**:409-416. DOI: 10.1017/S0007114510003685

[41] Rautava S, Salminen S, Isolauri E. Specific probiotics in reducing the risk of acute infections in infant: A randomised, double-blind, placebo-controlled study. The British Journal of Nutrition. 2009;**101**:1722-1726. DOI: 10.1017/S0007114508116282

[42] Olivares M, Díaz-Ropero MP, Sierra S, Lara-Villoslada F, Fonolla J, Navas M, et al. Oral intake of *Lactobacillus fermentum* CECT 5716 enhances the effect of influenza vaccination. Nutrition. 2007;**23**:254-260. DOI: 10.1016/j.nut.2007.01.004

[43] Tang ML, Lahtinen SJ, Boyle RJ. Probiotics and prebiotics: Clinical effects in allergic disease. Current Opinion in Pediatrics. 2010;**22**:626-634. DOI: 10.1097/MOP.0b013e32833d9728

[44] Osborn DA, Sinn JK. Probiotics in infants for prevention of allergic disease and food hypersensitivity. Cochrane Database of Systematic Reviews. 2007;**17**(4):CD006475. DOI: 10.1002/14651858.CD006475.pub2

[45] Pelucchi C, Chatenoud L, Turati F, Galeone C, Moja L, Bach JF, et al. Probiotics supplementation during pregnancy or infancy for the prevention of atopic dermatitis: A meta-analysis. Epidemiology. 2012;**23**:402-414. DOI: 10.1097/EDE.0b013e31824d5da2

[46] Berni Canani R, Nocerino R, Terrin G, Coruzzo A, Cosenza L, Leone L, et al. Effect of *Lactobacillus* GG on tolerance acquisition in infants with cow's milk allergy: A randomized trial. The Journal of Allergy and Clinical Immunology. 2012;**129**:580-582. DOI: 10.1016/j.jaci.2011.10.004

[47] Berni Canani R, Nocerino R, Terrin G, Frediani T, Lucarelli S, Cosenza L, et al. Formula selection for management of children with cow milk allergy influences the rate of acquisition of tolerance: A prospective multicenter study. The Journal of Pediatrics. 2013;**163**:771-777. DOI: 10.1016/j.jpeds.2013.03.008

[48] Thompson-Chagoyan OC, Vieites JM, Maldonado J, Edwards C, Gil A. Changes in faecal microbiota of infants with cow milk protein allergy – a Spanish prospective case-control six-month follow-up study. Pediatric Allergy and Immunology. 2010;**21**:e394-e400. DOI: 10.1111/j.1399-3038.2009.00961.x

[49] Thompson-Chagoyan OC, Fallani M, Maldonado J, Vieites JM, Khanna S, Edwards C, et al. Faecal microbiota and short-chain fatty acid levels in faeces from infants with cow's milk protein allergy. International Archives of Allergy and Immunology. 2011;**156**:325-332. DOI: 10.1159/000323893

[50] Berni Canani R, Sangwan N, Stefka AT, Nocerino R, Paparo L, Aitoro R, et al. *Lactobacillus rhamnosus* GG-supplemented formula expands butyrate-producing bacterial strains in food allergic infants. The ISME Journal. 2016;**10**:742-750. DOI: 10.1038/ismej.2015.151

[51] Isolauri E, Rautava S, Salminen S. Probiotics in the development and treatment of allergic disease. Gastroenterology Clinics of North

America. 2012;**41**:747-762. DOI: 10.1016/j.gtc.2012.08.007

[52] Fiocchi A, Pawankar R, Cuello-García C, Ahn K, Al-Hammadi S, Agarwal A, et al. World Allergy Organization-McMaster University Guidelines for Allergic Disease Prevention (GLAD-P): Probiotics. World Allergy Organization Journal. 2015;**8**:4. DOI: 10.1186/s40413-015-0055-2

[53] Maldonado LJ. Nuevos ingredientes en las fórmulas para lactantes nacidos a término (I): Probióticos, prebióticos y simbióticos. Acta Pediátrica Española. 2014;**72**:56-62

[54] Bertelsen RJ, Jensen ET, Ringel-Kulka T. Use of probiotics and prebiotics in infant feeding. Best Practice & Research. Clinical Gastroenterology. 2016;**30**:39-48. DOI: 10.1016/j.bpg.2016.01.001

[55] European Food Safety Authority (EFSA). EFSA Panel on dietetic products, nutrition and allergies (NDA). Scientific Opinion on the essential composition of infant and follow-on formulae. EFSA Journal. 2014;**12**(7):3760. DOI: 10.29013/j.efsa.2014.3760

[56] de Vrese M, Schrezenmeir J. Probiotics, prebiotics, and synbiotics. Advances in Biochemical Engineering/Biotechnology. 2008;**111**:1-66. DOI: 10.1007/10_2008_097

[57] Garrido D, Kim JH, German JB, Raybould HE, Mills DA. Oligosaccharide binding proteins from *Bifidobacterium longum* subsp. *infantis* reveal a preference for host glycans. PLoS One. 2011;**6**:e17315. DOI: 10.1371/journal.pone.0017315

[58] Thurl S, Munzert M, Boehm G, Matthews C, Stahl B. Systematic review of the concentrations of oligosaccharides in human milk. Nutrition Reviews. 2017;**75**:920-933. DOI: 10.1093/nutrit/nux044

[59] Donovan SM, Comstock SS. Human milk oligosaccharides influence neonatal mucosal and systemic immunity. Annals of Nutrition & Metabolism. 2016;**69**(Suppl. 2):42-51. DOI: 10.1159/000452818

[60] Reverri EJ, Devit AA, Kajzer JA, Baggs GE, Borschel MW. Review of the clinical experience of feeding infant formula containing the human milk oligosaccharide 2′-fucosyllactose. Nutrients. 2018;**10**:1436. DOI: 10.3390/nu10101346

[61] Vandenplas Y, De Greef E, Veeremen G. Prebiotics in infant formula. Gut Microbes. 2014;**5**:681-687. DOI: 10.4161/19490976.2014.972237

[62] Veereman-Wauters G, Staelens S, Van de Broeck H, Plaskic K, Wesling F, Roger LC, et al. Physiological and bifidogenic effects of prebiotic supplements in infancy formulae. Journal of Pediatric Gastroenterology and Nutrition. 2011;**52**:763-771. DOI: 10.1097/MPG.0b013e3182139f39

[63] Arslonoglu S, Moro GE, Schmitt J, Tandoi L, Rizzardi S, Boehm G. Early dietary intervention with a mixture of prebiotic oligosaccharides reduces the incidence of allergic manifestations and infections during the first two years of life. The Journal of Nutrition. 2008;**138**:1091-1095. DOI: 10.1093/jn/138.6.1091

[64] Bruzzese E, Volpicelli M, Squeglia V, Bruzzese D, Salvini F, Brisceglia M, et al. A formula containing galacto- and fructo-oligosaccharides prevents intestinal and extraintestinal infections: An observational study. Clinical Nutrition. 2009;**28**:156-161. DOI: 10.1016/j.clnu.2009.01.008

[65] Scholtens PA, Alliet P, Raes M, Allu MS, Kroes H, Boehm G, et al.

Fecal secretory immunoglobulin A in increased in healthy infants who receive a formula with short-chain galacto-ologosaccharides and long-chain fructo-oligosaccharides. The Journal of Nutrition. 2008;**138**:1141-1147. DOI: 10.1093/jn/138.6.1141

[66] Raes M, Scholtens PA, Allet P, Hemsen K, Jougen H, Boehm G, et al. Exploration of basal immune parameters in healthy infants receiving an infant milk formula supplemented with prebiotics. Pediatric Allergy and Immunology. 2010;**21**(2 Pt 2):e377-e385. DOI: 10.1111/j.1399-3038.2009.00957.x

[67] Knol J, Boehm G, Lidestri M, Negretti F, Jelinek J, Agosti M, et al. Increase of faecal bifidobacteria due to dietary oligosaccharides induces a reduction of clinically relevant pathogen germs in the faeces of formula-fed infants. Acta Paediatrica. 2005;**94**:S31-S33. DOI: 10.1111/j.1651-2227.2005.tb02152.x

[68] Sierra C, Bernal MJ, Blasco J, Martínez R, Dalmau J, Ortuño I, et al. Prebiotic effect during the first year of life in healthy infants fed formula containing GOS as the only prebiotic: A multicenter, randomized, double-blind and placebo-controlled trial. European Journal of Nutrition. 2015;**54**:89-99. DOI: 10.1007/s00384-014-689-9

[69] Borewicz K, Suarez-Diez M, Hechler C, Beijers R, de Weerth C, Arts I, et al. The effect of prebiotic fortified infant formulas on microbiota composition and dynamics in early life. Scientific Reports. 2434;**2019**:9. DOI: 10.1038/s41598-018-38268.x

[70] Moro G, Arslonoglu S, Stahl B, Jalinek WH, Boehm G. A mixture of prebiotic oligosaccharides during the first six months of age. Archives of Disease in Childhood. 2006;**91**:814-819. DOI: 10.1136/adc.2006.098251

[71] Osborn DA, Sinn JK. Prebiotics in infants for prevention of allergy disease and food hypersensitivity. Cochrane Database of Systematic Reviews. 2007;**17**(4):CD006474. DOI: 10.1002/14651858.CD006474.pub2

[72] Bozensky J, Hill M, Zelenka R, Skyba T. Prebiotics do not influence the severity of atopic dermatitis in infants: A randomized controlled trial. PLoS One. 2015;**10**(11):e0142897. DOI: 10.1371/journal.pone.0142697. eCollection2015

[73] Wopereis H, Sim K, Shaw A, Warner JO, Knol J, Kroll JS. Intestinal microbiota in infants at high risk for allergy: Effects of prebiotics and role in eczema development. The Journal of Allergy and Clinical Immunology. 2018;**141**:1334-1342. DOI: 10.1016/j.jaci.2017.05.054

[74] Markowiak P, Śliżewska K. Effects of probiotics, prebiotics, and synbiotics on human health. Nutrients. 2017;**9**(9):1021. DOI: 10.3390/nu9091021

[75] Radke M, Picaud JC, Loui A, Cambonie G, Faas D, Lafeber HN, et al. Starter formula enriched in prebiotics and probiotics ensures normal growth of infants and promotes gut health: A randomized clinical trial. Pediatric Research. 2017;**81**:622-631. DOI: 10.1036/pr.2016.270