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PREGLEDNI RAD

THE DIFFERENCE IN INTESTINAL MICROBIOTA BETWEEN BREAST-FED AND FORMULA-FED CHILDREN

RAZLIKA U CRIJEVNOJ MIKROBIOTI IZMEĐU DJECE HRANJENE MAJČINIM MLIJEKOM I DJECE HRANJENE ADAPTIRANIM MLIJEKOM

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SAŽETAK

Crijevna mikrobiota zajednica je mikroorganizama koja kolonizira probavni sustav čovjeka. U novije vrijeme, crijevna mikrobiota intenzivno se istražuje, odnosno, istražuje se njezina uloga u zdravlju domaćina. U ljudskom tijelu, između ostalog, crijevna mikrobiota ima ulogu središnjeg regulatora metabolizma i imunološke funkcije, a poremećaji u ravnoteži crijevne mikrobiote danas se povezuju s brojnim patološkim stanjima. Na sastav i raznolikost crijevne mikrobiote utječu brojni faktori, među kojima se kao najznačajniji navode prehrambene navike pojedinca, stoga se postavlja pitanje u kojoj mjeri prehrambene navike mogu utjecati na raznolikost i sastav crijevne mikrobiote te u kojoj mjeri promjene u crijevnoj mikrobioti uvjetovane prehrambenim navikama mogu utjecati na zdravlje domaćina. Crijevna mikrobiota razvija se od najranije dobi te neki autori sugeriraju da formiranje crijevne mikrobiote započinje već *in utero*, stoga ćemo u ovom radu ukratko nastojati prikazati recentne spoznaje na temu utjecaja načina prehrane na crijevnu mikrobiotu dojenčadi, odnosno ukazati na razlike u crijevnoj mikrobioti dojene djece i djece hranjene adaptiranom mliječnom formulom.

Ključne riječi: *crijevna mikrobiota, prehrambene navike, imunološki sustav, dojenje, adaptirane mliječne formule, oligosaharidi ljudskog mlijeka*

ABSTRACT

Intestinal (gut) microbiota is a community of microorganisms that colonize the human digestive system. In recent times, intestinal microbiota has been intensively researched, that is, its role in the host's health has been a research subject. In the human body, among other things, the intestinal microbiota plays the role of a central regulator of metabolism and immune function, and disturbances in the balance of the intestinal microbiota are associated today with numerous pathological conditions. The composition and diversity of the intestinal microbiota are influenced by numerous factors, among which the dietary habits of the individual are cited as the most significant. Therefore, the question arises to what extent dietary habits can influence the diversity and composition of the intestinal microbiota and to what extent changes in the intestinal microbiota conditioned by dietary habits can influence the host's health. The intestinal microbiota develops from an early age, and some authors suggest that its formation begins already in utero. In this paper, we will briefly present recent findings on the topic of the influence of diet on the intestinal microbiota of infants, pointing out the differences between breastfed children and those fed with adapted milk formula.

Keywords: *intestinal microbiota; gut microbiota; dietary habits; immune system; breastfeeding; adapted milk formulas; human milk oligosaccharides*

INTRODUCTION

Microbiota is the name for the community of microorganisms, which consists of bacteria, archaea, and fungi, as well as their viruses and phages. Human intestinal microbiota is no exception and consists of a densely populated ecosystem dominated by bacteria (1). The intestinal microbiota is the central regulator of the host's metabolism, and its composition and function are very dynamic and subject to various influences, such as dietary habits and lifestyle (2). In addition to the programming of metabolism, intestinal microbiota plays a central role in the programming of the host's immune function, with immediate and long-term effects on health (3).

When talking about infants, their intestinal microbiota is affected by numerous factors such as the method of delivery, exposure to antibiotics, and feeding method; some authors suggest that factors affecting the intestinal microbiota of infants could be already present in utero (4,5). The process of primary intestinal colonization of infants is very dynamic, and disturbances in the primary colonization of the digestive tract can result in lifelong health consequences and are associated with a number of host conditions. After birth, how infants are fed is one of the main factors that determine early microbial colonization, and the differences in the intestinal microbiota of breastfed children and children fed with adapted milk formula are well documented (6). Molecular tools based on the sequence of the 16S rRNA gene applied to the fecal microbiota indicated the existence of a difference in the intestinal microbiota of infants with regard to the feeding method (3). For example, increased levels of bifidobacteria are recorded in breastfed children, while in formula-fed infants, we find a more diverse intestinal microbiota dominated by *Staphylococcus, Bacteroides, Clostridium, Enterococcus, etc. (6).*

In this narrative review, the differences in intestinal microbiota between breastfed and formula-fed children will be presented, along with the latest findings from relevant recent studies dealing with this topic.

INFANT'S INTESTINAL MICROBIOTA - EARLY COLONIZATION AND FACTORS AFFECTING ITS COMPOSITION AND DIVERSITY

The human intestinal microbiota is a very diverse, densely populated ecosystem – it is a community of microorganisms consisting of archaea, bacteria, and fungi, as well as their viruses and phages (1)and it is becoming increasingly relevant to identify preventive or therapeutic solutions targeting it. The composition and function of the gut microbiota are relatively well described for infants (less than 3 years. A healthy intestinal microbiota is stable and plays a role in the metabolism of hard-to-digest polysaccharides and the detoxification of the organism. It also serves as a natural barrier against pathogens while helping the development of the host's immune system. Numerous studies have shown that intestinal bacteria and their metabolites, such as short-chain fatty acids, play an important role in the proliferation and differentiation of T cells, which speaks of their role in the immune system (7). Today, it is known that the composition of the intestinal microbiota is influenced by numerous factors, such as the method of delivery, exposure to animals (pets), and the use of antibiotics. Although the first major microbial colonization of newborns occurs during birth (exposure to maternal microbes, i.e., microbes of the vaginal and fecal flora), some experts believe that colonization and formation of the microbiota can begin already *in utero*, depending on changes in the mother's microbiota during pregnancy (5).

Colonization of the child's gastrointestinal system

The profile of a healthy intestinal microbiota of an infant includes the fecal microbiota of an exclusively breastfed infant delivered vaginally at term (8). The development of the intestinal microbiota in childhood occurs during a "critical period", and a disturbance in this process can favor the development of immune diseases such as atopic diathesis and asthma (7). The theory of the developmental origin of health and disease (DOHaD) proposes that the "critical period" is a period of 1000 days (the period from conception to the second year of life - the first 1000 days of life). This period is characterized by the rapid maturation of metabolic, endocrine, neural, and immune signaling pathways that support regulatory abilities. These pathways are influenced by the maternal milieu and exposures in the perinatal environment, which together may influence the future risk of disease development. During the "critical period", the fetus is exposed to microbial metabolites of the mother, and microbial colonization of the neonatal gastrointestinal system takes place as an integral part of postnatal maturation (4).

The gastrointestinal system was traditionally considered sterile. However, recent studies have revealed the presence of microorganisms in amniotic fluid, fetal membranes, umbilical cord, placenta, and meconium (7,9). Bacterial colonization of the child's gastrointestinal system begins when the fetus is in the lower part of the uterus, with the gut microbiota becoming established after birth. The establishment of a stable intestinal microbiota is influenced by two major transitions in infancy, and changes in the microbiome continue until around the third year of life, when a stable intestinal microbiota is generally acquired. The first transition occurs shortly after birth, during breastfeeding, and results in the dominance of *Bifidobacterium*. The second transition occurs during the weaning period and with the introduction of solid food, which results in the establishment of a complex adult microbiome dominated by Bacteroidetes and Firmicutes (1,4,7). It is considered that children younger than 36 months (3 years) have dynamic and highly individual microbial profiles with a lower diversity index (1).

Immediately after birth, the intestinal microbiota of a newborn is transiently dominated by facultatively anaerobic bacteria such as *Enterobacter, Enterococcus, Streptococcus,* and *Staphylococcus* strains. After breastfeeding starts, the intestinal microbiota is dominated by Bifidobacterium and Lactobacillus, and such flora is maintained until the introduction of solid food (around the sixth month of life). After weaning from breastfeeding, *Bifidus* flora is suppressed by *Bacteroides, Prevotella, Ruminococcus, Clostridium,* and *Veillonella* (4,7).

From birth onwards, the intestinal microbiota evolves together with the host and the host's metabolic and neurological programming, and the development of this microbial community is, therefore, of crucial importance for maintaining health in adulthood (1).

Factors affecting the composition and diversity of the infant's intestinal microbiota

As mentioned above, numerous factors influence the diversity of the intestinal microbiota in humans. In addition to the factors present i*n utero*, the composition and diversity of the intestinal microbiota are influenced by geographic origin, method of delivery (vaginal or C-section), the time of delivery (at term or before term), the way newborns are fed (breastfeeding or feeding with formula), and exposure to animals and antibiotics (5,7,10–12).

A number of studies have shown that the method of delivery affects the intestinal microbiota of newborns. Some studies even suggest that C-sections may contribute to the development of dysbiosis of the gut microbiome of the newborn. A lower level of total microbial diversity and reduced amounts of *Bifidobacterium, Bacteroides* and *Lactobacillus* and increased levels of *Clostridium difficile* were found in newborns born by C-section, while their intestinal microbiota was most similar to the skin microbiota, which is dominated by *Staphylococcus, Corynebacterium,* and *Propionibacterium*. In newborns born vaginally, dominate *Lactobacillus*, *Prevotella*, or *Sneathia*, and it is believed that, due to exposure to the mother's vaginal and fecal microbiota, the first species that colonize the intestines of newborns are facultatively anaerobic bacteria such as *Escherichia* and other members of the *Enterobacteriaceae* family (5–7,9). Newborns who were born before term have a less diverse microbiome and higher levels of *Enterobacter*, *Enterococcus*, *Klebsiella*, and *Staphylococcus* compared to term newborns, and these differences persist until at least the fourth year of the child's life (4).

The way an infant eats has a great influence on the composition and diversity of the intestinal microbiota. In breastfed children, the intestinal microbiota has a lower diversity but higher levels of *Bifidobacterium*, including *Bifidobacterium*

Table 1. A list of selected gut microbiota bacteria taxa and the impact of changes in the body's abundance on health in young children

Genus	Low relative abundance	High relative abundance	Ref.
Enterobacter	IBD	Cystic fibrosis	(14), (15)
Enterococcus	allergic sensitization in neonates	higher cumulative incidence of respiratory infections, acute appendicitis, autism spectrum disorder, infant colic, critical congenital heart disease, systemic inflammation, diarrhea, ADHD	(16),(17), (18), (19), (20), (21), (22), (23), (24)
Streptococcus	better joint attention in infants	IBD, autism spectrum disorder, severe acute malnutrition, diarrhea, cholangitis	(14), (19), (25), (26), (23), (27)
Staphylococcus		coeliac disease, obesity	(28), (29)
Bifidobacterium	Asthma, atopic wheeze, higher cumulative incidence of respiratory infection, compromised immune function, autism spectrum disorder, stunting, infant colic, coeliac disease, critical congenital heart disease, functional abdominal pain	Protection against sepsis and necrotizing enterocolitis in preterm infants, a protective effect on several neuropsychiatric disorders, better joint attention in infants, juvenile idiopathic arthritis, higher humoral and cellular vaccine response	(16), (30), (31), (32), (33), (19), (25), (26), (34), (21), (28), (35), (22), (36), (36), (37)
Lactobacillus	allergic sensitization in neonates, eczema, IBD, infant colic, coeliac disease	Inhibition of the adhesion and growth of GIT pathogens, autism spectrum disorder, obesity, functional abdominal pain	(17), (38), (14), (19), (39), (20), (34), (28), (36)
Bacteroides	Cystic fibrosis, severe acute malnutrition, H. pylori infection	Asthma, wheezing, eczema, obesity, diarrhea, type 1 diabetes	(17), (40), (29), (29), (29), (36), (41)
Prevotella	autism spectrum disorder, stunting, behavioral problems	IBD, infant colic, coeliac disease, functional abdominal pain, functional constipation	(14), (19), (26), (21), (28), (42), (36)
Ruminococcus	Asthma, atopic wheeze	negative correlation with atopic diathesis in childhood and obesity, autism spectrum disorder, infant colic	(13),(16), (19), (21)
Clostridium	IBD	Asthma, wheezing, eczema, acute appendicitis, autism spectrum disorder, obesity, severe intestinal inflammation, diarrhea and pseudomembranous colitis (C.difficile), H.pylori infection	(17), (31), (14), (18), (19), (39), (43), (36)
Veillonella	Increased risk for asthma, wheezing, acute appendicitis	Significantly increased in children with ADHD, infant colic, cystic fibrosis, cholangitis, functional abdominal pain	(16),(17), (33), (18), (21), (15), (35), (40), (27), (36)
Klebsiella		higher cumulative incidence of respiratory infection, higher risk of developing necrotizing enterocolitis, acute appendicitis, infant colic, cystic fibrosis	(16), (44), (18), (20), (34), (35)
Oscillospira		negative correlation with atopic diathesis in childhood and obesity	(13)
Pseudomonas		Cystic fibrosis, lower humoral and cellular responses to these vaccines	(15), (37)
Escherichia / Shigella		Eczema, IBD, severe acute malnutrition, stunting, infant colic, coeliac disease, cystic fibrosis, necrotizing enterocolitis, diarrhea, cholangitis, lower humoral and cellular responses to vaccines	(17), (14), (26), (20), (15), (34), (21), (28), (45), (23), (35), (27), (37)

breve, Bifidobacterium bifidum, and *Bifidobacterium longum* (1). Also, lower levels of bacteria *Escherichiae coli* and *Clostridium difficile*, as well as *Bacteroides* and *Lactobacillus* were found in breastfed children, while the intestinal microbiota of children fed with adapted milk formula had a higher proportion of *Bacteroides, Clostridium coccoides,* and *Lactobacillus* (5).

Exposure to pets early in life also has a certain impact on the intestinal microbiota, and pets generally contribute to microbial diversity in the household while reducing the risk of developing atopic diathesis. Nermes et al. (2015) state that there is a higher probability that the *Bifidobacterium pseudolongum* strain (of animal origin) will be found in the feces of infants who live with pets, while Tun et al. (2017) state that exposure to pets contributes to a significant increase in *Oscillospira* and *Ruminococcus*, which are in negative correlation with atopic diathesis in childhood and obesity (5,13).

Exposure to antibiotics can significantly affect the intestinal microbiota of newborns/infants due to its plasticity during the first years of life. The use of antibiotics is associated with a decrease in the levels of *Bifidobacterium* and *Bacteroides*, while the recovery or increase in *Bifidobacterium* levels is correlated with the duration of antibiotic therapy. Along with the decrease in the levels of Bifidobacterium, the dominance of Enterococcus was observed after the application of antibiotic therapy. When using antibiotic prophylaxis during childbirth (mothers positive for Group B *Streptococcus*), lower levels of *Bifidobacterium* were observed with an increase in the level of *Clostridium*, along with a reduced representation of *Actinobacteria* and *Bacterioidetes,* and an increased representation of bacteria from the *Proteobacteria* genus (4,5,7).

The research results on intestinal microbiota and the connection of its diversity and composition with different pathological conditions showed that the relative abundance of gut commensal bacteria genera could be associated with higher or lower risk of some health conditions, as stated below in Table 1. However, it is important to emphasize that each member of the intestinal microbiota has an important role in the body, and for the preservation of homeostasis, in the context of the intestinal microbiota, their optimal ratio is crucial.

Table 1. lists health conditions and changes in the relative abundance of bacterial taxa associated with them. It is important to emphasize that a clear cause-and-effect relationship between the abundance of some bacterial taxa and health conditions is still unknown, and the connection between intestinal microbiota and health is still being intensively investigated. What we could conclude from these data is that a greater or lesser risk of developing some diseases is related to the fact that the intestinal microbiota is directly involved in the modulation of the immune response of the host, but also in many other metabolic and signaling pathways in the body that play a role in disease pathogenesis. Intestinal microbiota and the association of changes in its composition and the relative abundance of certain bacterial taxa with different pathological conditions could also provide a new therapeutic direction for these conditions and may be used as potential early disease markers.

Environmental/geographical variance of infants' microbiota status

When interpreting and comparing the results of different studies, it is important to take into account that the diversity and composition of the intestinal microbiota are influenced by the geographical area and environmental factors; living in different parts of the world also implies different cultural areas that shape specific, culturally conditioned eating habits and diet, access to childbirth and medical treatment and influence many other aspects of life that in turn have an impact on the colonization and formation of the intestinal microbiota in early life (46–49). Although intrinsic (genetic) factors partially influence the composition of the intestinal microbiota, research indicates that, ultimately, the greatest role in shaping the intestinal microbiota is played by extrinsic environmental factors (47,50,51).

In a 2023 study, differences in the composition of breast milk and intestinal microbiota of infants were compared between five rural and urban regions in Vietnam. The research results showed a strong connection between the geographical region, breast milk composition, and infants' intestinal microbiota. The authors suggest that the found differences are most likely related to the mother's diet; the dietary patterns themselves were conditioned by the geographic region and the socioeconomic and nutritional status of the mother. The influence of the mother's diet on the composition of the breast milk microbiome and, consequently, on the intestinal microbiota of infants is also confirmed by other studies (46,52,53).

In a study that compared the development of the intestinal microbiota of infants from Western countries and India, Bharadia et al. state that the process of intestinal colonization is influenced by the conditions in which the infant grows – in Western, more developed countries, a more hygienic environment prevails, which, in contrast to developing countries, limits the infant's exposure to pathogenic microbes; exposure to pathogens at an early age, i.e., the hygienic environment in which the child grows has an impact on the process of forming the intestinal microbiota. For example, colonization of the child's intestines with enterobacteria such as E. coli has been delayed in developing countries (54). Conversely, when we talk about the earliest (infant) age, although there are some differences in the diversity and composition of the intestinal microbiota of infants from India and Western countries, their intestinal microbiota composition is very similar, which points to the importance of infant nutrition as a primary factor in shaping intestinal microbiota (54,55).

According to Stearns et al., ethnicity also has an impact on the composition and diversity of the gut microbiota. Their research found an association between bacterial genera such as *Streptococcus, Enterococcus, Lactobacillus, Ruminoccocus, Blautia,* and *Oscillospira* and ethnicity, suggesting the existence of different metabolic processes within the intestinal microbiome of South Asian and Caucasian infants. The authors suggest that in the first year of life, the intestinal microbiota is shaped by ethnicity and breastfeeding, while ethnic differences in the intestinal microbiome may be conditioned by differences in mother/child nutrition, which again speaks in favor of nutrition as the most important extrinsic factor in shaping the intestinal microbiota (56).

As mentioned earlier, the gut microbiota plays a central role in shaping the host's immune response; identifying geographic differences in gut microbiota and the factors that influence them could provide a new direction in improving health outcomes in children by optimizing population-specific interventions (57).

INTESTINAL MICROBIOTA OF BREASTFED CHILDREN

Breastfeeding is considered the gold standard of infant nutrition and, due to the numerous advantages of breastfeeding, the World Health Organization (WHO) recommends that breastfeeding should be the exclusive way of feeding infants for the first six months of life (58). Breastfeeding plays a significant role in shaping the infant's intestinal microbiota, which is crucial for the development of the immune system and can affect the host's physiology in different ways, for example, through the production of various metabolites that have a physiological function in the body (30).

Human milk oligosaccharides

The most common solid component of breast milk, along with lipids and lactose, are human milk oligosaccharides (HMOs), i.e., natural probiotics. Oligosaccharides of human milk are complex sugars, highly present in mother's milk, and serve as substrates for specific microbes, including certain types of *Bifidobacterium* - it is believed that the bifidobacteria in the intestines of infants are the most efficient in assimilating HMOs (30,59). HMOs provide a selective nutritional advantage to the beneficial microbes of the gastrointestinal system of infants - one such strain, adapted specifically to metabolizing HMOs, is *Bifidobacterium longum subspecies infantis (B. infantis)* (60). HMOs in infants stimulate the growth of strains of beneficial bacteria, such as *Bifidobacterium infantis*, and thereby contribute to the protection of the gastrointestinal tract (and thus the infant) from potential pathogens. HMOs have the ability to inhibit the adhesion and invasion of pathogenic bacteria, while having an antimicrobial effect on certain pathogens (38).

The coevolution of *Bifidobacterium* and the host, mediated by human milk oligosaccharides, greatly affects the colonization of the intestine in early life and has a very important, critical effect on the immune system and its development. It is believed that the depletion of specific microbes, including *Bifidobacterium*, can compromise immune function and lead to an increased risk of developing atopic diathesis, immune diseases such as asthma, and increased susceptibility to infectious diseases (30).

All HMOs are based on lactose (they contain lactose at the reducing end) and consist of five monosaccharides – glucose, galactose, N-acetylglucosamine, fucose, and sialic acid (61). HMOs are structurally very diverse, and more than 200 different human milk oligosaccharides have been described to date. The largest amount of HMOs is found in colostrum (up to 25 g/L), but they can also be found in mature breast milk (up to 10 g/L). Digestion of HMOs does not occur in the upper part of the digestive system, but the undigested ones reach the large intestine, where they serve as a substrate for intestinal microbes. The use of HMOs by the intestinal bacteria of infants results in the secretion of microbial metabolites such as short-chain fatty acids (SCFA) and lactate, which have a significant impact on intestinal homeostasis. HMOs can also prevent the adhesion of pathogens to the intestinal mucosa of infants and can directly modulate the immune and epithelial response (62–64).

HMOs contain numerous non-nutritive bioactive components that affect the health of infants, such as α-lactalbumin, lysozymes, lactoferrin, and secretory immunoglobulin A (sIgA). Secretory immunoglobulin A prevents the adhesion of pathogens such as *Escherichia coli, Vibrio cholerae*, rotavirus, cytomegalovirus, and *Candida albicans* to the intestinal mucosa, lactoferrin has a bacteriostatic and bactericidal effect, while lysozymes inhibit the reproduction of pathogenic bacteria by acting synergistically with lactoferrin. HMOs also contain cytokines, such as tumor necrosis factor α (TNF-α), interleukins, transforming growth factor β, and interferon γ, which have an immunomodulatory effect, provide passive protection and have the ability to influence the immune response through T cells, shifting their response to a balanced production of Th1/Th2 cytokines (38,65).

Breast milk microbiome

In addition to being a source of nutrients and bioactive substances, breast milk is also a source of commensal bacteria that contribute to improving infant health by preventing the adhesion of pathogens and promoting the colonization of beneficial microbes in the gastrointestinal system. Although in the beginning, it was considered that breast milk was sterile and that microbes isolated from it were considered contaminants (originating from the mother's skin, from the infant's oral cavity, or arising from improper handling of expressed milk), today, it is known and widely accepted that breast milk has its own unique microbiome (63). The most common bacteria found in human breast milk include *Staphylococcus, Streptococcus, Lactobacillus, Pseudomonas, Bifidobacterium, Corynebacterium, Enterococcus, Acinetobacter, Rothia, Cutibacterium, Veillonella,* and *Bacteroides*. Fitzstevens et al. suggest that Streptococcus and Staphylococcus are the dominant genera of bacteria in breast milk (65,66).

Huang et al. conducted a study in 2022 examining the influence of the breast milk microbiome on the intestinal microbiome of infants. Although it is now known that breast milk has its own microbiome, in some women, it has very low levels of bacteria and may even be sterile. A total of 17 samples were tested in this research, and in the breast milk samples that were not classified as sterile (11 of them), predominated *Firmicutes* (73.1%), *Proteobacteria* (9.2%), *Actinobacteria* (6.3%), and *Bacteroidetes* (2.2%). Specific intestinal bacteria, such as *Bifidobacterium* and *Bacteroides*, were present in 10 out of 11 tested samples. The other 6 samples that were used in this research were classified as sterile or with a very low number of bacteria. The results of this research showed that infants fed milk containing bacteria and those fed sterile milk had a similar composition of intestinal microbiota but differed in microbial diversity - the intestinal microbiota of infants fed milk with bacteria present was richer. The intestinal microbiota of infants was dominated by *Proteobacteria*

and *Actinobacteria*, while the most abundant bacteria were *Bifidobacterium, Escherichia-Shigella,* and *Bacteroides* (67).

Le Doare et al. state that the bacteria present in breast milk can have an immediate as well as a long-term role in reducing the frequency and severity of bacterial infections of the gastrointestinal tract. For example, *Lactobacillus* isolated from breast milk inhibits the adhesion and growth of pathogens of the gastrointestinal system, such as *Shigella, Pseudomonas and Salmonella,* and *Escherichia coli* (38).

Although the mechanisms are not yet fully elucidated, some authors suggest the existence of vertical transmission of bacterial species from mother to child, which includes *Bifidobacterium* typical of the intestines of infants *(B. adolescentis, B. angulatum, B. breve, B. dentium, B. longum, B. pseudolongum, and B. thermacidophilum)* but also bacteria that are usually found in the intestines of adults, such as *Ruminococcus bromii* and *Coprococcus comes* (68,69).

Intestinal microbiota of breastfed children

In order for the newborn to develop optimally, exclusive breastfeeding is recommended during the first 6 months of life because, in addition to the nutritional component, mother's milk also contains a number of non-nutritive but very valuable bioactive substances such as vitamins, minerals, HMOs, cytokines, lactoferrin, hormones, microRNA, lysozymes, etc. (62,70,71). Numerous studies have also proven the presence of bacteria in breast milk. Although this area is still intensively researched, they suggest that there is a vertical transfer of bacteria from mother to infant through the ingestion of breast milk (68,69).

When we consider all the components of breast milk, exclusive or partial breastfeeding is the most significant factor affecting infants' intestinal microbiota structure. In infants fed breast milk, higher levels of bacteria from the genus *Bifidobacterium* were found. They are the dominant bacteria in the gastrointestinal tract of infants during breastfeeding. In contrast, the cessation of breastfeeding is associated with a decrease in the number of *Bifidobacterium* and with a faster maturation of the intestinal microbiome, i.e., a higher prevalence of *Firmicutes*. Higher levels of *B. bifidum, B. breve, B. dentium, Lactobacillus rhamnosus, and Staphylococcus epidermidis and lower levels of Escherichia coli, Tyzzerella nexilis, Eggerthella lenta, Ruminococcus torques,* and *Roseburia intestinalis* were recorded in breastfed children (58,72). *B. breve, B. longum, B. dentium, B. infantis, and B. pseudocatenulatum* are the most frequently detected bacteria from the *Bifidobacterium* genus that influence the establishment of a healthy intestinal microbiome (9).

INTESTINAL MICROBIOTA OF FORMULA FED CHILDREN

Although mother's milk is the gold standard of nutrition for infants and the recommendations speak in favor of exclusive breastfeeding in the first 6 months of life, there are numerous situations when breastfeeding, i.e., feeding with mother's milk, is not an option. When breastfeeding is not possible, adapted milk formula becomes the primary way of feeding infants in the first 6 months of life (and sometimes longer). Today, there are many different formulas of infant formula, but although considerable efforts are made to make the infant formula resemble breast milk as much as possible, there are obvious differences in nutrients, components, and the effect on the development of intestinal microbiota (63).

Adapted milk formulas

Despite the recommendations, the sale and, consequently, the use of adapted milk formulas is on the rise. In the period from 2005 to 2019, the consumption of adapted milk formulas increased by 121.5% at the world level (73). Adapted milk formulas, despite being necessary for infant nutrition when breastfeeding is not an option, are not without risks. For example, Kong et al. suggest that nutrition with adapted milk formula is significantly associated with the prevalence of obesity in childhood, while Ratsika et al. state that adapted milk formula feeding is associated with a faster growth curve, i.e., greater weight gain, increased adiposity, and a higher risk of obesity (73–75).

Human breast milk is a dynamic liquid, unique in its composition. In addition to genetic factors, the composition of breast milk is influenced by the mother's diet and health, body composition, and geographic region (76). The goal of adapted milk formulas is to mimic breast milk's nutritional composition and functionality. Given that breast milk is a very dynamic liquid and its components change during the lactation period, it is very difficult to design a formula that would be equal in composition to breast milk, especially when we talk about bioactive, non-nutritive components (9,76).

Adapted milk formulas today are mostly derived from cow's or soy milk (9). Therefore, most of the proteins are derived from cow's milk and are of lower quality compared to proteins derived from human milk. These differences in protein quality are attributed to the limited amount of essential amino acids present in cow's milk (75).

Nowadays, industry and scientists are putting a lot of effort into designing an adapted milk formula that is most similar to mother's milk. The practice of enriching adapted milk formula with probiotics and prebiotics was adopted because infants fed with adapted milk formula lack the microbes that can be found in breast milk, thus encouraging the growth of commensal bacteria in the intestines of infants (9,63). Therefore, several new approaches have been developed in order to add bioactive ingredients such as lactoferrin, probiotics, prebiotics (fructooligosaccharides (inulin-type fructans), and galactooligosaccharides originating from cow's milk) and milk fermentation products (postbiotics) to the adapted milk formula (75,77–79). Synbiotic-adapted milk formulas have also been developed, which are based on the concept of the synergistic effect of probiotics and prebiotics on the optimal development of the intestinal microbiota of infants. Such formulas are enriched with probiotic bacteria and prebiotic oligosaccharides (80). Oligosaccharides in adapted milk formulas have a bifidogenic effect and stimulate the growth of beneficial bacteria from the genus *Bifidobacterium* while reducing the levels of *E.coli.* Probiotics such as *Bifidobacterium* and *Lactobacillus* are non-pathogenic microorganisms that stimulate the growth of beneficial intestinal flora (9).

Considering the significant role of HMOs in the development of the intestinal microbiota of infants, there are efforts to enrich adapted milk formulas with industrially produced HMOs. The European Union and the US Food and Drug Administration have approved the use of two HMOs for the purpose of fortification of adapted infant milk formulas: 20-fucosylactose (20-FL) and/or lacto-N-neotetraose (LNnT) (59,62).

Regardless of the efforts of science to bring the design of adapted milk formula closer to the composition of mother's milk and the fact that, in some cases, adapted milk formula is the only choice for infant nutrition, certain controversies are associated with adapted milk formulas. Most criticism of adapted milk formula focuses on its aggressive and often potentially misleading marketing and the prominent health and nutrition claims on the product. Chueng et al. conducted a cross-sectional study focused specifically on the health and nutritional claims made on adapted milk formulas and their support by scientific evidence. The results of this research showed that most of the products had at least one health and/or nutritional claim highlighted and that the said claims were not supported by solid scientific evidence, while the products did not provide relevant scientific references to support the said claims. The main problem of stating such claims on the packaging of adapted milk formulas is indirectly highlighting the advantages of adapted milk formulas in relation to breastfeeding and thus indirectly undermining breastfeeding (81).

Intestinal microbiota of children fed with adapted milk formula

Many studies have proven the existence of differences in the intestinal microbiota of children fed with mother's milk and children fed with adapted milk formulas. Exclusively breastfed children have a stable and more uniform microbiome with fewer microbial species present, and supplementary feeding with relatively small amounts of adapted milk formula can change it in the direction of a microbiome with a wider and more diverse spectrum of microbial organisms (82).

In breastfed children, there is a higher level of Bifidobacterium, while in children fed with adapted milk formula, the intestinal microbiota is more diverse and includes *Staphylococcus, Bacteroides, Enterococcus, Atopobium,* and *Clostridium*. In infants fed exclusively with adapted milk formula, there is a higher prevalence of bacteria such as *E. coli, B. fragilis, C. difficile,* and *Lactobacillus* (6,9). In children who were primarily breastfed but also received formula for a short period, a decrease in the number of bacteria from the *Bifidobacteriaceae* family was observed, along with an increase in the level of bacteria from the *Enterobacteriaceae* family (compared to the children who were never fed with adapted milk formula) (70).

In infants fed with adapted milk formula, a higher prevalence of opportunistic pathogens such as *Klebsiella pneumoniae, Klebsiella oxytoca, Staphylococcus epidermidis, Staphylococcus aureus,* and an abundance of *Clostridiaceae* (a class of bacteria involved in amino acid fermentation) was found (82,83).

The different compositions of adapted milk formulas can also affect the development and composition of the intestinal microbiota. Although depletion of *Bifidobacterium* is present when using any formula milk, formula milk with a reduced lactose content can lead to a greater reduction in the prevalence of bacteria from the *Bifidobacteriaceae* family compared to standard formula milk containing lactose. Also, formulas with a reduced lactose content can lead to a significant increase in the prevalence of *Acidaminococcaceae* and *Lachnospiraceae* families compared to standard formulas (84).

Synbiotic-adapted milk formulas enriched with *Bifidobacterium* breve showed success in stimulating colonization of the intestines of infants with *Bifidobacterium*, and their intestinal microbiota was comparable to that of children who were born vaginally and fed breast milk. Furthermore, such adapted milk formulas could have a positive long-term effect on the intestinal microbiota of both healthy infants and infants at risk of dysbiosis (85,86).

As stated earlier, one of the advantages of exclusive breastfeeding is maintaining the level of secretory IgA in the gastrointestinal tract, considering that in the first few months of life, the infant does not have the ability to synthesize endogenous sIgA. Secretory immunoglobulin A, as part of gut-associated lymphoid tissue (GALT), plays a significant role in the immune defense of the gastrointestinal tract and represents the first line of defense against pathogenic microorganisms. Adapted milk formulas containing bioactive compounds formed during the fermentation process, including the combined activity of *Bifidobacterium* breve C50 and *Streptococcus thermophilus* ST065, have a positive effect on the intestinal microbiota of infants in terms of increasing the level of *Bidfidobacterium* in stool, reducing stool pH, and reducing the number of *Clostridium* spores and *Bacteroides fragilis*. Adapted milk formulas containing these bioactive compounds, i.e., postbiotics, in combination with prebiotics, have positive effects on the intestinal microbiota of formula-fed infants. These effects include an increase in the number of *Bifidobacterium* and a rise in sIgA levels, thus bringing the intestinal microbiota of formula-fed infants closer to the intestinal microbiota of exclusively breastfed infants (87).

DISCUSSION

The intestinal microbiota is a diverse and dynamic community of microorganisms consisting of bacteria, archaea, fungi, and their viruses and phages. Although neglected for a long time, today, it is widely accepted that precisely the intestinal microbiota plays a role as a central regulator of the host's metabolism and immune function. Its composition and function, and, consequently, the host's health, are influenced by many factors, including nutrition, that is, eating habits and lifestyle.

Recent studies suggest intestinal microbiota development begins already in utero, under the influence of maternal microorganisms and their metabolites. When we talk about children, more specifically newborns and infants, in addition to factors present in utero, the early colonization of the intestinal

microbiota and its further development is influenced by the method of delivery (cesarean section or vaginal delivery), the term of delivery (delivery at term or before term/premature), exposure to antibiotics (in utero, during and after birth), and environmental factors such as exposure to pets.

The nutrition of newborns/infants is the most significant factor affecting their intestinal microbiota. Breast milk is a very dynamic liquid with a unique composition, which changes during the period of breastfeeding or lactation. In addition to being a source of nutrients and an ideal way of feeding infants and providing them with the optimal ratio of nutrients necessary for proper growth and development, breast milk also contains numerous bioactive components as well as commensal bacteria, which also play a role in the formation of the intestinal microbiota of infants. In addition to vitamins and minerals, other bioactive non-nutritive components of breast milk contribute significantly to infants' health by participating in shaping the infants' immune response.

Exclusive breastfeeding is the gold standard of infant nutrition, and adapted milk formulas are associated with slightly higher health risks in childhood and adulthood. The production and marketing industry also faces increasing criticism for promoting these formulas. However, there are situations when breastfeeding is simply not an option, making adapted milk formula the only means of feeding an infant in the first six months of life. There are numerous variations of adapted milk formulas, which primarily originate from cow's milk or soy milk. Many studies have proven differences in the intestinal microbiota of infants based on their feeding method, while some recent studies link adapted milk formulas to various health problems in both childhood and adulthood. Therefore, considerable efforts are made to make the composition of adapted milk formulas as similar as possible to the composition of mother's milk, and some of the newer technologies include the enrichment of such formulas with prebiotics, probiotics, synbiotics, postbiotics, or some of their combinations.

Generally speaking, the intestinal microbiota of exclusively breastfed children is stable and more uniform, with the predominance of *Bifidobacterium*, while children fed with adapted milk formula (exclusively or in combination with breast milk) have a more diverse intestinal microbiota, i.e., bacteria from the genera *Staphylococcus, Bacteroides, Enterococcus, Atopobium,* and *Clostridium*. Furthermore, the absence of breast milk nutrition in infants can result in atopic diathesis, an increased risk of developing immune diseases, susceptibility to infections of the gastrointestinal system of bacterial and/or viral origin, and an increased risk of developing obesity in childhood (and consequently in adulthood).

Further research is necessary in order to determine the exact extent of the influence of the intestinal microbiota and its alterations on the host health. Although numerous studies have been conducted with infants, they have certain limitations. These include, for example, variations in adapted milk formulas, marked individual specificity in breast milk composition, demographic variations depending on the geographical areas, and the impossibility of distinguishing the individual effects of different factors on growth, development, and immune response, etc. As a result, it is very difficult to draw universal conclusions about the influence of infant nutrition on the intestinal microbiota and the influence of its alterations on health both in childhood and adulthood.

In conclusion, although not all the mechanisms by which intestinal microbiota influences the development and health of the host are known, it is undeniable that nutrition is the most important factor affecting its composition and function from an early age. Furthermore, the results of numerous studies suggest that feeding with breast milk has a beneficial effect on the development of intestinal microbiota, which then has an immunoregulatory role but also a role in metabolism and other physiological functions that contribute to the host's growth, development, and health.

Knowledge from previous studies could be useful in promoting breastfeeding as the gold standard of infant nutrition, which has an optimal effect on the growth and development of the child. This may also reduce the amount and rational use of antibiotics during pregnancy and after childbirth, and encourage vaginal delivery whenever possible, thereby reducing the number of (non-indicated) cesarean deliveries. Certainly, both industry and science should continue striving to bring the composition of formula milk closer to that of breast milk. While the benefits of breast milk and the risks associated with formula are well known, there are cases where breastfeeding is not an option, and formula becomes the only available choice.

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