Probiotics and prebiotics in infant formula

Probiotiki in prebiotiki v mlečnih formulah za dojenčke

Yvan Vandenplas,[1] Abdallah Ghanma,[1,2] Johan Franckx,[3] Stefaan Peeters,[4] Michel Pletincx,[5] Bruno Hauser[1]

[1] *Pediatric Gastroenterology, UZ Brussel, Vrije Universiteit Brussel, Brussels, Belgium*

[2] *King Hussein Medical Center, Amman, Jordan*

[3] *Pediatrie, Campus Asse, Onze Lieve Vrouw Ziekenhuis, Aalst, Belgium*

[4] *Pediatrie, Aalsters Stedelijk Ziekenhuis, Aalst, Belgium*

[5] *Service de Pédiatrie, Clinique Saint Anne Saint Remy, Brussels, Belgium*

Korespondenca/ Correspondence:
Y. Vandenplas, UZ Brussel, Vrije Universiteit Brussel, Laarbeeklaan 101, 1090 Brussels, Belgium.
Tel: + 32.2.477.57.80; Fax: + 32.2.477.57.83; e-mail: yvanvandenplas@uzbrussel.be

Ključne besede:
hranjenje dojenčkov, formula za dojenčke, Lactobacillus, prebiotik, probiotik

Key words:
infant feeding, infant formula, lactobacillus, prebiotic, probiotic

Citirajte kot/Cite as:
Zdrav Vestn 2013; 82 supl 1: I-70–6

Abstract

The gastrointestinal flora of breast-fed infants and those on classic standard infant formula differs. While mother's milk is rich in prebiotic oligosaccharides and also contains some probiotics, a standard infant formula contains none of both.

There is evidence that addition of pro- and prebiotics bring the gastrointestinal (GI) flora composition of formula-fed infants closer to that of breastfed infants. However, there is only limited evidence that these changes in GI flora induce a health benefit. Almost no adverse effects have been shown.

Pre- and probiotics are added to infant formula because they are present in mother's milk, and since the risk for adverse events seems minimal to non-existent. The evidence of a relevant clinical benefit is limited. However, since most studies suggest a trend for beneficial effects (which differs from study to study and which is most of the time insignificant) and since these ingredients are very safe, most infant formula companies do add prebiotics or probiotics, or both, to infant formula.

Izvleček

Dojeni dojenčki imajo drugačno črevesno floro kot tisti, ki so hranjeni z mlečno formulo. Medtem ko je materino mleko bogato s prebiotičnimi oligosaharidi in vsebuje tudi probiotike, jih običajne mlečne formule nimajo.

Dokazano je, da dodatek pre- in probiotikov povzroči, da se črevesna flora dojenčkov, hranjenih z mlečnimi formulami, približa flori, ki jo imajo dojeni otroci. Vendar so dokazi, da so te spremembe tudi koristne za zdravje, omejeni. Stranskih učinkov praktično nimajo.

Pre- in probiotike dodajajo mlečnim formulam, ker so prisotni tudi v materinem mleku in ker je tveganje za stranske učinke zelo majhno ali ga celo ni. Dokazi za pomemben klinični učinek so omejeni. Vendar kaže večina raziskav trend koristnih učinkov (kar se sicer razlikuje med raziskavami in večinoma ni statistično pomembno) in ker so tovrstni dodatki zelo varni, večina podjetij dodaja prebiotike ali probiotike oz. kar oboje v mlečne formule za dojenčke.

Introduction

The gastro-intestinal (GI) flora can be considered an organ within an organ contributing to host nutrition, developmental regulation of intestinal angiogenesis, protection from pathogens and development of the immune response.[1] It is well known that GI flora develops differently in breastfed and formula-fed infants.[2] There are many aspects in the composition of mother's milk and cow's milk that contribute to these differences, such as the carbohydrate, protein, iron and phosphorus content. Prebiotic oligosaccharides are the third most prevalent component in mother's milk, and they are virtually absent in cow's milk. Prebiotic oligosaccharides are "bifidogenic".

Some selected probiotic strains are present in mother's milk in small amounts. Probiotics are non-pathogenic live micro-organisms that, when consumed in adequate amounts, have a positive effect on the health of the host. There are minimum require-

Prispelo: 16. maj 2013,
Sprejeto: 6. jun. 2013

ments for a status of "probiotic microorganism" which include:[3] i) the assessment of strain identity (genus, species, strain level); ii) *in vitro* tests to screen potential probiotic strain's activity; iii) assessment of safety and *in vivo* studies for substantiation of health effects in the target host. Probiotics are considered to be safe in ambulatory care in non-immunocompromised patients.

Only these commercialized products for which convincing data are available can be recommended for medical use. Since some commercialized products are combinations of different strains, clinical testing of each product or infant formula is mandatory. Moreover, dosage and duration of administration should be taken into account as different doses and different durations may have opposite effects.[4]

Prebiotics are non-digestible food ingredients that stimulate the growth and/or activity of bacteria in the digestive system in ways claimed to be beneficial to health. In infant formula, mostly galacto-oligosaccharides (GOS) and/or fructo-oligosaccharides (FOS) are used. The majority of research done on prebiotics is based on full-spectrum prebiotics.

A major shortcoming of infant formula studies is their different definitions of outcomes.[5] There is a need for well-designed and carefully conducted randomized controlled trials, with relevant inclusion/exclusion criteria and adequate sample sizes.[5] These studies should use validated clinical outcome measures.[5]

Probiotics or prebiotics?

The addition of prebiotics is intended to result in a bifidogenic effect on the GI flora of the host. This effect is per definition global. However, not all prebiotics are the same. Short-chain prebiotics are mainly fermented in the caecum and colon ascendens. Long chain oligosaccharides are fermented in the entire colon. As a consequence, prebiotics change the GI flora composition. Probiotics, on the contrary, are specific strains that are additional to the GI flora of the host.

Alterations of the maternal GIT microbiota composition via supplementation with probiotics and prebiotics have been shown; however, transfer of these benefits to the offspring remains to be demonstrated.[6] This review focuses on the influence of maternal GIT microbiota during the pre- and postpartum periods on the colonization of the infant GIT.[6] Not only pre- and probiotics determine the GI flora composition, but. Lipids (β-palmitate) do too.[7] Protein, as well as the source (whey, alpha-lactalbumin) quantity, lactose, low phosphor and iron have a bifidogenic effect.

There are a number of studies on the effect of probiotics added to infant formula. They do not lower the incidence of diarrhoea, colic, spitting up / regurgitation, crying, restlessness or vomiting.[8] Probiotics in formula also fail to have any significant effect on growth, stool frequency or consistency.

Prebiotics in formula increase weight gain but had no impact on length or head circumference gain.[8] Prebiotics increase stool frequency but have no impact on stool consistency, the incidence of colic, spitting up / regurgitation, crying, restlessness or vomiting.[8] The quality of evidence is compromised by imprecision, inconsistency of results, use of different study designs and publication bias.[8] As a consequence, the evidence for benefit of synbiotics added to infant formula is equally limited.

Necrotizing enterocolitis (NEC)

NEC is a severe condition occurring especially in preterm babies. Abnormal gastrointestinal flora development has been hypothesized as one of the possible etiologic factors. The first publication reporting that L. acidophilus and B. infantis reduced NEC dates back to 1999.[9] Shortly afterwards, oligofructose was not shown to decrease NEC,[10] which was followed by a negative study showing that seven days of L. GG supplementation starting with the first feed was not effective in reducing the incidence of urinary tract infection, NEC and sepsis in preterm infants.[11] Then, several randomized trials with different lactobacilli and bifidobacteria showed a significant reduction in the development of NEC.[12,13] Although *S. boulardii* was shown to ameliorate hypoxia/

reoxygenation-induced NECs in young mice,[14] it did not protect against NEC in infants.[15] A Cochrane review in 2008 concluded that enteral probiotic supplementation reduced the incidence of NEC Stage II or more and mortality rates.[16] No systemic infections or serious adverse events were directly attributed to the administered probiotic microorganism.[16] According to the published trials, the number need to treat or to prevent one case of NEC is 21 and 27 respectively.[16] However, the centers in which these trials have been performed have a much higher incidence of NEC than most European or North American centers. The recommendation may be different in centers with a high incidence of NEC in which the other measures to decrease NEC are difficult to apply. The updated Cochrane review from 2011 comes to different conclusions: enteral supplementation of probiotics prevents severe NEC and reduces all-cause mortality in preterm infants.[17] The updated review of the available evidence supports a change in practice. More studies are needed to assess efficacy in ELBW infants and assess the most effective formulation and dose to be utilized.[17] The debate whether probiotics should be given to preterms routinely or not is still going on. The American Pediatric Surgical Association Outcomes and Clinical Trials Committee systematic review concluded in 2012 acknowledges that recent Cochrane reviews support the use of prophylactic probiotics in preterm infants weighing less than 2500 grams to reduce the incidence of NEC, as well as the use of human breast milk rather than formula when possible. There is no clear evidence to support delayed initiation or slow advancement of feeds.[18] However, an expert group of nutritionists and neonatologists concluded that there is insufficient evidence to recommend routine use of probiotics to decrease NEC.[19] According to this group, there is encouraging data, which justifies further investigations regarding the efficacy and safety of specific probiotics in the circumstances of a high local incidence of severe NEC.[19] According to others, the available evidence is still too limited to recommend probiotics to reduc NEC.[20] Other experts suggest that it may be unethical not to give probiotics to preterm babies to decrease NEC.[21]

Allergy and atopic dermatitis

The use of GOS/FOS in dietary products was shown to possibly provide an opportunity to stimulate the adaptive immune response in a Th1-direction and subsequently inhibit infections and Th2-related immune disorders in humans, eg. allergies.[22] Prebiotics were shown to reduce immunoglobulin--free light-chain concentrations in infants at risk for allergy.[23] Simultaneous pro- and prebiotic treatment (a mixture of 4 strains and GOS) given to pregnant women during 2–4 weeks before delivery and to infants during 6 months compared with placebo showed no effect on the cumulative incidence of allergic diseases at the age of 2 years but tended to reduce IgE-associated (atopic) diseases since a significant reduction of (atopic) eczema was noticed.[24] However, Taylor and coworkers challenge the role of probiotics in allergy prevention since they recorded that early probiotic supplementation with L acidophilus did not reduce the risk of AD in high-risk infants and was even associated with increased allergen sensitization in infants receiving supplements.[25] A Cochrane review from 2007 concluded that there was insufficient evidence to recommend the addition of probiotics to infant feeds for prevention of allergic disease or food hypersensitivity.[26] Although there was a reduction in clinical eczema in infants, this effect was not consistent between studies and caution was advised in view of methodological concerns regarding the studies included.[26] However, the efficacy of probiotic intervention to reduce atopic dermatitis and/or allergic disease may depend on the moment of intervention. Preventive administration of probiotics may only be effective if given during pregnancy. Probiotics given to nonselected mothers reduced the cumulative incidence of AD, but had no effect on atopic sensitization.[27] A recent meta-analysis showed that the administration of lactobacilli during pregnancy prevented atopic eczema in children aged 2 to 7 years.[28] However, a mixture of various bacterial strains does not affect the deve-

lopment of atopic eczema, independent of whether they contain lactobacilli or not.[28] Lactobacillus rhamnosus HN001 was reported effective against eczema in the first 2 years of life, the effect persisting upto 4 years of age, while Bifidobacterium animalis subsp lactis HN019 had no effect.[29] Therefore, not only timing of administration but also strain specificity seems to be important. However, timing of administration and strain specificity were then again contradicted in the meta-analysis by Pelucchi and coworkers, being in support of a moderate role of probiotics in the prevention of atopic dermatitis and IgE-associated atopic dermatitis in infants, but regardless of the time of probiotic use (pregnancy or early life) or the subject(s) receiving probiotics (mother, child, or both).[30] Being somewhat contradictory, the data on probiotics and allergy require further clarification. It might be that geographical or genetic differences play a detrimental role, especially for atopic dermatitis.

In a large study of 259 high risk infants, prebiotic supplements to formula feeding reduced the development of atopic dermatitis at the age of 6 months.[31] However, systematic reviews failed to prove the efficiency of prebiotics in atopic dermatitis prevention.[32]

Recently, in a double-blind, placebo--controlled multi-centre trial, 90 infants with atopic dermatitis, aged < 7 months, were randomized to receive an infant formula with B. breve M-16V and a mixture of short-chain GOS and long-chain FOS, or the same formula without synbiotics during 12 weeks.[33] There were no significant differences between the synbiotic and the placebo group.[33] The same group showed that synbiotics prevent asthma-like symptoms in infants with atopic dermatitis.[34] At the same time, another group reported that a synbiotic combination of L. salivarius plus FOS is superior to the prebiotic alone for treating moderate to severe childhood AD.[35]

While some studies with probiotics as a treatment for atopic dermatitis show a benefit,[36] most studies are negative.

Colic

Colic is a frequent problem in infants and often parents are desperate for a solution. In this indication, the effect of L. reuteri has been exhaustively studied in breastfed infants.[39-41] However, there are no data with L. reuteri in formula fed babies. Dupont et al. reported efficacy of another probiotic strain in formula fed infants.[42] There are very limited data with prebiotics in the prevention or treatment of colic. One study suggests oligosaccharides may contribute to an improved intestinal comfort.[43]

Safety and side effects

Probiotics have a long record of safety, which relates primarily to lactobacilli and bifidobacteria.[44] Experience with other forms of probiotic is more limited. There is no such thing as zero risk, particularly in the context of certain forms of host susceptibility.[44] Probiotics are "generally regarded as safe" and side-effects in ambulatory care have almost not been reported. Large-scale epidemiological studies in countries where probiotic use is endemic demonstrate (in adults) low rates of systemic infection, between 0.05 and 0.40 %.[45] Administration during pregnancy and early infancy is considered safe.[46] Probiotic compounds may contain hidden allergens of food and may not be safe for subjects with allergy to cow's milk or hen's eggs.[47] Documented invasive infections have been primarily noted to occur in immuno-compromised adults.[48] Invasive infections in infants and children are extremely rare.[48-50] Two cases of bacteriemia attributable to Lactobacillus supplementation, with identical molecular clinical and supplement isolates, were recently reported in an infant and a child without underlying gastrointestinal disease or immuno-compromised status.[51] Sepsis with probiotic lactobacilli has been reported in children with short gut. Recently, the occurrence of plasmid transfer of antibiotic resistance has been shown to be clinically possible. Long-term use of probiotics under antibiotic selection pressure could cause antibiotic resistance, and the resistance gene could be transferred to other bac-

teria.[52] Translocation from the gastro-intestinal tract into the systemic circulation has not been reported. There is poor public understanding of the concept of risk, in general, and risk/benefit analysis, in particular.[44] Uncertainty persists regarding the potential for transfer of antibiotic resistance with probiotics, but the risk seems to be low with currently available probiotic products.[44] As with other forms of therapeutics, the safety of probiotics should be considered on a strain-by-strain basis.[44] The potential benefits of supplementation should be weighed against the risk of development of an invasive infection resulting from probiotic therapy.

Conclusion

There is not enough evidence to state that supplementation of term infant formula with synbiotics, probiotics or prebiotics does result in relevant clinical benefit.[8] However, many studies show some advantages. Since pro- and prebiotics are present in mothers' milk, and since their addition to infant formula is safe, there seems to be no reason to not add them in infant formula, although–as said–the evidence for a benefit of doing so is limited. Future research has to focus on specificity, safety, dosage, and combinations.

References

1. Johnson CL, Versalovic J. The human microbiome and its potential importance to pediatrics. Pediatrics 2012, 129: 950–960.
2. Harmsen HJ, Wildeboer-Veloo AC, Raangs GC, Wagendorp AA, Klijn N, Bindels JG, Welling GW. Analysis of intestinal flora development in breast-fed and formula-fed infants by using molecular identification and detection methods. J Pediatr Gastroenterol Nutr. 2000 ; 30: 61–7
3. Reid G. Microbiology: Categorize probiotics to speed research. Nature 2012, 485: 446.
4. Ritchie ML, Romanuk TN. A meta-analysis of probiotic efficacy for gastrointestinal diseases. Plos One 2012, 7:e34938.
5. Szajewska H. Supplementation of infant formula with probiotics/prebiotics: lessons learned with regard to documenting outcomes. J Clin Gastroenterol 2012; 46 Suppl:S67–8.
6. Thum C, Cookson AL, Otter DE, McNabb WC, Hodgkinson AJ, Dyer J, Roy NC. Can nutritional modulation of maternal intestinal microbiota influence the development of the infant gastrointestinal tract? J Nutr. 2012; 142: 1921–8.
7. Yaron S, Shachar D, Abramas L, Riskin A, Bader D, Litmanovitz I, Bar-Yoseph F, Cohen T, Levi L, Lifshitz Y, Shamir R, Shaoul R. Effect of high β-palmitate content in infant formula on the intestinal microbiota of term infants. J Pediatr Gastroenterol Nutr. 2013; 56: 376–81
8. Mugambi MN, Musekiwa A, Lombard M, Young T, Blaauw R. Synbiotics, probiotics or prebiotics in infant formula for full term infants: a systematic review. Nutr J. 2012; 11: 81
9. Hoyos AB. Reduced incidence of necrotizing enterocolitis associated with enteral administration of Lactobacillus acidophilus and Bifidobacterium infantis to neonates in an intensive care unit. Int J Infect Dis 1999, 3: 197–202.
10. Butel MJ, Waligora-Dupriet AJ, Szylit O. Oligofructose and experimental model of neonatal necrotising enterocolitis. Br J Nutr 2002, 87 Suppl 2:S213–219.
11. Dani C, Biadaioli R, Bertini G, Martelli E, Rubaltelli FF. Probiotics feeding in prevention of urinary tract infection, bacterial sepsis and necrotizing enterocolitis in preterm infants. A prospective double-blind study. Biol Neonate 2002, 82: 103–108.
12. Lin HC, Su BH, Chen AC, Lin TW, Tsai CH, Yeh TF, Oh W. Oral probiotics reduce the incidence and severity of necrotizing enterocolitis in very low birth weight infants. Pediatrics 2005, 115: 1–4.
13. Bin-Nun A, Bromiker R, Wilschanski M, Kaplan M, Rudensky B, Caplan M, Hammerman C. Oral probiotics prevent necrotizing enterocolitis in very low birth weight neonates. J Pediatr 2005, 147: 192–196.
14. Akisu M, Baka M, Yalaz M, Huseyinov A, Kultursay N. Supplementation with Saccharomyces boulardii ameliorates hypoxia/reoxygenation-induced necrotizing enterocolitis in young mice. Eur J Pediatr Surg 2003, 13: 319–323.
15. Costalos C, Skouteri V, Gounaris A, Sevastiadou S, Triandafilidou A, Ekonomidou C, Kontaxaki F, Petrochilou V. Enteral feeding of premature infants with Saccharomyces boulardii. Early Hum Dev 2003, 74: 89–96.
16. Alfaleh K, Bassler D. Probiotics for prevention of necrotizing enterocolitis in preterm infants. Cochrane Database Syst Rev 2008, (1):CD005496
17. Alfaleh K, Anabrees J, Bassler D, Al-Kharfi T. Probiotics for prevention of necrotizing enterocolitis in preterm infants. Cochrane Database Syst Rev 2011, (3):CD005496.
18. Downard CD, Renaud E, St Peter SD, Abdullah F, Islam S, Saito JM, Blakely ML, Huang EY, Arca MJ, Cassidy L, Aspelund G; For the 2012 American Pediatric Surgical Association Outcomes Clinical Trials Committee. Treatment of necrotizing enterocolitis: an American Pediatric Surgical Association Outcomes and Clinical Trials Committee systematic review. J Pediatr Surg 2012, 47: 2111–2122.
19. Mihatsch WA, Braegger CP, Decsi T, Kolacek S, Lanzinger H, Mayer B, Moreno LA, Pohlandt F, Puntis J, Shamir R, Stadtmüller U, Szajewska H, Turck D, van Goudoever JB. Critical systematic review of the level of evidence for routine use of probiotics for reduction of mortality and preven-

tion of necrotizing enterocolitis and sepsis in preterm infants. Clin Nutr 2012, 31: 6–15.
20. Fallon EM, Nehra D, Potemkin AK, Gura KM, Simpser E, Compher C; American Society for Parenteral and Enteral Nutrition (A.S.P.E.N.) Board of Directors, Puder M. A.S.P.E.N. clinical guidelines: nutrition support of neonatal patients at risk for necrotizing enterocolitis. JPEN J Parenter Enteral Nutr. 2012; 36: 506–23.
21. Janvier A, Lantos J, Barrington K. The Politics of Probiotics: Probiotics, necrotizing enterocolitis, and the ethics of neonatal research. Acta Paediatr 2012 (in press)
22. Vos AP, Haarman M, Buco A, Govers M, Knol J, Garssen J, Stahl B, Boehm G, M'Rabet L. A specific prebiotic oligosaccharide mixture stimulates delayed-type hypersensitivity in a murine influenza vaccination model. Int Immunopharmaco. 2006, 6: 1277–1286.
23. Schouten B, Van Esch BC, Kormelink TG, Moro GE, Arslanoglu S, Boehm G, Knippels LM, Redegeld FA, Willemsen LE, Garssen J. Non-digestible oligosaccharides reduce immunoglobulin free light-chain concentrations in infants at risk for allergy. Pediatr Allergy Immunol 2011, 22: 537–542.
24. Kukkonen K, Savilahti E, Haahtela T, Juntunen-Backman K, Korpela R, Poussa T, Tuure T, Kuitunen M. Long-term safety and impact on infection rates of postnatal probiotic and prebiotic (synbiotic) treatment: randomized, double-blind, placebo-controlled trial. Pediatrics 2008, 122: 8–12.
25. Taylor AL, Dunstan JA, Prescott SL. Probiotic supplementation for the first 6 months of life fails to reduce the risk of atopic dermatitis and increases the risk of allergen sensitization in high-risk children: a randomized controlled trial. J Allergy Clin Immuno. 2007, 119: 184–191.
26. Osborn DA, Sinn JK. Probiotics in infants for prevention of allergic disease and food hypersensitivity. Cochrane Database Syst Rev 2007, (4):CD006475.
27. Dotterud CK, Storrø O, Johnsen R, Oien T. Probiotics in pregnant women to prevent allergic disease: a randomized, double-blind trial. Br J Dermatol 2010, 163: 616–23.
28. Doege K, Grajecki D, Zyriax BC, Detinkina E, Zu Eulenburg C, Buhling KJ. Impact of maternal supplementation with probiotics during pregnancy on atopic eczema in childhood—a meta-analysis. Br J Nutr 2012, 107: 1–6.
29. Wickens K, Black P, Stanley TV, Mitchell E, Barthow C, Fitzharris P, Purdie G, Crane J. A protective effect of Lactobacillus rhamnosus HN001 against eczema in the first 2 years of life persists to age 4 years. Clin Exp Allergy 2012, 42: 1071–1079.
30. Pelucchi C, Chatenoud L, Turati F, Galeone C, Moja L, Bach JF, La Vecchia C. Probiotics supplementation during pregnancy or infancy for the prevention of atopic dermatitis: a meta-analysis. Epidemiology 2012, 23: 402–414.
31. Moro G, Arslanoglu S, Stahl B, Jelinek J, Wahn U, Boehm G. A mixture of prebiotic oligosaccharides reduces the incidence of atopic dermatitis during the first six months of age. Arch Dis Child 2006, 91: 814–819.
32. Williams HC, Grindlay DJ. What's new in atopic eczema? An analysis of systematic reviews published in 2007 and 2008. Part 2. Disease prevention and treatment. Clin Exp Dermatol 2010, 35: 223–227.
33. van der Aa LB, Lutter R, Heymans HS, Smids BS, Dekker T, van Aalderen WM, Sillevis Smitt JH, Knippels LM, Garssen J, Nauta AJ, Sprikkelman AB; Synbad Study Group. No detectable beneficial systemic immunomodulatory effects of a specific synbiotic mixture in infants with atopic dermatitis. Clin Exp Allergy 2012, 42: 531–539.
34. van der Aa LB, van Aalderen WM, Heymans HS, Henk Sillevis Smitt J, Nauta AJ, Knippels LM, Ben Amor K, Sprikkelman AB; Synbad Study Group. Synbiotics prevent asthma-like symptoms in infants with atopic dermatitis. Allergy 2011, 66: 170–177
35. Wu KG, Li TH, Peng HJ. Lactobacillus salivarius plus fructo-oligosaccharide is superior to fructo-oligosaccharide alone for treating children with moderate to severe atopic dermatitis: a double-blind, randomized, clinical trial of efficacy and safety. Br J Dermatol 2012, 166: 129–136.
36. Han Y, Kim B, Ban J, Lee J, Kim BJ, Choi BS, Hwang S, Ahn K, Kim J. A randomized trial of Lactobacillus plantarum CJLP133 for the treatment of atopic dermatitis. Pediatr Allergy Immunol 2012, 23: 667–673.
37. Savino F, Pelle E, Palumeri E, Oggero R, Miniero R. Lactobacillus reuteri (American Type Culture Collection Strain 55730) versus simethicone in the treatment of infantile colic: a prospective randomized study. Pediatrics 2007, 119:e124–130.
38. Savino F, Cordisco L, Tarasco V, Palumeri E, Calabrese R, Oggero R, Roos S, Matteuzzi D. Lactobacillus reuteri DSM 17938 in infantile colic: a randomized, double-blind, placebo-controlled trial. Pediatrics 2010, 126:e526–533.
39. Szajewska H, Gyrczuk E, Horvath A. Lactobacillus reuteri DSM 17938 for the management of infantile colic in breastfed infants: a randomized, double-blind, placebo-controlled trial. J Pediatr 2012 (in press)
40. Dupont C, Rivero M, Grillon C, Belaroussi N, Kalindjian A, Marin V. Alpha-lactalbumin-enriched and probiotic-supplemented infant formula in infants with colic: growth and gastrointestinal tolerance. Eur J Clin Nutr 2010, 64: 765–767.
41. Vivatvakin B, Mahayosnond A, Theamboonlers A, Steenhout PG, Conus NJ. Effect of a whey-predominant starter formula containing LCPUFAs and oligosaccharides (FOS/GOS) on gastrointestinal comfort in infants. Asia Pac J Clin Nutr 2010, 19: 473–480.
42. Shanahan F. A commentary on the safety of probiotics. Gastroenterol Clin North Am 2012, 41: 869–876.
43. Fedorak RN, Madsen KI. Probiotics and prebiotics in gastrointestinal disorders. Curr Opin Gastroenterol 2004, 20: 146–155.
44. Allen SJ, Jordan S, Storey M, Thornton CA, Gravenor M, Garaiova I, Plummer SF, Wang D, Morgan G. Dietary supplementation with lactobacilli and bifidobacteria is well tolerated and not associated with adverse events during late pregnancy and early infancy. J Nutr 2010, 140: 483–488.
45. Martín-Muñoz MF, Fortuni M, Caminoa M, Belver T, Quirce S, Caballero T. Anaphylactic reac-

tion to probiotics. Cow's milk and hen's egg allergens in probiotic compounds. Pediatr Allergy Immunol 2012, 23: 778–784.
46. Borriello SP, Hammes WP, Holzapfel W, Marteau P, Schrezenmeir J, Vaara M, Valtonen V. Safety of probiotics that contain Lactobacilli or Bifidobacteria. Clin Infect Dis 2003, 36: 775–780.
47. Mackay AD, Taylor MB, Kibbler CC, Hamilton-Miller JM. Lactobacillus endocarditis caused by a probiotics organism. Clin Microbiol Infect 1999, 5: 290–292.
48. Rautio M, Jousimies-Somer H, Kauma H, Pietarinen I, Saxelin M, Tynkkynen S, Koskela M. Liver abcess due to a Lactobacillus rhamnosus strain indistinguishable from a L. rhamnosus strain GG. Clin Infect Dis 1999, 28: 1159–1160.
49. Cabana MD, Shane AL, Chao C, Oliva-Henker M. Probiotics in primary care pediatrics. Clin Pediatr 2006, 45: 405–410.
50. Dai M, Lu J, Wang Y, Liu Z, Yuan Z. In vitro development and transfer of resistance to chlortetracycline in Bacillus subtilis. J Microbiol 2012, 50: 807–812.