PROBIOTICS & INFANT NUTRITION

Little babies, big claims

by Naomi Pocrnic, PhD;
Mal Evans PhD;
Najla Guthrie, CEO, KGK Science

Breast milk is the optimal nutrition source for infants and exclusive breastfeeding from birth to 4-6 months is encouraged by pediatric societies worldwide. Infant formula plays a positive role in providing adequate nutrition when breastfeeding is not possible, or mothers have elected not to do so. Parents' selection of infant formulas is driven by stronger awareness that infancy is the most critical window of opportunity to shape a child's long-term health outcomes. The plethora of infant nutrition products available results in mounting concern for selecting the "right" product. Which formulation will provide targeted health benefits such as cognitive, bone, immune or digestive health? Furthermore, parents are becoming increasingly aware of the health benefits of probiotics. How can I give my baby probiotics? What makes one probiotic better than others?

Worldwide probiotic consumption is on the rise; with the global probiotic market projected to reach $74.7 billion over the next decade (1). Probiotic consumption via food is the largest piece of the proverbial pie at 70%, however the fastest growth in the probiotic market is seen in supplements (2). According to Lux Research, infant formula containing probiotics is projected to attain a market size of $17.3 billion by 2024 (3).Infant probiotic products are a unique and relatively untapped domain within this industry, paving the way for exciting opportunities in product development. The market for infant formula has expanded over the past decade and competition is high amongst leading manufacturers to make claims that differentiate their products from others. Ingredients like probiotics that were previously considered components of specialty formulas are now included in commercially-available formulations and the need for well-substantiated claims is vital. This white paper will examine the regulatory climate, the evidence base and current claims surrounding probiotics in infant formula and present the gaps in knowledge for probiotic supplementation in infants.

> "We have to make sure that what we are doing in that first year of life is beneficial down the road."
>
> — *Gregor Reid, Canadian Research and Development Centre for Probiotics*

Regulatory Bodies: North America and Europe

It is important to first understand that claims are evaluated against how they may influence breastfeeding practices, a viewpoint on which all international pediatric societies are aligned in promoting breastfeeding as the gold standard of infant nutrition. Any claim that implicitly or explicitly suggests a product is superior or equivalent to breast milk violates the 1981 World Health Organization's International Code of Marketing of Breast-Milk Substitutes (4). Furthermore, marketing claims that are perceived to promote early weaning or interfere with breastfeeding are also not permitted. Many developed nations adhere to this code, however according to the latest update of UNICEF's "state of the code by country", the United States has taken no official action to implement the code (5). National and international standards render the infant food product sector one of the most rigorously regulated food sectors in the world. Claims on probiotic products are under a great deal of scrutiny to ensure benefits are not overstated as the "newborn" is an especially vulnerable stage where critical growth and developmental processes are occurring.

> "While we have come to understand that companies manipulate consumers' emotional responses to sell a variety of products, this behavior is especially unethical when it comes to the health of vulnerable babies"
>
> – Nusa Urbancic, campaigns director for the Changing Markets Foundation

In September 2016, the Food and Drug Administration (FDA) released a Draft Guidance for Industry: Substantiation for Structure/Function Claims Made in Infant Formula Labels and Labeling (6), which highlights the need for appropriately designed clinical trials of sufficient sample size to demonstrate meaningful clinical outcomes, for the indication studied, for claim substantiation. This followed a report stating that the majority of claims on infant formula products, including probiotic-containing formulas, available in the United States were not based on evidence deemed acceptable by the FDA (7). Although pre-approval for structure/function claims for infant formula is not required, the claim must be truthful and not misleading (7). In Canada, infant formula is subject to the specific mandatory nutrient requirements set out under Division 25 of the Food and Drug Regulations, which states: all infant formulas meet basic nutrient requirements and therefore one cannot be superior to the other (8). Regarding probiotics, Health Canada (HC) currently has not accepted any strain-specific claims but has published a list of acceptable structure/function probiotic claims (9).

The European Food Safety Authority (EFSA) does not provide any recommendations pertaining to probiotics in infant formula in the current 2017 Guidance on the risk assessment of substances present in food intended for infants below 16 weeks of age (10). Currently, probiotics have failed to achieve health claims in Europe, both on infant and adult products alike. Some European infant formula brands, for example HiPP, have formulations containing probiotics but are marketed without associated claims.

The classification of a probiotic is determined mainly by its intended use; food or food ingredient, medical food, dietary supplement, and drug or biological product (11). With regards to current products and classifications, Infloran and FloraBaby are probiotics used in neonatal intensive care units in Australia and Canada respectively and are classified as a drug as they are required to be

administered under the supervision of a physician. Infant formulas with an added probiotic are classified as a food and commercially-available probiotic drops for babies are categorized as a dietary supplement.

Making claims on the foundation of research

Successful claims have been made for probiotic-containing infant formulas. "Strengthen the body's defense system", "caring for the digestive system" "probiotic that contributes to healthy gut flora" are examples of some of the current claims in the probiotic marketplace in Canada and the US. Mead Johnson Nutramigen with Enflora™ LGG® contains L. rhamnosus and claims to help avoid an immune system response "for the dietary management of cow's milk allergy"; Gerber contains L. reuteri with the claim that it "will reduce crying time"; and Nestle Good Start contains B. Lactis with the claim that the "Probiotic contributes to your baby's healthy gut flora. A healthy gut flora is part of an overall part of a healthy gut".

High quality scientific research serves as the foundation on which a successful claim is built. Taking Mead Johnson Nutramigen with Enflora™ LGG® for example, the first claim relating to allergy, "Help infants consume milk protein without an allergic response in as early as 6 months of feeding" is substantiated by citing a randomized controlled trial (RCT) in 69 infants that evaluated the effect of that particular probiotic strain on results of skin prick tests, atopy patch tests, and a full clinical evaluation (12). The second allergy-related claim, "Helps reduce the likelihood of other allergic manifestations by ~50% at 3 years of age" was attributed to results of a 3-year RCT in 220 infants (13). Both studies were published in peer reviewed medical journals. Other infant probiotic products that have successful claims are those that are not coupled with an infant formula product (i.e. Jamieson and BioGaia oral probiotic drops). Liquid probiotic drops can be provided to infants directly through the mouth, dropped onto the breast before a feeding or added to infant formula. In this sense, they are considered complimentary feeding products and thus do not promote infant formula over breastfeeding, thereby abiding by the 1981 WHO code. Successful claims on oral probiotic drops include "probiotic drops for colic and digestive comfort" on BioGaia ProTectis Baby drops and "helps support gastrointestinal health" on Jamieson Probiotic Baby drops.

What does the evidence say about probiotics?

The complex and dynamic ecosystem of microorganisms in our gut, known as the microbiome, play a major role in directing bodily processes and overall health from birth. The neonatal period represents a critical window for development of the gut microbiota in infants. The developing infant microbiome is extremely sensitive to disruptions in the first year of life that have a lasting impact on health in later life (14). Probiotics, the "good" bacteria, help to promote a balance of bacteria in the intestines and combat the growth of "unfriendly" organisms capable of causing infections and inflammation. The role of probiotics as part

of an infant nutrition regimen is to repair deficiencies in gut microbiota and restore the protective effect of beneficial bacteria.

Scientific evidence exists to support a beneficial role for probiotics in early infant nutrition regimens (15) however whether the positive health effects are mediated via the presence or absence of specific species, overall diversity or both deserves further exploration. The gaps in knowledge described below can inform the design of clinical trials for targeted probiotic products in the infant arena.

Allergies. Recent research suggests that the microbiome of children with allergies is different from that of those who do not demonstrate allergies. The development and direction of the immune response in infants is largely dictated by exposure to microbes (16,17). Given that the immune system is involved in the allergic response, there may be an association between the bacterial species present in the gut and in the development of allergies. The role for probiotics in early infancy has not been elucidated yet, however some initial results of development of eczema and probiotic supplementation and indicate potential benefits for use in allergies (18,19).

Infant colic. Probiotics, specifically a strain of L. reuteri, has been shown to reduce inconsolable crying and fussing in breastfed, colicky infants (20–22). The mechanism underlying this effect is not fully understood and is a relatively underexplored area with great promise.

Infections. L. fermentum is a probiotic strain isolated from human milk and has been evaluated as a preventive measure against infection in infants. In an RCT, infant formula supplemented with L. fermentum in 215 infants 6-12 months of age had significantly less gastrointestinal and upper respiratory tract infections (23). Similar results were obtained with the same probiotic strain in a formula administered to 126 infants aged 1-6 months old in that the rate of gastrointestinal infections was higher in the group that did not receive the probiotic (24). Further investigation into how L. fermentum supplementation reduces gastrointestinal and upper respiratory tract infections is warranted.

Necrotizing enterocolitis (NEC) in preterm infants. NEC, an inflammatory bowel disease, is one of the leading causes of death in premature infants (25). NEC is characterized by a low bacterial diversity and a lower proportion of beneficial bacteria such as Lactobacilli and Bifidobacteria. Probiotics have been reported to reduce the incidence of NEC as well as all-cause mortality (25,26). Probiotic administration is part of standard clinical practice in neonatal intensive care units in some countries such as Canada and Australia. The gastrointestinal benefits of probiotics for both preterm and full-term infants remain to be determined, specifically

which strains exert benefits and if there is a gestational age-dependent effect.

Overall health. Currently, the bulk of research is focused on the efficacy of probiotics on specific health outcomes or singular health focuses, such as "digestive health" or "immune support". Given the multi-factorial effects of probiotic supplementation, it is surprising that such a paucity of research has been conducted on the efficacy of probiotics in a more global context. Feeding habits, growth, digestive symptoms and cognition are all aspects of infant health that are likely to be influenced by the microbiome and thus, a probiotic and therefore deserve more attention. One study in the US provides some insight into probiotics and general infant health, where two levels of probiotic supplementation (high and low) and a formula without probiotics were evaluated in 118 infants for 18 months (27). Less colic and irritability and fewer prescriptions for antibiotics were reported in the infants receiving probiotics, and no adverse events were reported for any of the formulas. The overall health effect of probiotics in the absence of a condition such as colic, allergies or infection remains to be explored.

From a claims perspective, probiotics are a burgeoning area in the infant nutrition market. High quality clinical trial data generated through RCTs, the gold standard in research, are urgently needed to provide safe and efficacious infant probiotic products into the infant market.

Factors shaping the infant microbiome

While it has long been accepted that development of the human microbiome begins from birth, this belief has been challenged by studies reporting the presence of microbes in the placenta (28–30). Research is required to determine firstly which microorganisms are residents versus invaders, secondly what defines a "healthy placental microbiome" and thirdly the role that the placental microbiome plays in colonization of the infant microbiome. This knowledge may aid in the future development of probiotic products specific to pregnancy that contribute to a healthy placental flora and beneficial outcomes for the infant in the long term.

Colonization of the infant's microbiome continues immediately after birth; in as soon as twenty minutes after delivery, the baby's microbiome either resembles the mother's vaginal microbiota if delivered vaginally or it contains organisms commonly found on skin or in a hospital environment if the infant was born via Caesarean section (31–34). Diversity increases linearly with exposure to the mother's body, to pets and other human contacts, objects present in the infant's environment and their diet (35,36). Fecal sample collection as part of an infant case study revealed that significant changes in gut microbiota composition were apparent at five major time points over a 2.5 year period (35). These major changes corresponded with the ingestion of breast milk, a fever at day 92, first introduction of rice cereal at day 134, formula feeding and table foods at day 161, and antibiotic treatment and adult-like diet at day 371 (35). Each of these events was accompanied by an enrichment of bacterial species and corresponding genes associated with different digestive functions such as vitamin biosynthesis and polysaccharide digestion (35). While individual infants display differences in presence and abundance of species over time, the consortia is not random which suggests microbial plasticity. The species within the microbiota depend both on each other and on the environment.

Twenty-four-hour patterns, known as circadian rhythms, regulate behavior, organs, cellular function and can even mediate the influence of the microbiome on the body (37). The purpose of circadian rhythms is to line up biological functions with regular and predictable environmental habits, such as sleeping and eating. Aligning them in a diurnal rhythm is the body's way of optimizing biological function and physical health. Recently research has revealed that the microbiome may be affected not only by diet but also by time of feeding, as it too is regulated by circadian rhythms (38). This is especially important for newborn infants in whom the microbiome is still being established, as feeding occurs frequently for these babies. The effect of probiotic supplements on infant circadian rhythms and sleep patterns represents an exciting area of research in the infant supplement arena.

Even the healthiest of infants may encounter factors capable of disrupting their delicate microbiomes, thus the need for probiotic products that can protect against long-lasting disturbances is very real. The microbiome converges toward an adult-like microbiota between the first 2.5-5 years of life (28,29,39) and is relative stable throughout adulthood yet still sensitive to external perturbations (14). Although a great deal of research has been conducted on probiotics, much remains to be discovered. Knowledge of the factors that disrupt the microbiome in infancy can provide a springboard for the development of probiotic products- which effect is desired and which strain is safe and likely to achieve a protective effect?

What makes a claim successful?

It is widely accepted that early life nutrition shapes growth and development and can have long-term impact on health. Current recommendations from pediatric societies and committees regarding probiotic use in infancy are cautious and accordingly, have mandated that well-designed and well-controlled studies support all claims. The vulnerability of this population demands high quality data to support the efficacy and most importantly, safety, of all products intended for infant ingestion.

"To subvert health needs to marketing needs is unconscionable."

- Elisabeth Sterken, Director of INFACT Canada

Although rigid, the current structure of the regulatory processes adopted by FDA, EFSA and HC is one that encourages scientific investigation (11,40). Thus, the goal for claims-based research is very clear; to provide strong data generated from rigorous and carefully designed trials with clearly defined measurable outcomes to support probiotic products is of value.

Considering the regulatory requirements and enhanced scrutiny, a one-system, one-outcome approach is not sufficient to appropriately capture the multi-factorial effects of probiotic-containing products for claims substantiation purposes. In the absence of long-term data on the efficacy of probiotics as part of early-life nutrition regimens, we propose that the short-term evaluations of probiotics in infancy must be comprehensive and contain measures by which to assess efficacy and safety at various stages of development. A single endpoint specific to a claim is still acceptable, however, we propose that it should be evaluated in tandem with a developmental stage-specific, global index to determine efficacy. A global index allows researchers to answer questions that might not have otherwise been possible with a single endpoint. Furthermore, it has the added advantage of an increase in both the efficiency and clinical relevance of a study. A Global Index for infant nutrition is currently being formulated with intent for more robust claim substantiation. The Global Index allows for a better quantification of efficacy of supplements in RCTs with infants.

In summary, the most effective means to achieve an infant probiotic claim is firstly, to meet the evidentiary burden required by regulatory agencies through data generated from well-designed, "gold-standard" RCT. Secondly, it is imperative that the marketing claim refrain from comparing non-breast milk products to breast milk itself. Lastly, a global index that captures the multi-faceted effects of probiotics, will provide more comprehensive and robust data on which to substantiate probiotic claims and demonstrate benefit.

Highlights

1. In countries that abide by the World Health Organization's International Code of Marketing of Breast-Milk Substitutes, formulating standalone probiotic formulas as opposed to probiotic-containing infant formulas may help to avoid the added scrutiny stemming from the worldwide initiative to promote breastfeeding
2. Ensure that clinical trial results are publicly available (especially applicable for FDA)
3. Infants are not simply little adults- a specific global index for probiotic use in infants will provide a comprehensive and targeted knowledge foundation on which to substantiate claims

References

1. Global Probiotics Market Analysis & Trends - Industry Forecast to 2025 [Internet]. Accuray Research. 2018 [cited 2018 Apr 20]. Available from: http://www.accurayresearch.com/food-and-beverages/probiotics-market-analysis-size-report-share-trends

2. Klein D. How Marketers Can Stay Ahead of the Booming Probiotic Market [Internet]. Macromark. [cited 2018 Apr 20]. Available from: https://www.macromark.com/blog/how-marketers-can-stay-ahead-of-the-booming-probiotic-market

3. Fisher E. Probiotic Infant Formula to Claim 76% Share of $22.9 Billion Market in 2024. Lux Research; 2015 Apr.

4. World Health Organization. International Code of Marketing of Breast-milk Substitutes [Internet]. 1981 [cited 2018 Apr 18]. Available from: http://www.who.int/nutrition/publications/code_english.pdf

5. UNICEF. National Implementation of the International Code of Marketing of Breastmilk Substitutes (April 2011) [Internet]. 2011. Available from: https://www.unicef.org/nutrition/index_24805.html

6. U.S. Food & Drug Administration. Draft Guidance for Industry: Substantiation for Structure/Function Claims Made in Infant Formula Labels and Labeling [Internet]. 2016 [cited 2018 Apr 18]. Available from: https://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ucm514640.htm

7. Belamirich P, Bochner R, Racine A. A Critical Review of the Marketing Claims of Infant Formula Products in the United States. Clin Pediatr Phila. 2016;55(5):437–42.

8. Canadian Food Inspection Agency. Labelling Requirements for Infant Foods, Infant Formula and Human Milk: Infant Formula [Internet]. 2018. Available from: http://www.inspection.gc.ca/food/labelling/food-labelling-for-industry/infant-foods-infant-formula-and-human-milk/eng/1393069958870/1393070130128?chap=3#s10c3

9. Canadian Food Inspection Agency. Probiotic Claims [Internet]. Government of Canada; 2018. Available from: http://inspection.gc.ca/food/labelling/food-labelling-for-industry/health-claims/eng/1392834838383/1392834887794?chap=10

10. European Food Safety Authority. Guidance on the risk assessment of substances present in food intended for infants below 16 weeks of age [Internet]. 2017. Available from: https://efsa.onlinelibrary.wiley.com/doi/epdf/10.2903/j.efsa.2017.4849

11. Degnan F. The US Food and Drug Administration and probiotics: regulatory categorization. Clin Infect Dis. 2008;46(Suppl 2):S133–6.

12. 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. J Allergy Clin Immunol. 2012;129(2):580–2.

13. Berni Canani R, Di Costanzo M, Bedogni G, Amoroso G, 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. J Allergy Clin Immunol. 2017;139(6):1906–13.

14. Thursby E, Juge N. Introduction to the human gut microbiota. Biochem J. 2017;474(11):1823–36.

15. Thomas D, Greer F. Clinical Report—Probiotics and Prebiotics in Pediatrics. Pediatrics. 2010;126(6).

16. Fulde M, Hornef M. Maturation of the enteric mucosal innate immune system during the postnatal period. Immunol Rev. 2014;260(1):21–34.

References

17. Ribet D, Cossart P. How bacterial pathogens colonize their hosts and invade deeper tissues. Microbes Infect. 2015;17(3):173–83.

18. Heine R. Food Allergy Prevention and Treatment by Targeted Nutrition. Ann Nutr Metab. 2018;72(Suppl 3):27–39.

19. Osborn D, Sinn J. Probiotics in infants for prevention of allergic disease and food hypersensitivity. Cochrane Database Syst Rev. 2007;17(4):CD006475.

20. Bergmann H, Rodriguez J, Salminen S, Szajewska H. Probiotics in human milk and probiotic supplementation in infant nutrition: a workshop report. Br J Nutr. 2014;112(7):1119–28.

21. Chau K, Lau E, Greenberg S, Jacobson S, Yazdani-Brojeni P, Verma N, et al. Probiotics for infantile colic: a randomized, double-blind, placebo-controlled trial investigating Lactobacillus reuteri DSM 17938. J Pediatr. 2015;166(1):74–8.

22. Baldassarre M, Di Mauro A, Tafuri S, Rizzo V, Gallone M, Mastromarino P, et al. Effectiveness and Safety of a Probiotic-Mixture for the Treatment of Infantile Colic: A Double-Blind, Randomized, Placebo-Controlled Clinical Trial with Fecal Real-Time PCR and NMR-Based Metabolomics Analysis. Nutrients. 2018;10(2).

23. Maldonado J, Canabate F, Sempere L, Vela F, Sanchez A, Narbona E, et al. Human milk probiotic Lactobacillus fermentum CECT5716 reduces the incidence of gastrointestinal and upper respiratory tract infections in infants. J Pediatr Gastroenterol Nutr. 2012;54(1):55–61.

24. Gil-Campos M, Lopez M, Rodriguez-Benitez M, Romero J, Roncero I, Linares M, et al. Lactobacillus fermentum CECT 5716 is safe and well tolerated in infants of 1-6 months of age: a randomized controlled trial. Pharmacol Res. 2012;65(2):231–8.

25. Rees C, Hall N, Fleming P, Eaton S. Probiotics for the prevention of surgical necrotising enterocolitis: systematic review and meta-analysis. BMJ Paediatr Open. 2017;1(1):e000066.

26. Tarnow-Mordi W, Wilkinson D, Trivedi A, Brok J. Probiotics reduce all-cause mortality and necrotizing enterocolitis: it is time to change practice. Pediatrics. 2010;125(5):1068–70.

27. Saavedra J, Abi-Hanna A, Moore N, Yolken R. Long-term consumption of infant formulas containing live probiotic bacteria: tolerance and safety. Am J Clin Nutr. 2004;79(2):261–7.

28. Aagaard K, Ma J, Antony K, Ganu R, Petrosino J, Versalovic J. The placenta harbors a unique microbiome. Sci Transl Med. 2014;6(237):237ra65.

29. Rodriguez J, Murphy K, Stanton C, Ross P, Kober O, Juge N, et al. The composition of the gut microbiota throughout life, with an emphasis on early life. Microb Ecol Health Dis. 2015;26:26050.

30. Zhu L, Luo F, Hu W, Han Y, Wang Y, Zheng H, et al. Bacterial Communities in the Womb During Healthy Pregnancy. Front Microbiol. 2018;9:2163.

31. Falana K, Knight R, Martin CR, Goldszmid R, Greathouse KL, Gere J, et al. Short Course in the Microbiome. J Circ Biomark. 2015 Dec;4:8.

References

32. Munyaka P, Khafipour E, Ghia J. External influence of early childhood establishment of gut microbiota and subsequent health implications. Front Pediatr. 2014;2:109.

33. Song S, Dominquez-Bello M, Knight R. How delivery mode and feeding can shape the bacterial community in the infant gut. CMAJ. 2013;185(5):373–4.

34. Rutayisire E, Huang K, Liu Y, Tao F. The mode of delivery affects the diversity and colonization pattern of the gut microbiota during the first year of infants' life: a systematic review. BMC Gastroenterol. 2016;16(1):86.

35. Koenig JE, Spor A, Scalfone N, Fricker AD, Stombaugh J, Knight R, et al. Succession of microbial consortia in the developing infant gut microbiome. Proc Natl Acad Sci U S A. 2011 Mar 15;108 Suppl 1:4578–85.

36. Ursell LK, Metcalf JL, Parfrey LW, Knight R. Defining the human microbiome. Nutr Rev. 2012 Aug;70 Suppl 1:S38-44.

37. Marcinkevicius EV, Shirasu-Hiza MM. Message in a biota: gut microbes signal to the circadian clock. Cell Host Microbe. 2015 May 13;17(5):541–3.

38. Cubero J, Narciso D, Aparicio S, Garau C, Valero V, Rivero M, et al. Improved circadian sleep-wake cycle in infants fed a day/night dissociated formula milk. Neuro Endocrinol Lett. 27(3):373–80.

39. Davis E, Wang M, Donovan S. The role of early life nutrition in the establishment of gastrointestinal microbial composition and function. Gut Microbes. 2017;8(2):143–71.

40. Saldanha L. US Food and Drug Administration regulations governing label claims for food products, including probiotics. Clin Infect Dis. 2008;46(Suppl 2):119–21.