

Literature Review: Infant formulas with human milk oligosaccharides on gut microbiome and intestinal immune system

Literature Review: Dampak susu formula bayi pada mikrobiota dan sistem kekebalan usus

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Abstract

Background: Infant formula has a similar composition to breast milk, especially on key components like proteins, fats, and functional oligosaccharides. However, significant differences exist between bovine milk-based formula and human breast milk, particularly in the abundance and diversity of human milk oligosaccharides (HMOs). HMOs are pivotal in shaping the infant gut microbiome and immune system, with potential implications for infant health and development.

Objectives: The literature review aimed to explore the role of HMOs in shaping the gut microbiome and strengthening the intestinal immune system in infants.

Methods: Descriptive research was carried out in May-August 2023 using the literature review method. The design and implementation of this study referred to the guidelines outlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) for Randomized Controlled Trials (RCTs). Literature sources were obtained from PubMed, Elsevier, Medline, Nature, PLOS, MPDI, JAMA, and Scopus publication databases with a limitation of the last ten years (2013-2023). One hundred twenty articles were obtained with these keywords and then selected in stages according to predetermined criteria. Six articles that met the criteria were retrieved from 2013-2023. The articles obtained were compiled, analyzed, and concluded by looking for similarities and dissimilarities, giving views, comparing, and summarizing.

Results: The review revealed that HMOs significantly influenced the composition and function of the infant's intestinal microbiota, inhibiting harmful pathogens in the intestine. Therefore, HMOs as prebiotics were crucial in promoting intestinal immune system health in infants.

Conclusion: Human Milk Oligosaccharides (HMOs) in infant formula substantially impacted the gut microbiome composition and the infant's intestinal immune system. Studies consistently showed that HMOs influenced the growth and diversity of gut bacteria, leading to outcomes similar to those seen in breastfed infants.

Keywords

Formula, Intestinal immune, Microbiota, Oligosaccharides

Abstrak

Latar Belakang: Susu formula bayi memiliki komposisi yang mirip dengan Air Susu Ibu (ASI), terutama pada komponen-komponen utama seperti protein, lemak, dan oligosakarida fungsional. Namun, terdapat perbedaan signifikan antara susu formula berbasis susu sapi dan ASI, terutama dalam kelimpahan dan keragaman *Human Milk Oligosaccharides* (HMOs). HMOs memainkan peran penting dalam membentuk mikrobiota usus bayi dan sistem kekebalan tubuh, dengan potensi implikasi bagi kesehatan dan perkembangan bayi.

Tujuan: Tinjauan literatur ini bertujuan untuk mengeksplorasi peran HMOs dalam membentuk mikrobiota usus dan memperkuat sistem kekebalan usus pada bayi.

Metode: Penelitian deskriptif, menggunakan metode kajian literatur dilaksanakan bulan Mei-Agustus 2023. Dalam desain dan pelaksanaan penelitian ini, digunakan pedoman yang diuraikan dalam *Preferred Reporting Items for Systematic Reviews*

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and Meta-Analysis (PRISMA) untuk *Randomized Controlled Trials* (RCTs). Sumber literatur diperoleh dari basis data publikasi PubMed, Elsevier, Medline, Nature, PLOS, MPDI, JAMA, dan Scopus dengan batasan waktu 10 tahun terakhir (2013-2023). Sebanyak 120 artikel diperoleh dengan kata kunci tersebut, kemudian dipilih secara bertahap sesuai kriteria yang telah ditetapkan. Enam artikel yang memenuhi kriteria berhasil diambil dari periode 2013 hingga 2023. Artikel yang diperoleh dikompilasi, dianalisis, dan disimpulkan dengan mencari persamaan, perbedaan, memberikan pandangan, membandingkan, dan merangkum hasilnya.

Hasil: Studi literatur ini mengungkapkan bahwa HMOs secara signifikan mempengaruhi komposisi dan fungsi mikrobiota usus bayi, menghambat patogen berbahaya di usus. Oleh karena itu, HMO sebagai prebiotik sangat penting dalam mempromosikan kesehatan sistem kekebalan usus pada bayi.

Kesimpulan: *Human Milk Oligosaccharides* (HMOs) dalam formula bayi memiliki dampak signifikan pada komposisi mikrobioma usus dan sistem kekebalan usus bayi. Studi secara konsisten menunjukkan bahwa HMOs mempengaruhi pertumbuhan dan keragaman bakteri usus, menghasilkan hasil yang mirip dengan bayi yang diberi ASI.

Kata Kunci

Formula, Kekebalan Usus, Mikrobiota, Oligosakarida

Introduction

The gold standard in commercial infant formula milk resembles breast milk's composition. It is accomplished by carefully designing and optimizing key components such as proteins, fats, and functional oligosaccharides. Although bovine milk, which serves as the foundational ingredient in formula milk, can fulfill the primary energy and nutritional requirements for infant growth, it differs significantly from human breast milk in terms of its primary constituents and proportions. Specifically, human milk oligosaccharides (HMOs) are the third most abundant group of components in mature breast milk, ranging from 5 to 15 grams per liter, following lactose (55–70 g/L) and lipids (16–39 g/L). The diversity of HMO compositions exceeds that of oligosaccharides found in the milk of other mammals. Over 200 distinct molecular structures of HMOs have been identified (Petschacher & Nidetzky, 2016).

The formula utilization in Indonesia exhibited a notable increase, rising from 15% in 2003 to 79,8% in 2013. In Central Java, the prevalence of formula usage was considerably high, standing at approximately 89%, placing it sixth among all provinces in Indonesia (Fitriani et al., 2015). Long-term epidemiological studies have shown that formula-fed infants are more prone to various childhood disorders, including necrotizing enterocolitis (NEC), irritable bowel syndrome (IBS), obesity, allergies, and eczema, compared to breastfed infants (Yu et al., 2016). These health issues prevalent among formula-fed infants are closely linked to deviations in their gut

microbiota. HMOs are widely believed to play a crucial role in the differences observed in the microbial composition of the gut microbiota between formula-fed and breastfed infants (Marcobal et al., 2010).

Despite the wealth of information available regarding HMO molecular specificity and their health-promoting functions (Bode, 2012; Triantis et al., 2018; Walsh et al., 2020), limited information exists regarding HMO utilization strategies by typical infant bacteria, particularly *Bacteroides* and *Lactobacillus*, and the roles of HMOs in the development of the infant microbiome and gut immune function. Additionally, there is limited information about the recent application of artificial HMOs in infant formula and the regulatory framework surrounding them.

This review aims to critically summarize current knowledge and understanding of the potential mechanisms and strategies governing the cellular uptake and metabolism of HMOs by gut bacteria and their associated effects on the infant gut microbiome and immune functions. It also provides an overview of the key structural characteristics of HMOs and a brief survey of their commercial applications in infant formulas.

Method

In the design and implementation of this study, we adhered to the guidelines outlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) for Randomized Controlled Trials (RCTs) (Moher et al., 2009).

Inclusion criteria

Type of studies: All published full-text RCTs that investigated the development of the Intestinal Immune System and Gut Barrier Function in Infants were included in this review. However, conference abstracts, unpublished RCTs, questionnaires, randomized trials, and observational studies were excluded.

Type of participants: Infants aged 0-12 months, regardless of gestational age or health status, who had been exposed to HMOs through breast milk or HMO-supplemented infant formulas were eligible for inclusion in this review.

Type of interventions: Studies that investigated the supplementation of HMOs, either through breast milk or as an additive in infants' formulas, to enhance the development of the intestinal immune system and gut microbiome in infants were considered for inclusion.

Type of outcomes measured: Studies that reported outcomes related to the development and functionality of the intestinal immune system and gut microbiome in infants, such as markers of the intestinal immune response, gut microbiota composition, or gut barrier function.

Search approaches for identification of studies: PubMed, Elsevier, Medline, Nature, PLOS, MPDI, JAMA, and Scopus databases were searched between 2013 and 2023. In addition, we hand-searched the references of the selected studies to locate other potentially eligible studies. Some of the MeSH terms used in this literature review were "Human Milk Oligosaccharides" OR "HMOs" AND "Intestinal Mucosa" OR "Intestinal Immune" AND "Gastrointestinal Barrier" OR "Intestinal Barrier" OR "Intestinal Permeability" OR "Gut Permeability" AND "Randomized Controlled Trials" OR "Clinical Trials" AND "Infant Nutrition" OR "Infant Health" OR "Infant Immunity" OR "Infant Gut Health" OR "Infant Microbiota".

Data collection and analysis procedures followed the guidelines outlined in the Cochrane Handbook for Systematic Reviews of Interventions. Initially, the research team screened the study titles and abstracts to identify potentially eligible studies. The same authors independently assessed the full text for studies that met the inclusion criteria to determine their eligibility.

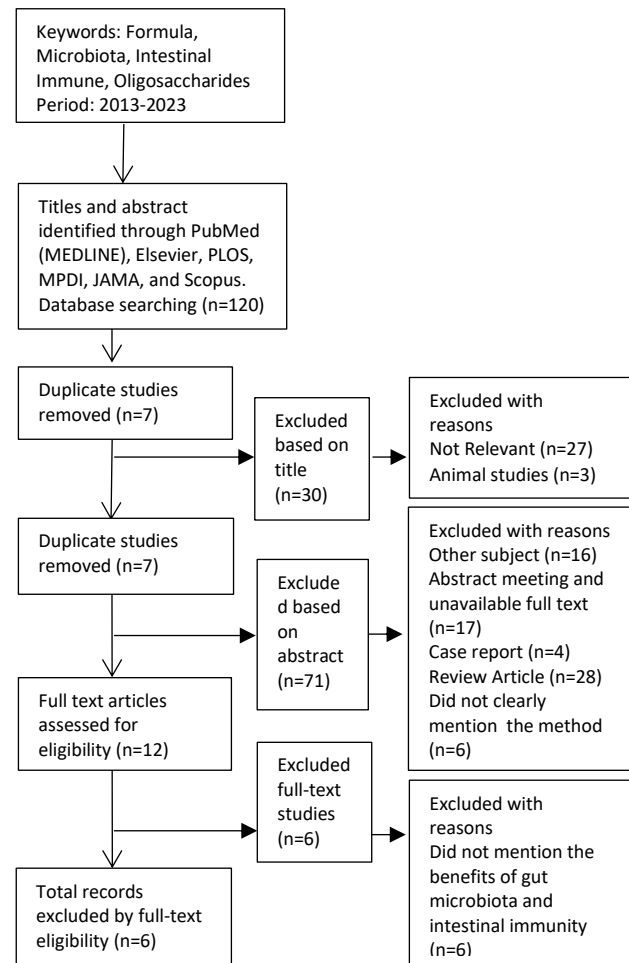


Figure 1. Flowchart of the search and publication selection

Data analysis was conducted by employing various analytical techniques, such as thematic, content, and statistical, to thoroughly assess and synthesize the findings from the six selected articles. In addition, throughout the design and implementation of this study, we adhered to the guidelines outlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) for Randomized Controlled Trials (RCTs).

Result

The studies for HMOs were randomized controlled trials (RCTs) that assessed the intestinal immune system, gut barrier function, and microbial composition (Figure 1). Relevant papers were identified based on their titles, and non-human studies, non-intervention studies, and review articles were excluded. Subsequently, the titles of the articles were used to identify non-relevant studies and exclude them. The abstracts of the remaining articles were then reviewed to

determine their suitability. Ultimately, six articles were included in this review.

Characteristics of the included studies

(Table 1) summarizes the characteristics of the six included trials. These studies employed the rigorous research design of Randomized Controlled Trials (RCT), focusing on healthy infants and systematically investigating the effects of formula with Human Milk

Oligosaccharides (HMOs) on various health outcomes, including the gut microbiota and intestinal immune system.

The incorporation of RCT allowed for the controlled and randomized allocation of participants into test and control groups, reducing bias and confounding variables and providing a more reliable assessment of the effects of HMOs in infant formulas.

Table 1. Characteristics of included trials

Study	Population	Study Groups	Duration	Outcomes
(Storm et al., 2019)	Healthy, full-term (≥ 37 weeks gestation; ≥ 2500 and ≤ 4500 g birth weight), singleton infants, ages 14 ± 5 days, who had been exclusively formula-fed for at least three days N=78	Test formula: infant formula with lactose, corn maltodextrin (70/30), and 0.25 g/L 2'FL Control formula: infant formula with lactose, corn maltodextrin (70/30)	Six weeks	An infant formula with 100% whey, partially hydrolyzed, as the protein source with the addition of 0.25 g/L of the HMO 2'FL and probiotic <i>Bifidobacterium lactis</i> is tolerated well based on a comprehensive tolerance assessment tool and is tolerated similarly to an otherwise identical formula without 2'FL.
(Berger et al., 2020)	Healthy, full-term infants ≤ 14 days of age N=175	Test formula: infant formula with 1.0 g/L 2'-fucosyllactose (2'FL) and 0.5 g/L LNnT Control formula: infant formula without HMOs Breastfed reference group	Six months	At three months of age, the microbiota of infants fed formula with HMOs was closer to that of the breastfed reference group with a fecal community type highly abundant in <i>Bifidobacteriaceae</i> . Specifically, an abundance of the genera <i>Escherichia</i> , <i>Bifidobacterium</i> , unclassified <i>Peptostreptococcaceae</i> , and <i>Streptococcus</i> in infants fed formula with HMOs was closer to that of the breastfed reference group. Formula-fed infants with abundant <i>Bifidobacteria</i> at three months of age were significantly less likely to require antibiotics during the first year of life.
(Bosheva et al., 2022)	Healthy and full-term infants with birth weight between 2,500 and 4,500 g, and aged 7-21	Test formula: infants formula with 1.5 g/L HMOs (test group 1, TG1); or with 2.5 g/L HMOs (test group 2, TG2)	15 months	An infant formula that included a specific blend of five HMOs was found to support the development of the intestinal immune system and gut barrier function.

	days N=185	Control formula: infant formula with standard cow's milk-based		Furthermore, it resulted in a gut microbiome resembling breastfed infants, with higher levels of <i>Bifidobacteria</i> , particularly <i>Bifidobacterium infants</i> , and lower levels of toxigenic <i>Clostridioides difficile</i> .
(Donovan et al., 2022)	Healthy term 37–42 weeks of gestation infants with a birth weight between the 5th and 95th percentiles and APGAR scores of 7 or greater at birth N=221	Test formula: the same formula also contains HMO 20FL at one g/L Control formula: the formulas consisted of a commercial product	16 weeks	Incorporating a physiologic level of 20FL did not affect the growth or occurrence of adverse effects in formula-fed infants. This observation provides additional evidence supporting the safe use of this HMO in infant formula. The presence of 20FL resulted in minor alterations in the microbiome, resembling those found in breastfed infants. This suggests that <i>Bifidobacterium</i> possesses enhanced metabolic capabilities when interacting with HMOs.
(Alliet et al., 2022)	Healthy infants < 14 days old N = 289	Test formula: same formula with added 1.0 g/L 2'FL Control formula: bovine milk-based formula containing <i>L. reuteri</i> DSM 17938 at one × 10 ⁷ CFU/g	6 months	<i>L. reuteri</i> -containing infant formula with 2'FL supports age-appropriate growth, is well-tolerated, and may play a role in shifting the gut microbial pattern towards that of breastfed infants, including increasing <i>Bifidobacteria</i> abundance.
(Hill & Buck, 2023)	Healthy singleton infants age 0–5 days and with birth weight > 2490 g N = 201	Test formula: infants formula with human milk oligosaccharide (HMOs) 20-fucosyllactose (20-FL) and galactooligosaccharides (GOS) Control formula: infant formula with 2.4 g/L GOS	Six weeks	An infant formula was fortified with HMO 20-FL, leading to a dose-dependent recovery of specific metabolites from microbial activity in the gastrointestinal tract, particularly secondary bile acids. These secondary bile acids are believed to potentially contribute to the development and maintenance of immune balance Our data suggests a connection between elevated levels of circulating secondary bile acids and the activation of systemic immune mediators.

Discussion

Definition and Types of Human Milk Oligosaccharides

Breast milk was unique because it contained more than 150 different structural variations of oligosaccharides at high concentrations, unlike other mammalian milk. The total concentration of oligosaccharides in breast milk, or Human Milk Oligosaccharides (HMOs), often exceeded the total protein concentration in mature milk, ranging from 5-15 g/L. HMOs are the third most abundant component after lactose and lipids, excluding water (Bode, 2012; Wicinski et al., 2020).

The three most abundant types of oligosaccharides in breast milk were non-fucosylated neutral HMOs, which accounted for 42-55% of total HMOs, with their derivatives being lacto-N-neotetraose (LNnT). Fucosylated neutral HMOs comprised 35-50% of total oligosaccharides in breast milk, including 2'-Fucosyllactose (2'-FL) or a combination of fucose and lactose. The third most abundant oligosaccharide was Sialylated acidic HMOs, which accounted for 12-14% of total HMOs, and their derivative was sialyl lactose (SL), a combination of N-acetylneuraminic acid and lactose (Dinleyici et al., 2023; Soyylimaz et al., 2021; Vandenplas et al., 2018)

Structures Identical to HMOs Added to Infant Formulas

Since 2016, specific infant formulas have included 2'-fucosyllactose (2'-FL) and lacto-N-neotetraose (LNnT). The safety of adding 2'-FL and LNnT alone or together in infant follow-on and young child formula was independently confirmed by both the European Food Safety Authority (EFSA) and the Food and Drug Administration (FDA) (EFSA NDA Panel, 2015), which are reputable institutions providing expertise on food-related matters. Furthermore, other HMOs were also accessible (Table 2)(Bych et al., 2019).

Table 2. Examples of commercially available structures identical to HMO in breast milk

2'-fucosyllactose	2'-FL
Lacto-N-neotetraose	LNnT
Lacto-N-tetraose	LNT
3-fucosyllactose	3-FL
Difucosyllactose	DiFL (or DFL or LDFT)
Lacto-N-triose II	LNT-II

Lacto-N-fucopentaose I	LNFP I
Lacto-N-fucopentaose III	LNFP III
Lacto-N-fucopentaose V	LNFP V
6'-sialyl lactose	6'-FL
3'-sialyl lactose	3'-FL

The Influence of Human Milk Oligosaccharides on the Composition of Infant Gut Microbiome

Bacterial community changes in the gut due to formula administration with HMOs in infants (Berger et al., 2020). They compared the types and levels of bacteria influenced by HMOs in formula-fed infants with those in the breastfed group. The alterations in the gut microbiome composition in infants fed with formula-containing HMOs and compared with breastfed infants (Bosheva et al., 2022).

Human Milk Oligosaccharides (HMOs) in formula led to changes in the gut microbiota, resembling those observed in breastfed infants. They observed higher Bifidobacteria and other beneficial bacteria levels in formula-fed infants with HMOs. The gut microbiome of infants fed with HMO-containing formula was more similar to breastfed infants, with increased levels of *Bifidobacteria*, particularly *B. infantis*, and lower levels of toxigenic *Clostridioides difficile* (Bosheva et al., 2022). Hill and Buck (2023) identified a correlation between the administration of HMO 20-FL and changes in the gut microbiome, indicating the potential role of HMOs in shaping the infant gut microbial pattern. These findings collectively suggest that HMOs played a significant role in modulating the composition of the infant gut microbiome, favoring the growth of beneficial bacteria and potentially providing health benefits for the infant's immune system and overall development.

The gut microbiota is a population of bacteria that lives in the gut and plays a role in digestion and influencing the immune system. HMOs help to increase the diversity and balance of gut microbiota, thus preventing the growth of pathogenic bacteria that can cause infection and disease (Ruhaak et al., 2014).

Human Milk Oligosaccharides and Their Role in Enhancing the Gut Barrier Function in Formula-Fed Infants

The gut barrier function was a critical aspect of the intestinal immune system that served as a protective barrier against harmful substances,

pathogens, and toxins. The presence of HMOs in infant formulas has been studied for its potential impact on enhancing the gut barrier function in formula-fed infants. Research indicated that HMOs played a prebiotic role in promoting the growth of beneficial bacteria, especially *Bifidobacteria*, in the infant gut. The fermentation of HMOs by these beneficial bacteria produces short-chain fatty acids (SCFAs) and creates an acidic environment that inhibits the growth of pathogenic bacteria. This microbial balance reinforced the gut barrier, preventing the attachment and colonization of harmful pathogens (Akkerman et al., 2019).

Furthermore, HMOs possessed unique properties that supported the gut barrier function. They could bind to the C-type lectin receptor on dendritic cells, preventing the uptake of viruses and potentially enhancing the immune response against infections. HMOs also acted as pathogen decoy receptors by binding to glycan structures on epithelial cells, effectively hindering pathogen attachment and invasion (Dinleyici et al., 2023; Soyilmaz et al., 2021; Vandenplas et al., 2018).

Studies have shown that infants fed with formula-containing HMOs exhibited gut microbiota composition and activities that resembled those of breastfed infants (Storm et al., 2019)—suggesting that HMOs might have contributed to establishing a robust and functional gut barrier, similar to the protective effects seen in breastfed infants (Bode, 2012; Wicinski et al., 2020).

Understanding the role of HMOs in supporting the gut barrier function was crucial, as a solid and intact gut barrier was essential for preventing gastrointestinal infections and promoting overall gut health in infants. The findings from these studies collectively emphasized the potential of HMO-supplemented formulas in mimicking the protective effects of breast milk on the gut barrier function, thereby contributing to the development of improved infant formulas with enhanced health benefits. Further research could advance infant nutrition and immune system development, ultimately benefiting infant health and well-being.

Impact of human milk oligosaccharides on the intestinal immune system of infants

HMOs significantly increased the local immune response and enhanced antibody production in the infant's intestinal immune system (Donovan et al., 2022). Additionally, HMOs were crucial in

influencing intestinal immune mediators and maintaining immunological balance in infants' gut linings (Hill & Buck, 2023). These findings provided compelling evidence for the beneficial impact of HMOs on the intestinal immune system of infants, suggesting that HMOs can effectively support the overall health and growth of infants through their positive influence on the intestinal immune response (Berger et al., 2020; Donovan et al., 2022; Hill & Buck, 2023).

HMOs act as a prebiotic agent that favors the growth of beneficial microbes over pathogens, thus safeguarding infants against infectious diseases (Markowiak & Ślizewska, 2017). Because pathogenic bacteria are less proficient in metabolizing HMO species, symbiotic bacteria can thrive and outcompete harmful invaders through competitive exclusion (Hoeflinger et al., 2015). Moreover, the metabolism of HMOs by *Bifidobacteria* produces organic acids that generate an acidic environment that inhibits pathogenic bacteria's growth (Thongaram et al., 2017; Walsh et al., 2020). HMOs deflect pathogens indirectly and directly impede pathogen entry by acting as soluble receptor decoys. Pathogenic viruses, bacteria, and protozoa require adherence to the 'glycocalyx' to attack the host and cause disease, which HMOs can obstruct (Kavanaugh et al., 2015).

The glycocalyx is the glycan-rich layer covering the epithelial cell surface and comprises glycans linked with protein or lipid (Kavanaugh et al. 2015; Kavanaugh et al. 2017). HMOs have a chemical structure similar to pathogenic glycans that attach to the epithelial cell surface (Newburg & Grave, 2014). When pathogens and toxins recognize and bind to HMOs instead of surface glycans, they traverse the digestive tract without causing any infection (Dorothy et al., 2018).

Conclusion

In conclusion, HMOs were consistently shown to have influenced the growth and diversity of gut bacteria resembling breastfed infants. Formula with HMOs increased beneficial *Bifidobacteria* and reduced harmful bacteria like *Clostridioides difficile*. HMOs acted as prebiotics, fostering the growth of beneficial bacteria and creating an acidic environment that inhibited harmful bacteria, providing infection protection.

Health institutions are expected to enhance healthcare services by providing information to the public through health education regarding using formula milk with HMOs for infants in need.

Conflict of Interest Declaration

This research does not include conflict between the author's interests and potential conflict agency interests in connection with research that has been done based on authorship and publication.

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