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BEYOND BREASTFEEDING: A NARRATIVE REVIEW OF EVIDENCE-BASED NUTRITION APPROACHES TO SUPPORT HEALTHY GUT MICROBIOTA IN NON-BREASTFED INFANTS

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Abstract

Background: Early-life gut microbiota development plays a critical role in immune, metabolic, and neurodevelopment, yet infants who are not exclusively breastfed may experience different patterns of microbial colonization, highlighting the need for strategies to support healthy gut microbiota development in this population. **Methods**: This narrative review synthesizes current evidence on interventions that support healthy gut microbiota development in non-breastfed infants. The keywords included “infant gut microbiota”, “prebiotic”, “probiotic”, “synbiotic”, “human milk oligosaccharides”, “infant formulas”, and “complementary feeding”. **Results:** Among the reviewed interventions, formulas supplemented with prebiotics and oligosaccharides have been investigated for their ability to modulate the infant gut microbiota. Synbiotic-containing formulas also demonstrated beneficial microbiota modulations, such as increased *Bifidobacterium* and reduced *Clostridioides difficile* abundance. Commonly reported effects from the interventions included increased abundance of selected bacterial bacterial taxa and lower faecal pH, although the magnitude of these changes varied across formulations and study populations. **Conclusion:** Formulas supplemented with prebiotics or synbiotics may modulate the developing gut microbiota of non-breastfed infants. Further studies are needed to evaluate long-term outcomes and effectiveness across diverse populations.

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Keywords: Gut microbiota, formula feeding, human milk oligosaccharides (HMOs), prebiotics, probiotics

Introduction

The first two years of life, often referred to as the "first 1,000 days," are a critical period for the establishment of the gut microbiota.[1] During this time, the gut microbiota plays an important role in immune development, metabolic programming, and neurodevelopment.[1-3] Disruptions to early microbial colonization have been associated with an increased risk of allergic diseases, obesity, and immune dysregulation later in life.[4-6] To address this, several strategies have been investigated for their potential to support or modulate early-life gut microbiota development, including prebiotics, probiotics, and their combination, known as synbiotics.

Among the factors shaping early microbiota development, early feeding stands out as one of the most influential. Breastfeeding is considered the biological reference standard for infant nutrition because of its well-established ability to promote a *Bifidobacterium*-dominant gut microbiota through human milk oligosaccharides (HMOs) and other bioactive components.[7, 8] The beneficial effects of breastfeeding on the infant gut microbiota have been extensively reviewed and are therefore not the primary focus of this article.[9, 10]

Relatively less attention has been given to infants who are not exclusively breastfed. Formula feeding may be necessary for a variety of reasons, including medical contraindications, insufficient milk supply, adoption, maternal illness or death, work-related challenges, or parental preference.[11-14] In the United States, approximately 16% of infants receive no human milk, while globally only 43% of infants under six months of age are exclusively breastfed.[15, 16] These figures indicate that formula-fed and mixed-fed infants constitute a substantial proportion of the global infant population. However, recommendations on how to support healthy gut microbiota development in these infants remain limited.

This narrative review aimed to summarize current evidence on how the gut microbiota develops in infants who are not breastfed, and strategies to support healthy gut microbiota development in non-breastfed infants.

Methods

This review summarizes current evidence on strategies to support gut microbiota

development in non-breastfed infants. Non-breastfed infants defined as infants receiving formula as part or all of their milk feeding, either from birth or after breastfeeding has ceased. Findings from studies involving preterm or other high-risk infants were also included (e.g. neonatal intensive care setting). A narrative approach was chosen to integrate findings from basic science and clinical studies, with an emphasis on clinical relevance. When available, systematic reviews and meta-analyses were prioritized. Studies were identified through searches of PubMed/MEDLINE, Scopus, and Web of Science using the combination of the following terms: “infant gut microbiota”, “prebiotic”, “probiotic”, “synbiotic”, “human milk oligosaccharides”, “infant formulas”, and “complementary feeding”. This review summarizes current evidence on strategies to support gut microbiota development in non-breastfed infants. Non-breastfed infants defined as infants receiving formula as part or all of their milk feeding, either from birth or after breastfeeding has ceased. Findings from studies involving preterm or other high-risk infants were also included (e.g. neonatal intensive care setting). A narrative approach was chosen to integrate findings from basic science and clinical studies, with an emphasis on clinical relevance. When available, systematic reviews and meta-analyses were prioritized. Studies were identified through searches of PubMed/MEDLINE, Scopus, and Web of Science using the combination of the following terms: “infant gut microbiota”, “prebiotic”, “probiotic”, “synbiotic”, “human milk oligosaccharides”, “infant formulas”, and “complementary feeding”.

Results and Discussion

Gut Microbiota Development in Infancy: Influence of Feeding Pattern and Other Early-Life Factors

Gut colonization begins at birth and proceeds through a broadly predictable succession, from facultative anaerobes in the first days of life toward obligate anaerobes such as *Bifidobacterium* and *Bacteroides* over the following weeks to months, with a further shift toward an adult-like, *Firmicutes*- and *Bacteroidetes*-dominant community after the introduction of solid foods.[2, 7] In healthy breastfed infants, this process is strongly shaped by HMOs, which selectively nourish specific *Bifidobacterium* species such as *B. breve*, producing a microbiota with comparatively lower diversity but a clear *Bifidobacterium* dominance in the

first months of life.[7, 17] Beyond feeding type, mode of delivery, gestational age, and antibiotic exposure are among the best-established modifiers of early colonization.[18, 19] These factors interact with feeding type rather than acting independently, which complicates efforts to isolate the specific contribution of formula feeding itself in observational studies.

Non-exclusively-breastfed infants showed higher predicted microbial pathways related to carbohydrate metabolism, alongside lower representation of pathways related to lipid metabolism, vitamin metabolism, and detoxification, compared with exclusively breastfed infants in the first six months of life.[8] Systematic reviews and cohort studies indicate that feeding type is associated with distinct patterns of gut microbiota composition. Compared with breastfed infants, formula-fed infants have been reported to exhibit different relative abundances of *Bifidobacterium*, *Lactobacillus*, *Enterobacteriaceae*, *Clostridiaceae*, and *Bacteroidaceae*.[18,20,21] Interestingly, formula-fed infants actually show higher alpha-diversity in the first months than breastfed infants, likely because formula lacks HMOs' *Bifidobacterium*-selecting effect, a pattern argued as an earlier shift toward an adult-like microbiome.[7, 8, 22]

Formula feeding has also been shown to drive microbial metabolic adaptation to specific dietary components. Formula-fed infants demonstrated approximately six-fold higher microbial fructoselysine degradation activity than breastfed infants, indicating functional adaptation of the gut microbiota to formula-derived substrates.[23] Clinically, several longitudinal studies have reported that formula-fed infants exhibit different *Bifidobacterium* abundance than breastfed infants during the first four to six months of life, although these differences are not consistently observed across all cohorts.[7, 18, 24] Associations between early microbial characteristics and later health outcomes have been reported in observational studies, including associations involving Bifidobacterium and allergic disease or growth. However, these findings do not establish that differences in a specific bacterial group are causal determinants of later disease or growth.[4, 18, 24]

Evidence-Based Approaches to Support Gut Microbiota in Non-Breastfed Infants

Formula composition strategies

Current evidence on formula composition strategies to support gut microbiota

development in non-breastfed infants is summarized in Table 1. HMOs have been investigated for their ability to selectively support *Bifidobacterium* and modulate gut microbiota composition, as well as for their potential effects on markers of intestinal immune development.[25-27] A randomized controlled trial evaluating a specific blend of five HMOs reported dose-related changes in gut microbiota development and selected markers of intestinal maturation.[27] Studies evaluating 2'-FL added to a galacto-oligosaccharides (GOSs) and fructo-oligosaccharides (FOSs)-containing base formula, have reported differences in bifidogenic responses compared with the study control formula.[22] 2'-FL has also been studied in combination with probiotic-containing formula, with available trials reporting acceptable gastrointestinal tolerance and growth outcomes in healthy term infants.[25, 28] An exploratory analysis investigated associations between microbiota and metabolite changes associated with 2'-FL/LNnT supplementation and respiratory tract illness. These findings provide hypotheses for future research but should not be interpreted as establishing a preventive effect of HMO supplementation against respiratory illness.[29] In a randomized controlled trial, supplementation of infant formula with 2'-FL and *Limosilactobacillus reuteri* DSM 17938 supported normal growth and was well tolerated, with reported changes in gut microbiota composition compared with the control formula.[30]

Other prebiotic ingredients have also been developed to modulate the infant gut microbiota. A double-blind randomized controlled trial involving 244 healthy term infants demonstrated that formulas supplemented with GOS prebiotics supported equivalent growth, similar tolerance, and comparable safety to a standard intact cow's milk protein formula during the first year of life.[31] The combination of short-chain galacto-oligosaccharides and long-chain fructo-oligosaccharides (scGOS/lcFOS), commonly provided in a 9:1 ratio, has been investigated extensively in formula-fed infants. Studies have reported a well-established bifidogenic effect in formula-fed infants compared with standard formula and might modify overall gut microbial colonization.[32-34] This prebiotic blend has also been evaluated in very preterm infants, where supplementation with scGOS/lcFOS (9:1) and glycomacropeptide was associated with changes in microbial functional pathways related to nutrient metabolism and other metabolic processes, without compromising growth or clinical tolerance.[35] Added inulin

has also been shown to modify the gut microbiota of healthy, fully formula-fed infants aged 3–5 months in a dose-dependent manner. A randomized double-blinded controlled clinical study compared control formula (n = 48) or a formula with native chicory inulin at 0.2 g/100 mL (n = 47) or 0.4 g/100 mL (n = 47) for 4 weeks. This study found that the 0.4 g/100 mL group was associated with prebiotic response, with a significant within-group increase in *Bifidobacterium* and a fall in faecal pH; whereas the 0.2 g/100 mL group showed a pH reduction without a significant bifidogenic effect.[36]

Both probiotic- and synbiotic-supplemented formulas have been shown to exert benefits in non-breastfed infants. Single-strain probiotic supplementation has demonstrated strain-specific effects on microbial colonization and community composition, though a substantial proportion of this evidence comes from extremely low birth weight or preterm infant trials aimed at preventing necrotizing enterocolitis and sepsis, rather than healthy term formula-fed infants.[37, 38] In term infants, a randomized controlled trial demonstrated that the combination of 0.8 g/100 mL scGOS/lcFOS (9:1) and *Bifidobacterium breve* M-16V promoted a *bifidobacteria*-dominated gut microbiota, lowered *Clostridioides difficile* abundance, and created a faecal metabolic environment more closely resembling that of breastfed infants.[39] In cesarean-delivered infants, the supplementation of scGOS/lcFOS combined with *Bifidobacterium breve* M-16V for 6 weeks significantly increased the relative abundance of intestinal *Bifidobacterium* and reduced both the prevalence and abundance of *Clostridioides difficile*. Compared with the control formula, the synbiotic formulas also resulted in a lower faecal pH and higher L-lactate concentrations and acetate proportions.[40] These findings indicate that this synbiotic combination can influence *Bifidobacterium* abundance and selected microbial and metabolic characteristics in infants receiving formula, including those born by cesarean section. More recent trials combining partially hydrolyzed whey protein with multiple HMOs and probiotic strains (*B. infantis, B. lactis*) acceptable safety and tolerance, together with changes in selected gut-health markers.[19, 41]

Feeding Practice and Complementary Feeding Transition

Partial or mixed breastfeeding may help preserve a more favorable gut microbiota than exclusive formula feeding. Cohort studies have shown that differences in *Bifidobacterium*

abundance according to feeding type remain detectable at four to five months of age, shortly before the introduction of complementary foods.[24] These findings suggest that maintaining any amount of breastfeeding, when feasible, may still support early gut microbial development rather than viewing infant feeding as a strict choice between exclusive breastfeeding and exclusive formula feeding.

The introduction of complementary foods, typically between four and six months of age, marks the next major inflection point in microbiota development regardless of prior feeding type, generally increasing microbial diversity and richness while reducing the relative dominance of *Bifidobacterium* in favor of adult-associated taxa such as *Lachnospiraceae* and *Ruminococcaceae*.[2, 42, 43] Diet composition also appears to influence this transition, with foods higher in fiber and protein associated with greater microbial diversification and an increased capacity to degrade complex plant polysaccharides.[2] Evidence on the effect of timing of complementary feeding introduction (before versus after 4–6 months) on diversity outcomes remains inconsistent across cohorts, with some studies reporting higher diversity following earlier introduction and others finding no significant difference.[43, 44] Evidence specific to non-breastfed infants is particularly limited, as most studies do not analyse outcomes according to previous feeding type, making it difficult to determine whether complementary feeding recommendations should differ for formula-fed infants.

Limitations and Future Research Directions

Several limitations should be considered when interpreting the current evidence. As this was a non-systematic narrative review, the literature search and study selection were not conducted using a predefined systematic methodology, and the possibility of selection bias and omission of relevant studies cannot be excluded. In terms of studies included in this review, probiotic and synbiotic studies vary widely in the strains, doses, and outcomes evaluated, making direct comparisons difficult.[37-39] Most intervention studies, including those of HMO-supplemented formulas, also have relatively short follow-up (3–6 months), so the long-term clinical relevance of microbiota changes remains uncertain. In addition, much of the probiotic evidence comes from preterm or very-low-birth-weight infants in neonatal intensive care settings and may not be directly applicable to healthy term formula-fed infants.[37, 38]

Because several interventions contain multiple bioactive components, the contribution of individual ingredients or strains cannot always be separated from the overall formulation effect. The available evidence is also dominated by studies from North America, Europe, and parts of East Asia, with relatively few data from Southeast Asia and other low- and middle-income countries, where feeding practices, environmental exposures, and disease burden may differ.

Conclusion

Non-breastfed infants represent a clinically important population whose gut health has received less research attention than breastfeeding promotion itself. The evidence synthesized in this review indicates that selected prebiotic- and synbiotic-containing formulas can modulate the developing gut microbiota of non-breastfed infants, with several studies reporting increased *Bifidobacterium* abundance and changes in microbial composition or function. Some interventions produce selected microbiota characteristics that have also been described in breastfed reference groups; however, microbiota similarity should not be interpreted as evidence of equivalent clinical outcomes. Since the introduction of solid food drives microbiota maturation on its own regardless of feeding history, diverse complementary diet is generally encouraged as complementary feeding begins, although evidence specific to non-breastfed infants remains limited. However, more research is needed to clarify which formulation characteristics and complementary feeding practices are associated with clinically meaningful outcomes in non-breastfed infants.

Acknowledgment

None.

Conflict of Interest

The authors declare no conflicts of interest related to this work.

Table 1. Selected Formula Composition Strategies Investigated for Their Effects on Gut Microbiota in Non-Breastfed Infants

Specific Strategy	Key Findings	Population (Study Design)	Ref
1. Prebiotics			
Human Milk Oligosaccharide (HMO) Analogs			
2'-FL + LNnT	Associated with changes in gut microbiota composition and selected markers of intestinal immune defense in randomized controlled trials. Exploratory analyses have also investigated associations between microbiota changes and respiratory outcomes.	Term infants partly or fully formula-fed (RCTs)	26, 29
HMO blend (2'-FL, DFL, LNT, 3'-SL, 6'-SL)	Associated with dose-related changes in gut microbiota development and selected markers of intestinal maturation.	Term infants partly or fully formula-fed (RCT)	27
2'-FL added to prebiotic base	Associated with increased bifidogenic response compared with the study control formula.	Fully-formula fed term infants (RCT)	22
2'-FL added to probiotic base	Reported acceptable gastrointestinal tolerance and growth outcomes, with no significant gastrointestinal safety signal in the reported trial.	Fully-formula fed term infants (RCT)	28, 30
Prebiotic Oligosaccharides (GOS/FOS)			
scGOS/lcFOS	Associated with increased *Bifidobacterium* abundance and changes in the pattern of microbial colonization compared with control formulas.	Term infants (RCT and Cohort study)	32, 33
scGOS/lcFOS + glycomacropepti	Associated with changes in microbiota composition and function in a more	Very preterm infants receiving minimal or no	35

Specific Strategy	Key Findings	Population (Study Design)	Ref
de (GMP)	vulnerable population. Because this population is clinically distinct from healthy term infants, these findings should not be directly extrapolated to the general infant population.	breastmilk (Non-randomized trial)	
Inulin			
Oligofructose-enriched inulin	Associated with changes in gut microbiota composition, including a trend toward higher *Bifidobacterium* counts, together with changes in stool characteristics.	Formula fed healthy term newborns (RCT)	45
Chicory inulin	Significantly increased B*ifidobacterium* and reduced faecal pH compared with control formula in a dose-dependent manner after 4 weeks of supplementation with native chicory inulin at 0.4 g/100 mL (n = 47), but not 0.2 g/100 mL (n = 47),	Fully formula-fed infants aged 3-5 months (RCT)	36
2. Probiotic- and Synbiotic-Supplemented Formulas			
Single-strain probiotics	Strain-specific changes in microbial colonization and community composition have been reported, although evidence is predominantly from ELBW/preterm populations.	Mostly ELBW/preterm infants (NEC/sepsis prevention focus) (RCTs)	37, 38
Synbiotic	Significant increase in *Bifidobacteria* proportions within 6 weeks in formula containing *B. breve* M-16V + scGOS/lcFOS in term infants and C-section delivered infants.	Fully formula-fed term infants, C-section delivered infants (RCTs and Cohort studies)	39-41

Specific Strategy	Key Findings	Population (Study Design)	Ref
	Synbiotic formula containing six HMOs + 2 probiotics maintained normal growth and GI tolerance while increasing beneficial *Bifidobacteria* during the first 4 months of life in term infants.		

Abbreviations: 2'-FL, 2'-fucosyllactose; LNnT, lacto-N-neotetraose; DFL, 2',3-difucosyllactose; LNT, lacto-N-tetraose; 3'-SL, 3'-sialyllactose; 6'-SL, 6'-sialyllactose; GOS, galacto-oligosaccharides; FOS, fructo-oligosaccharides; scGOS/lcFOS, short-chain GOS / long-chain FOS; ELBW, extremely low birth weight; NEC, necrotizing enterocolitis; NICU, neonatal intensive care unit; PHF, partially hydrolyzed formula; RCT, randomized controlled trial.

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