Review Article **Open Access**

The influence of *2'-fucosyllactose* on intestinal cell function: A comprehensive review of experimental studies

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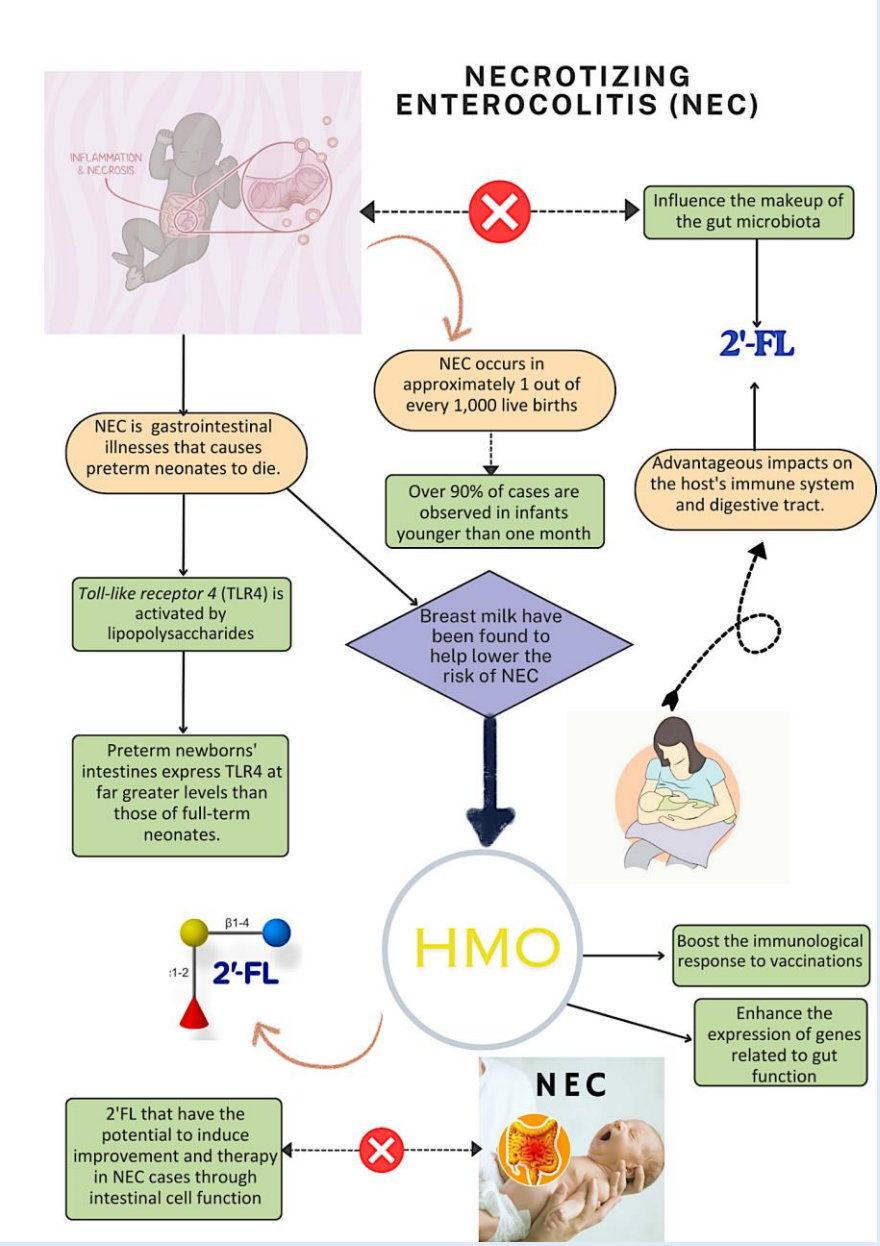
Submission Date: July 30th, 2025, **Acceptance Date:** September 29th, 2025, **Publication Date:** October 6th, 2025

Please cite this article as: Sulistijono E., Fitri L. E., Subandiyah K., Khotimah H. The influence of 2'-fucosyllactose on intestinal cell function: A comprehensive review of experimental studies. *Functional Foods in Health and Disease* 2025; 15(10): 701 – 711. DOI: https://doi.org/10.31989/ffhd.v15i10.1675

ABSTRACT

Premature new-borns confront considerable GI problems due to the immaturity of their digestive systems, one of which is Necrotizing enterocolitis (NEC). NEC is one of the most common gastrointestinal illnesses that causes preterm neonates to die. Certain elements in breast milk have been found to help lower the risk of NEC and previous research reveals that consuming supplement of human milk oligosaccharides (HMOs) could be a useful additional treatment for NEC and other gastrointestinal disease. This systematic review aims to analyze various experimental studies related to the administration of *2'-Fucosyllactose* (2'FL) that have the potential to induce improvement and therapy in NEC and other digestive disorder cases through intestinal cell function. There have not been many systematic reviews that specifically highlight the therapeutic effects of 2'FL on NEC, most of which still address the role of HMOs. The results show that the search flow uses the PRISMA Flowchart; after screening 133 titles and abstracts, 55 studies were accessed to verify the feasibility of the full text. Overall, there were 5 studies selected in this study for a comprehensive review. According to the study's findings, 2'-FL, one of the HMOs, has a number of advantageous impacts on the host's immune system and digestive tract. Among many other advantages, HMOs have been shown to boost the immunological response to vaccinations, influence the makeup of the gut microbiota, and enhance the expression of genes related to gut function.

Keywords: NEC, HMOs, 2'-FL, Intestine, microbiota.



Graphical Abstract: The influence of *2'-fucosyllactose* on intestinal cell function of NEC

INTRODUCTION

The most frequent cause of gastrointestinal illness-related death in premature newborns is *necrotizing enterocolitis* (NEC). There is intestinal tissue death with this syndrome, which often leads to sepsis and death. Since NEC was first identified, survival rates have not altered despite improvements in newborn care (1,2). According to research, the condition is brought on by lipopolysaccharide-induced activation of TLR4, which preterm children's intestines express at far higher levels than those of full-term infants (3).

Necrotizing enterocolitis (NEC) affects approximately 1 in every 1,000 live births, with an incidence rate between 5% and 10%, and over 90% of cases occur in newborns under one month old. In 2020, the Perinatology Division at the Department of Pediatric,

Dr. Saiful Anwar at Hospital Malang, recorded 82 NEC cases out of approximately 1,278 births. According to research, 7 out of 100 newborns with extremely low birth weights admitted to the Neonatal Intensive Care Unit are at high risk of developing NEC (4).

One of the typical components of breast milk is human milk oligosaccharides (HMOs). Oligosaccharides with different types and concentrations are the main differences between human breast milk and formula milk. Compared to human breast milk, the concentration of oligosaccharides in formula milk is lower and the type is less (5). To date, approximately 200 structurally diverse oligosaccharide types have been identified in human milk, whereas cow's milk contains only around 50 varieties (6,7).

Human breast milk contains oligosaccharides at concentrations ranging from 10-15 g/L, which is significantly higher than the levels found in cow's milk. Colostrum contains 20-25g/L of HMO, whereas mature breast milk has 10-15g/L. Based on an average energy content of 64 kcal per 100 mL of milk, this amounts to around 1.5–2.3 g per 100 kcal (5,8). The three primary forms of HMOs found in breast milk are *non-fucosylated* neutral HMOs (42–55%), *sialylated* (acidic) HMOs (12–14%), and *fucosylated* neutral HMOs (35–50%). More than 75% of the overall HMO content is composed of *fucosylated* neutral HMOs. However, 2'-FL is a member of the *fucosylated* group, *lact-N-neotetraose* (LNnT) is classified as a *non-fucosylated* neutral HMO (9,10).

Fasting, antibiotic medication, and surgery are the standard therapies for NEC; supportive measures including nursing, probiotics, and fecal microbiota transplantation have also been successful. The interplay between the host and microbial populations throughout the early prenatal and postnatal stages is a crucial element in the development of NEC. It has been shown that methods to improve the gut microbiota's composition, such tailored feeding schedules, probiotic use, and the responsible use of antibiotics, lower the risk of NEC (11–13). Furthermore, new research suggests that taking supplements of HMOs might be a useful supplementary treatment for NEC (14,15). This systematic review analyzes a variety of experimental studies related to the administration of 2'FL that have the potential to induce enhance treatment and outcomes in cases of NEC and other digestive disorder by influencing intestinal cell function.

Design Research: This research use of the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) guiding procedure is relevant to this investigation (16). To avoid bias, the entire procedure for the implementation of this study was carried out by two researchers.

Criteria for Inclusion and Exclusion: The requirements for inclusion in this study were based on *Population, Intervention, Comparator, Outcome*/PICO (Evidence-Based medicine), which consisted of [1]; Population: Laboratory experimental study conducting research on intestinal cell activity after administration of 2'FL [2]; Intervention: *2'-Fucosyllactose* (2'FL) [3]; Comparison: administration of other therapies other than 2'FL [4]; Outcome: intestinal cell activity. The exclusion criteria in this study were: 1. Inconsistency with the results of with the inclusion criteria; 2. Incomplete information; 3. Qualitative study; 4. Duplication of articles.

Search Strategy and Study Selection: A comprehensive search of journals was conducted across international databases including PubMed/Medline, Science Direct, Cochrane Library, ProQuest, and Google Scholar on literature published in the last 10 years. The keywords used consisted of the phrases "*2'-Fucosyllactose*", "intestinal cell function", "experimental studies", and other synonyms such as "gut epithelial cells", "effects of 2'-FL on intestines", and "*fucosyllactose and gut health*". Keywords were combined using Boolean operators (AND, OR), and experimental papers that incorporate *in vitro*

and *in vivo* were highlighted. The study selection was conducted by considering the research design, sample size, experimental methodology, and relevance of the results in exploring the influence of 2'FL on intestinal cell function.

Assessment of study quality: The experimental methods of this study were evaluated to ensure that the experimental procedures met the standards and were reliable in exploring the mechanisms and effects of 2'FL on intestinal cell function. Evaluation also includes consideration of adequate sample sizes, control of relevant variables, appropriate statistical analysis, and recognition of potential biases and confounding factors. The review also looked for consistency of results between included studies, focusing on the clinical relevance of the reported findings.

Data Extraction: In the process of extracting data from identified publications, we recorded information regarding the study design, type of intervention, control group, and relevant research results. This information is compiled in a table (Table 1) and each data is described in detail. The writing of the information is carried out with attention to clarity and specificity to facilitate a comprehensive examination of 2'-FL's impact on intestinal cell activity from a professional medical standpoint.

RESULTS

Figure 1 illustrates a search flow chart using the PRISMA Flowchart. After screening 133 titles and abstracts, 55 studies were accessed to verify the feasibility of the full text. Overall, there were 5 studies selected in this study for a comprehensive review. Table 1 shows all the literatures that met the inclusion criteria in this study.

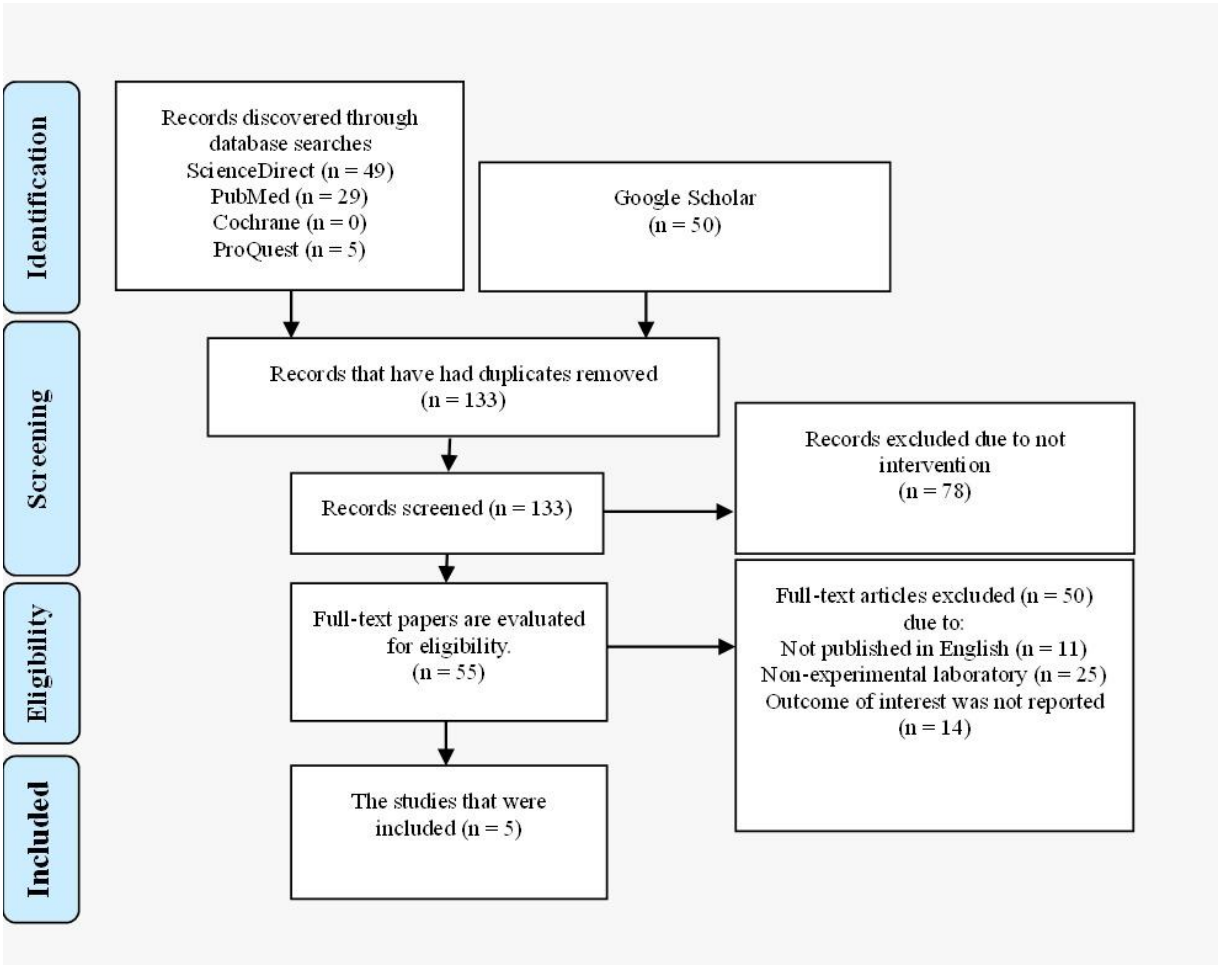


Figure 1. PRISMA Flowchart for Literature Inclusion.

Table 1. Study Inclusion Characteristics.

Author, Year	Country	Research Design	Intervention	Control Group	Results
(17)	USA	*In vivo*	Mice with NEC given 2'Fl	*Breastfed group* (Control), *Formula-fed group* (FF)	By maintaining mesenteric blood flow and upregulating the production of e-NOS, HMOs-2'-FL protects against NEC.
(18)	USA	*In vitro and In vivo*	5-fluorouracil (5-FU) was used to induce apoptosis in human gastrointestinal cancer cell lines (HT29 and AGS), *mouse small intestinal epithelial* cell lines, and mouse enteroid cultures. The anti-apoptotic properties of 2'-FL were assessed.	Control group (without treatment)	By shielding intestinal epithelial cells from 5-FU-induced apoptosis, 2'-FL may be able to stop intestinal mucositis brought on by 5-FU.
(19)	USA	*In vitro epithelial model*	Using preconfluent HT-29, preconfluent Caco-2Bbe, and postconfluent Caco-2Bbe cell lines, high doses (1.0g/L 3'SL, 1.0g/L 6'SL, and 2.0g/L 2'-FL) and low doses (3'SL, 0.4g/L 6'SL, and 0.2g/L 2'-FL) were investigated in crypt-villus axis models.	Lipopolysaccharide (LPS)	*Human milk oligosaccharides* (HMOs) promote the differentiation of preconfluent Caco-2Bbe and HT-29 cells. In postconfluent Caco-2Bbe cells, 3'SL, 6'SL, and 2'-FL increase alkaline phosphatase activity while maintaining disaccharidase activity. Furthermore, these cells exhibit much lower levels of necrosis and apoptosis ($P<0.001$).
(20)	USA	Laboratory experiments on mouse germ cells (*In vivo*)	Isolated HMO 15 mg/day for 7 and 14 days in mice and euthanasia on day 35 or 50	Placebo	Human milk oligosaccharides (HMOs) upregulate genes associated with various biological functions. In the HMO-treated group, CD4+ and CD8+ T cell populations increased in the spleen and mesenteric lymph nodes by day 50 compared to the control group. Plasma cell numbers rose in the mesenteric lymph nodes on days 28 and 35, and in the spleen on day 28 only. Meanwhile, macrophage/monocyte and neutrophil counts were reduced in the spleen on days 28, 35, and 50, and in the mesenteric lymph nodes, neutrophils were notably lower on day 50 following 14 days of HMO administration.
(21)	China	Laboratory experiments in dextran sulfate induced sodium (DSS) mice (*In vivo)*	Induction of mice with DSS + 2'-FL. Euthanasia on the 14th and 21st days.	Control group (without DSS grant) and DSS group without 2'-FL grant.	*2'-Fucosyllactose* (2'-FL) helps alleviate intestinal inflammation in colitis models by modulating gut microbiota, preserving goblet cell integrity, and promoting mucus production.

DISCUSSION

This study focuses on one of the most prominent Human Milk Oligosaccharides (HMOs), namely 2'FL. There have not been many systematic reviews that specifically highlight the therapeutic effects of 2'FL on Necrotizing Enterocolitis (NEC), yet most of studies still address the role of HMOs. This systematic review aims to analyze various experimental studies related to the administration of 2'FL that have the potential to induce improvement and therapy in gastrointestinal illness especially NEC cases through intestinal cell function. Breast milk is a nutrient-dense food contains a rich source of vitamins, proteins, lipids, probiotics, and prebiotics. It is crucial for supporting a newborn's development throughout the first few months of life and acts as their primary food. While the main ingredients of formula and breast milk are identical, their functional effects are very different. The amount of structurally varied HMOs in breast milk is primarily responsible for the reduced prevalence of inflammation seen in breastfed infants as opposed to formula-fed ones. *Fucosylated, disialylated*, and *non-fucosylated* neutral kinds are among the more than 200 varieties of HMOs that have been found to date (22). Due to their *fucosyl* and *sialyl* groups, instead of being digested by gastrointestinal tract enzymes, HMOs are fermented by the gut microbiota in the colon (23).

Nutrition during the early years of life is crucial for optimal development in babies, as this stage establishes important physiological processes such as growth, brain maturation, formation of the immune system, and the food absorption system. While breastfeeding is considered the ideal option, in some circumstances infant formulas are used as a supplement or substitute (24).

About 25% of the total HMO content in breast milk is made up of the most prevalent HMO, 2'-FL. The Food and Drug Administration (FDA) of the United States has authorized its usage as a nutritional health product and dietary supplement. Studies have demonstrated that 2'-FL is essential for bolstering the newborn immune system by encouraging the development of advantageous gut microorganisms like *Lactobacillus* and *Bifidobacteria.* Additionally, 2'-FL is known to prevent certain pathogens from attaching to epithelial cell surface glycans, thereby reducing their ability to cause infection. Although 2'-FL is known to regulate the makeup of the gut microbiota, it is still unclear if the gut flora is required for 2'-FL to perform its physiological functions (25,26).

In a study conducted by Yao in 2022 in China, it was stated that mice induced with dextran sulphate sodium (DSS) + 2'-FL had longer colons, slower weight loss, and a lower disease activity index (DAI) than the DSS group that did not receive 2'-FL. ($p < 0.05$). It is well documented that 2'-FL lowers the quantity of mucin-degrading bacteria, including *Bacteroides*, *Lachnospiraceae HK4A136*, *Lachnospiraceae*, and *Bacteroides vulgatus*, that are higher in the DSS group (21). However, it is still unknown whether the gut flora is affected by the disruptions in gut microbiota brought on by DSS treatment.

The regeneration of goblet cells is aided by the administration of 2'-FL, this also raises NOD-like receptor protein 6 (NLRP6) expression. and *mucin-2* (MUC2). NLRP6, a recognized negative regulator of the TLR4/myeloid differentiation protein 8/*nuclear factor-kappa B* (NF-kB) signaling cascade, is increased by 2'-FL in colonic tissue. According to these findings, 2'-FL effectively lowers colitis, most likely via changing the gut flora. Its protective effect appears to be linked to improved MUC2 synthesis and goblet cell population recovery through mechanisms involving TLR4 (21).

A newly discovered member of the NOD-like receptor family, NLRP6 is mostly expressed in the liver and gastrointestinal tract and forms the NLRP6 inflammasome. NLRP6 is crucial for regulating the production of IL-8 in the gut, goblet cell MUC2 expression, and microbial homeostasis. Research has shown that metabolic and microbiological cues, such as spermine, histamine, taurine, and unsaturated fatty

acids, impact intestinal epithelial cells' NLRP6 expression. Furthermore, some bacterial species, such as TM7 and Prevotella, function as secondary signals that combine with NLRP6 to initiate the activation and production of inflammasomes. Furthermore, TLR ligands can activate the MyD88 reactive oxygen species (ROS) pathway, which in turn can activate the NLRP6 inflammasome (27–29). Previous studies have suggested that 2'-FL may counteract the decline in NLRP6 and MUC2 caused by DSS. It was demonstrated that 2'-FL treatment increased NLRP6 while lowering TLR4, MyD88, and NF-kB expression, indicating that NLRP6 could function as a negative regulator of TLR4-related signaling pathways (21).

According to experimental findings by Good in 2016, administration of HMO and 2'-FL protected neonatal mice against NEC-related damage by reducing proinflammatory markers and preserving the structure of the intestinal mucosa. This protective effect is attributed to the restoration of intestinal blood flow, achieved by upregulating the vasodilatory enzyme endothelial nitric oxide (eNOS). While 16S rRNA analysis indicated that HMO-2'FL does influence the gut microbiota in neonatal mice, these microbial changes are not considered the main protective mechanism. Additionally, increased eNOS expression was observed in cultured endothelial cells treated with HMO-2'FL, suggesting a direct relationship between HMO and endothelial function. Overall, the findings indicate that HMO and 2'-FL help protect against NEC, at least in part, by enhancing mesenteric blood flow through eNOS upregulation (17).

Studies have shown that HMOs can directly influence cellular activity by modulating immune-related responses. Specifically, HMOs regulate gene expression associated with the immune system in the neonatal intestinal lining. 2'-FL supports cellular differentiation and helps suppress inflammation by downregulating CD4, a component of the LPS receptor complex, which in turn reduces inflammation triggered by both LPS and TNF. Additionally, protein glycosylation is essential for proper protein folding and molecular recognition processes. Receptors and other cell surface proteins may be directly impacted by 2'-FL, changing their structure and how they interact with one another. Through these mechanisms, it can influence receptor function, downstream signaling, and various cellular responses (30–32).

Prior research has shown that 2'-FL can lessen the inhibition of 5-FU-induced cell growth in mouse small intestinal epithelial (MSIE) cells. Furthermore, 2'-FL can inhibit the apoptosis that *5-fluorouracil* (5-FU) causes in enteroid and MSIE cells. The study also showed that 2'-FL treatment prior to 5-FU administration was able to protect against weight loss as well as improve inflammation scores, proinflammatory cytokine production, and prolong the short, small intestinal villi. Furthermore, in comparison to 5-FU therapy alone, 2'-FL may also lessen goblet cell loss, thigh junctional complex dysfunction, and epithelial cell death in the small intestine. However, concomitant treatment with 2'-FL had a lower impact on intestinal mucositis (18).

The direct effects of HMO on intestinal and host immune system function have also been studied in the past. In this research, intestinal tissue was assessed through histomorphometric and transcriptomic analyses, while flow cytometry was used to evaluate the spleen and mesenteric lymph nodes. The results demonstrated that HMO administration led to a reduction in small intestinal crypt depth on days 28 and 35 compared to the control group. Furthermore, mice undergoing HMO therapy showed a decrease in the depth of the glands in the large intestine and a decrease in the height of the villus in the small intestine on day 35. After receiving HMO therapy, gene expression analysis showed notable alterations in a number of intestinal tissue locations, particularly in genes related to extracellular matrix, nuclear transport, mononuclear cell differentiation, and protein ubiquitination. By day 50, CD4+ T cells had

proliferated in the spleen and the mesenteric lymph nodes, but CD8+ T cells had exclusively formed in the spleen. The number of plasma cells in the spleen and mesenteric lymph nodes increased in the HMO group on days 28 and 35. The group that received 14 days of HMO treatment had fewer neutrophils in the mesenteric lymph nodes only on day 50, although the spleen had less neutrophils and macrophages/monocytes on days 28, 35, and 50. Additionally, the HMO group had more cells secreting antibodies against tetanus and diphtheria toxoids than the control group. These results imply that even in the absence of host microbiota, HMOs can directly affect immunological responses and gastrointestinal metabolism (19,20).

The small intestine's epithelium is made up of crypts that reach into the lamina propria, which includes stem cells and Paneth cells at its base, and villi that contain enterocytes and goblet cells. In animal models of NEC, HMOs made from breast milk have been demonstrated to enhance mucus production by upregulating mucin 2 (MUC2) expression. Furthermore, dextran permeability assays revealed a reduction in intestinal permeability. When compared to untreated controls, male C57BL/6 mice given 2'-FL for seven days had greater crypt depth and villus height at 56 days of age. HMOs also increased the expression of several genes involved in the movement, absorption, and secretion of nutrients in small intestine tissue, regardless of when they were given. The enzyme alpha-2,6-sialytransferase 6 (ST6GALNAC1) is crucial for sialylating glycans in intestinal mucus, which is necessary for preserving gut homeostasis, according to recent research. Following HMO supplementation, the expression of a similar enzyme, ST6GALNAC1, also changed in the small intestine, suggesting that HMOs contribute to the enhancement of enzyme activity associated with intestinal balance and mucus function. The majority of HMOs wind up in the large intestine, where they are either eliminated unaltered or used as substrates for microbial fermentation (33–35).

Infants who are naturally breastfed frequently exhibit a stronger immune response to vaccinations than those who are fed formula. In animal models, recent studies have shown that supplementing with 2-'FL improves the immunological response to influenza vaccinations (36). Specifically, the spleen has a greater number of cells that produce antibodies against *tetanus* (TT) and *diphtheria* (DT), and mice who get HMO supplementation have higher levels of TT-IgG antibodies than controls. These results appear to be influenced by the animals' age, the length of time they have been consuming HMO, and the activity of their lymphoid organs. Although human breast milk interacts with the gut flora and contains a variety of bioactive components, previous studies have concentrated on HMO supplementation. These factors may together affect immunological responses both locally in the stomach and across the body. All things considered, the evidence points to a direct involvement of HMOs in regulating intestinal and systemic immunological activity. Future research is required to examine the functional importance of immune cell population alterations brought on by HMOs, whether or not microbiota is present. To fill up existing knowledge gaps, a better understanding of additional bioactive substances found in breast milk, such as miRNAs and IgA, and how they influence immune cell activity and vaccination response is also necessary (20).

Research on 2'-FL and HMOs shows promising results but still has limitations in the form of heterogeneity in the study design, population, and type of HMOs studied that make it difficult to compare directly. In addition, potential bias can arise from sample selection factors, environmental conditions, and the use of animal models that limit generalization to humans. The number of studies, especially long-term clinical trials, is

also still limited, so further studies with more standardized methodologies and a more diverse population are needed to corroborate the existing evidence, so further research is needed.

CONCLUSION

Overall, the study demonstrates that the administration of *2'-Fucosyllactose* (2'FL) has the potential to induce improvement and therapeutic effects in NEC cases through intestinal cell function. 2'-FL and other HMOs have several advantageous impacts on the host's immune system and digestive system. It has been demonstrated that human milk oligosaccharides (HMOs) affect the makeup of the gut microbiota, increase the expression of genes linked to gut function, and improve the response of the immune system to immunizations. By modulating the expression of MUC2 and NLRP6, HMOs also contribute significantly to protection against intestinal diseases like colitis. These results demonstrate how HMOs may be used as a supplement to strengthen the immune system and digestive health, although interactions with gut microbiota and other bioactive in human breast milk need to be further researched for a deeper understanding. There have not been many systematic reviews that specifically highlight the therapeutic effects of 2'FL on Necrotizing Enterocolitis (NEC), most of which still address the role of HMOs.

Abbreviations: Human milk oligosaccharides (HMOs); diphtheria (DT); tetanus (TT); ST6 N-*acetylgalactosaminide alpha-2,6-sialytransferase* 6 (ST6GALNAC1); Mucin 2 (MUC2); mouse small intestinal epithelial (MSIE); *5-fluorouracil* (5-FU); endothelial nitric oxide synthase (eNOS); reactive oxygen species (ROS); 2'-*Fucosyllactose* (2'-FL); disease activity index (DAI); population, intervention, comparator, and outcome (PICO); Preferred Reporting Items for Systematic Review and Meta Analyses (PRISMA); necrotizing enterocolitis (NEC); toll-like receptor 4 (TLR4); NOD-like receptors Protein-6 (NLRP6).

Competing Interests: The research funding for this study was granted by the Faculty of Medicine, Universitas Brawijaya's Research and Community Service Program in 2025, for which the authors are really grateful.

Author's contribution: All authors contributed to this study.

Acknowledgements: The authors would like to thank to Yohana Hartya Dwi Frastari and Annisatul Hakimah for their help in completing this review article.

REFERENCES

1. Sodhi CP, Wipf P, Yamaguchi Y, Fulton WB, Kovler M, Niño DF, et al. The human milk oligosaccharides 2'-fucosyllactose and 6'-sialyllactose protect against the development of necrotizing enterocolitis by inhibiting toll-like receptor 4 signaling. Pediatr Res [Internet]. 2021 Jan 1 [cited 2025 Apr 10];89(1):91–101. DOI: https://doi.org/10.1038/s41390-020-0852-3
2. Stoll BJ, Hansen NI, Bell EF, Walsh MC, Carlo WA, Shankaran S, et al. Trends in Care Practices, Morbidity, and Mortality of Extremely Preterm Neonates, 1993-2012. JAMA [Internet]. 2015 Sep 8 [cited 2025 Apr 10];314(10):1039–51. DOI: https://doi.org/10.1001/jama.2015.10244
3. Jilling T, Simon D, Lu J, Meng FJ, Li D, Schy R, et al. The roles of bacteria and TLR4 in rat and murine models of necrotizing enterocolitis. J Immunol [Internet]. 2006 Sep 1 [cited 2025 Apr 10];177(5):3273–82. DOI: https://doi.org/10.4049/jimmunol.177.5.3273
4. Sidauruk RJM, Amir I, Kadim M, Said M. Faktor Risiko yang Memengaruhi Kolonisasi Mikroflora Saluran Cerna Neonatus Kurang Bulan dengan Enterokolitis Nekrotikans. Sari Pediatr [Internet]. 2016 Nov 9 [cited 2024 Feb 29];15(6):353. DOI: https://doi.org/10.14238/sp15.6.2014.353-60
5. Bode L. Human milk oligosaccharides: every baby needs a sugar mama. Glycobiology [Internet]. 2012 Sep [cited 2025 Apr 10];22(9):1147–62. DOI: https://doi.org/10.1093/glycob/cws074
6. Albrecht S, Lane JA, Mariño K, Al Busadah KA, Carrington SD, Hickey RM, et al. A comparative study of free oligosaccharides in the milk of domestic animals. Br J Nutr [Internet]. 2014 Apr 14 [cited 2025 Apr 10];111(7):1313–28. DOI: https://doi.org10.1017/S0007114513003772
7. Huang Z, Li Y, Luo Y, Guo H. Human milk oligosaccharides 3'-sialyllactose and 6'-sialyllactose protect intestine against

necrotizing enterocolitis damage induced by hypoxia. J Funct Foods. 2021 Nov 1; 86:104708.
DOI: https://doi.org/10.1016/j.jff.2021.104708

8. Zivkovic AM, German JB, Lebrilla CB, Mills DA. Human milk glycobiome and its impact on the infant gastrointestinal microbiota. Proc Natl Acad Sci U S A [Internet]. 2011 Mar 15 [cited 2025 Apr 10];108 Suppl 1(Suppl 1):4653–8.
DOI: https://doi.org/10.1073/pnas.1000083107
9. Vandenplas Y, Berger B, Carnielli VP, Id JK, Id HL, Sanchez M, et al. in Infant Formula. 2018;
DOI: https://doi.org/10.20944/preprints201807.0416.v1
10. van Niekerk E, Autran CA, Nel DG, Kirsten GF, Blaauw R, Bode L. Human milk oligosaccharides differ between HIV-infected and HIV-uninfected mothers and are related to necrotizing enterocolitis incidence in their preterm very-low-birth-weight infants. J Nutr [Internet]. 2014 [cited 2025 Apr 10];144(8):1227–33.
DOI: https://doi.org/10.3945/jn.113.187799
11. Hackam DJ, Sodhi CP. Bench to bedside - new insights into the pathogenesis of necrotizing enterocolitis. Nat Rev Gastroenterol Hepatol [Internet]. 2022 Jul 1 [cited 2025 Apr 10];19(7):468–79.
DOI: https://doi.org/10.1038/s41575-022-00594-x
12. Kolba N, Tako E. Effective alternatives for dietary interventions for necrotizing enterocolitis: a systematic review of *in vivo* studies. Crit Rev Food Sci Nutr [Internet]. 2025 [cited 2025 Apr 10];65(4).
DOI: https://doi.org/10.1080/10408398.2023.2281623
13. Alshaikh BN, Sproat TDR, Wood C, Spence JM, Knauff M, Hamilton C, et al. A Quality Improvement Initiative to Reduce Necrotizing Enterocolitis in Very Preterm Infants. Pediatrics [Internet]. 2023 Dec 1 [cited 2025 Apr 10];152(6).
DOI: https://doi.org/10.1542/peds.2023-061273
14. Liu K, Guo J, Yang J, Su Y. The Association of Human Milk Proportion with the Clinical Outcomes of Necrotizing Enterocolitis in Preterm Infants: A Retrospective Study. Nutrients [Internet]. 2023 Sep 1 [cited 2025 Apr 10];15(17).
DOI: https://doi.org/10.3390/nu15173796
15. Natividad JM, Marsaux B, Rodenas CLG, Rytz A, Vandevijver G, Marzorati M, et al. Human Milk Oligosaccharides and Lactose Differentially Affect Infant Gut Microbiota and Intestinal Barrier *In vitro*. Nutrients [Internet]. 2022 Jun 1 [cited 2025 Apr 10];14(12).
DOI: https://doi.org/10.3390/nu14122546
16. Shamseer L, Moher D, Clarke M, Ghersi D, Liberati A, Petticrew M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. BMJ [Internet]. 2015 Jan 2 [cited 2025 Apr 10];350.
DOI: https://doi.org/10.1136/bmj.g7647
17. Good M, Sodhi CP, Yamaguchi Y, Jia H, Lu P, Fulton WB, et al. The human milk oligosaccharide 2 ′ -fucosyllactose attenuates the severity of experimental necrotising enterocolitis by enhancing mesenteric perfusion in the neonatal intestine. 2016; 4:1175–87.
DOI: https://doi.org/10.1017/S0007114516002944
18. Zhao G, Williams J, Washington MK, Yang Y, Long J, Townsend SD, et al. 2'-Fucosyllactose Ameliorates Chemotherapy-Induced Intestinal Mucositis by Protecting Intestinal Epithelial Cells Against Apoptosis. Cell Mol Gastroenterol Hepatol [Internet]. 2022 Jan 1 [cited 2025 Apr 10];13(2):441–57.
DOI: https://doi.org/10.1016/j.jcmgh.2021.09.015
19. Holscher HD, Bode L, Tappenden KA. Human Milk Oligosaccharides Influence Intestinal Epithelial Cell Maturation *In vitro*. J Pediatr Gastroenterol Nutr [Internet]. 2017 Feb 1 [cited 2025 Apr 10];64(2):296–301.
DOI: https://doi.org/10.1097/MPG.0000000000001274
20. Rosa F, Sharma AK, Gurung M, Casero D, Matazel K, Bode L, et al. Human Milk Oligosaccharides Impact Cellular and Inflammatory Gene Expression and Immune Response. Front Immunol [Internet]. 2022 Jun 29 [cited 2025 Apr 10];13. DOI: https://doi.org/10.3389/fimmu.2022.907529
21. Yao Q, Fan L, Zheng N, Blecker C, Delcenserie V, Li H, et al. 2'-Fucosyllactose Ameliorates Inflammatory Bowel Disease by Modulating Gut Microbiota and Promoting MUC2 Expression. Front Nutr [Internet]. 2022 Feb 17 [cited 2025 Apr 10];9. DOI: https://doi.org/10.3389/fnut.2022.822020
22. Doherty AM, Lodge CJ, Dharmage SC, Dai X, Bode L, Lowe AJ. Human milk oligosaccharides and associations with immune-mediated disease and infection in childhood: A systematic review. Front Pediatr. 2018;6(April).
DOI: https://doi.org/10.3389/fped.2018.00091
23. Bode L. The functional biology of human milk oligosaccharides. Early Hum Dev [Internet]. 2015 Nov 1 [cited 2025 Apr 10];91(11):619–22.
DOI: https://doi.org/10.1016/j.earlhumdev.2015.09.001
24. Gonzalez CAY, Soler AJY. Infant nutrition: Breast milk substitutes and gut-brain axis improved by microalgae. Funct Food Sci. 2024;4(12):479–94.
25. Facinelli B, Marini E, Magi G, Zampini L, Santoro L, Catassi C, et al. Breast milk oligosaccharides: effects of 2'-

fucosyllactose and 6'-sialyllactose on the adhesion of Escherichia coli and Salmonella fyris to Caco-2 cells. J Matern Fetal Neonatal Med [Internet]. 2019 Sep 2 [cited 2025 Apr 10];32(17):2950–2.
DOI: https://doi.org/10.1080/14767058.2018.1450864

26. Manthey CF, Autran CA, Eckmann L, Bode L. Human milk oligosaccharides protect against enteropathogenic Escherichia coli attachment *in vitro* and EPEC colonization in suckling mice. J Pediatr Gastroenterol Nutr [Internet]. 2014 Feb [cited 2025 Apr 10];58(2):165–8.
DOI: https://doi.org/10.1097/MPG.0000000000000172
27. Elinav E, Strowig T, Kau AL, Henao-Mejia J, Thaiss CA, Booth CJ, et al. NLRP6 inflammasome regulates colonic microbial ecology and risk for colitis. Cell [Internet]. 2011 May 27 [cited 2025 Apr 10];145(5):745–57.
DOI: https://doi.org/10.1016/j.cell.2011.04.022
28. Levy M, Thaiss CA, Zeevi D, Dohnalová L, Zilberman-Schapira G, Mahdi JA, et al. Microbiota-Modulated Metabolites Shape the Intestinal Microenvironment by Regulating NLRP6 Inflammasome Signaling. Cell [Internet]. 2015 Dec 3 [cited 2025 Apr 10];163(6):1428–43.
DOI: https://doi.org/10.1016/j.cell.2015.10.048
29. Wang G, Wang Q, Bai J, Zhao N, Wang Y, Zhou R, et al. Upregulation of Intestinal NLRP6 Inflammasomes After Roux-en-Y Gastric Bypass Promotes Gut Immune Homeostasis. Obes Surg [Internet]. 2020 Jan 1 [cited 2025 Apr 10];30(1):327–35.
DOI: https://doi.org/10.1007/s11695-019-04152-4
30. He Y, Liu S, Leone S, Newburg DS. Human colostrum oligosaccharides modulate major immunologic pathways of immature human intestine. Mucosal Immunol [Internet]. 2014 Nov 25 [cited 2025 Apr 10];7(6):1326–39.
DOI: https://doi.org/10.1038/mi.2014.20
31. Moremen KW, Tiemeyer M, Nairn A V. Vertebrate protein glycosylation: diversity, synthesis and function. Nat Rev Mol Cell Biol [Internet]. 2012 Jul [cited 2025 Apr 10];13(7):448–62. DOI: https://doi.org/10.1038/nrm3383
32. Cheng L, Kong C, Wang W, Groeneveld A, Nauta A, Groves MR, et al. The Human Milk Oligosaccharides 3-FL, Lacto-N-Neotetraose, and LDFT Attenuate Tumor Necrosis Factor-α Induced Inflammation in Fetal Intestinal Epithelial Cells *In vitro* through Shedding or Interacting with Tumor Necrosis Factor Receptor 1. Mol Nutr Food Res [Internet]. 2021 Apr 1 [cited 2025 Apr 10];65(7).
DOI: https://doi.org/10.1002/mnfr.202000425
33. Seo K, Seo J, Yeun J, Choi H, Kim YI, Chang SY. The role of mucosal barriers in human gut health. Arch Pharm Res [Internet]. 2021 Apr 1 [cited 2025 Apr 10];44(4):325–41.
DOI: https://doi.org/10.1007/s12272-021-01327-5
34. Perdijk O, Baarlen P Van, Fernandez-Gutierrez MM, Brink E Vanden, Schuren FHJ, Brugman S, et al. Sialyllactose and Galactooligosaccharides Promote Epithelial Barrier Functioning and Distinctly Modulate Microbiota Composition and Short Chain Fatty Acid Production *In vitro*. Front Immunol [Internet]. 2019 [cited 2025 Apr 10];10(FEB).
DOI: https://doi.org/10.3389/fimmu.2019.00094
35. Wu RY, Li B, Koike Y, Määttänen P, Miyake H, Cadete M, et al. Human Milk Oligosaccharides Increase Mucin Expression in Experimental Necrotizing Enterocolitis. Mol Nutr Food Res [Internet]. 2019 Feb 1 [cited 2025 Apr 10];63(3).
DOI: https://doi.org/10.1002/mnfr.201800658
36. Xiao L, Leusink-Muis T, Kettelarij N, van Ark I, Blijenberg B, Hesen NA, et al. Human Milk Oligosaccharide 2'-Fucosyllactose Improves Innate and Adaptive Immunity in an Influenza-Specific Murine Vaccination Model. Front Immunol [Internet]. 2018 Mar 9 [cited 2025 Apr 10];9(MAR).
DOI: https://doi.org/10.3389/fimmu.2018.00452