Research Article Open Access

Ultra-low-dose lactulose modulates the gut microbiota in healthy adults: A double-blind, randomized, placebo-controlled crossover trial

Ryo Sakiyama*, Seiya Miyashita, Manabu Nakano, Miyuki Tanaka

Food Function Research Institute, R&D Division, Morinaga Milk Industry Co., Ltd., 5-1-83, Higashihara, Zama, 252-8583, Kanagawa, Japan.

***Corresponding author:** Ryo Sakiyama, Food Function Research Institute, R&D Division, Morinaga Milk Industry Co., Ltd., 5-1-83, Higashihara, Zama, 252-8583, Kanagawa, Japan

Submission Date: November 11th, 2025; **Acceptance Date:** December 4th, 2025; **Publication Date:** December 8th, 2025

Please cite this article as: Sakiyama R., Miyashita S., Nakano M., Tanaka M. Ultra-low-dose lactulose modulates the gut microbiota in healthy adults: A double-blind, randomized, placebo-controlled crossover trial. *Functional Foods in Health and Disease* 2025; 15(12): 907 – 920. DOI: https://doi.org/10.31989/ffhd.v15i12.1838

ABSTRACT

Background: Gut health, particularly the composition and function of the gut microbiota, is increasingly recognized as essential for overall well-being. Non-digestible oligosaccharides are established prebiotics that stimulate beneficial gut bacteria; however, excessive intake may cause gastrointestinal discomfort. This study aimed to determine the minimum effective dose of lactulose using 16S rRNA gene sequencing to support safe and sustainable dietary applications.

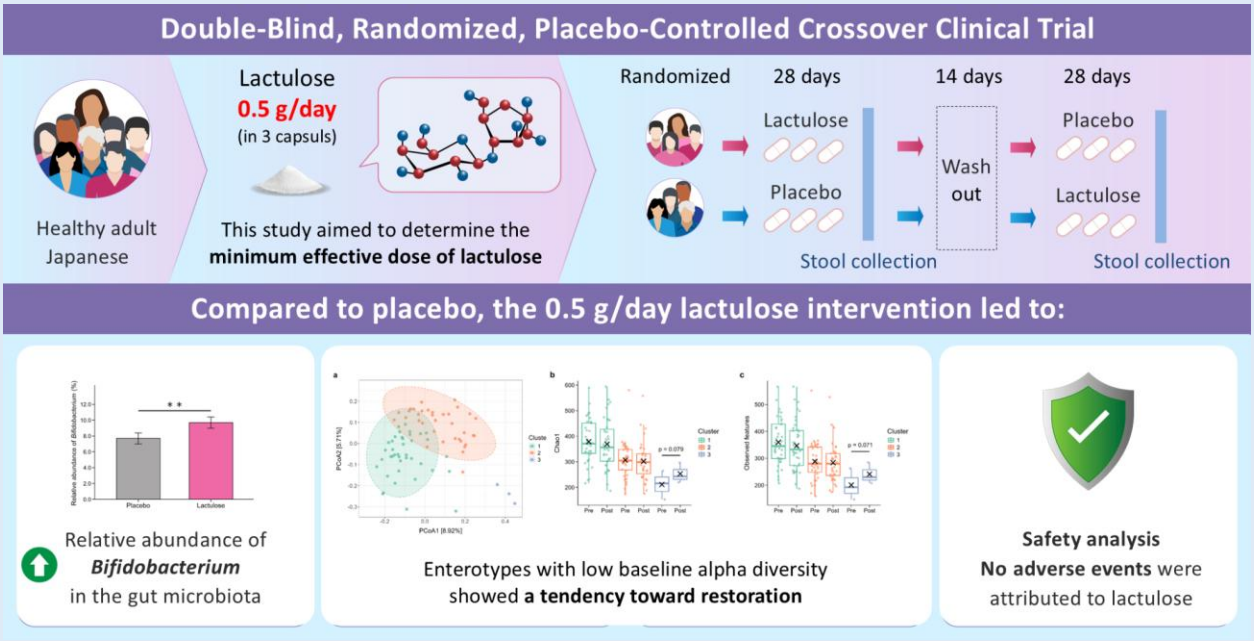
Methods: In a double-blind, randomized, placebo-controlled crossover trial, 100 healthy Japanese adults received lactulose (500 mg/day) or placebo for 28 days, separated by a 14-day washout period. The relative abundance of *Bifidobacterium* was analyzed using a linear mixed model with centered log-ratio transformation.

Results: As a result of the analyses conducted on the PPS (Per Protocol Set, n = 78) and FAS (Full Analysis Set, n = 99), lactulose intake significantly increased the relative abundance of *Bifidobacterium*. Overall alpha diversity remained unchanged, although participants with low baseline alpha diversity enterotypes showed a trend toward restoration of alpha diversity. No adverse events or gastrointestinal symptoms, such as bloating or diarrhea, were observed, confirming the safety of lactulose.

Conclusions: These findings provide novel evidence that even minimal doses of lactulose can beneficially modulate the gut microbiota, notably by increasing the relative abundance of *Bifidobacterium* without inducing laxative effects. Ultra-low-dose lactulose may serve as a practical and safe functional food ingredient to support long-term gut health, and continued research on its incorporation into functional food matrices will be important.

Trial registration: The study protocol was registered with UMIN-CTR (ID: UMIN000055862).

Keywords: Lactulose; Prebiotics; *Bifidobacterium*; Improving gut microbiota, Gastrointestinal health



Graphical Abstract: Ultra-low-dose lactulose modulates the gut microbiota in healthy adults

INTRODUCTION

The health of the large intestine, especially the gut microbiota, has gained international recognition as a fundamental pillar for maintaining overall well-being [1-4]. One contributing factor is the increasing awareness that changes in the gut microbiota are associated with an increased risk of various health conditions, including gut disorders and metabolic diseases [5-7]. This growing interest is driven by lifestyle changes such as the westernization of dietary habits, including increased consumption of ultra-processed foods and fast food [8-10]. Consequently, diets targeting the gut microbiota have attracted considerable attention [11]. Among functional food ingredients, non-digestible oligosaccharides (NDOs) are recognized for their prebiotic properties, selectively modulating the composition and activity of the gut microbiota [12,13]. Representative NDOs, including fructooligosaccharides (FOS), galactooligosaccharides (GOS), and lactulose, are largely resistant to digestion by human gastrointestinal enzymes, reaching the gut almost intact [6,14]. In the gut, they serve as fermentation substrates for microbiota, particularly *Bifidobacterium*. This fermentation promotes the production of acetic acid, short-chain fatty acids, and lactic acid, which suppress the proliferation of pathogenic microorganisms by lowering gut pH.

Furthermore, these metabolites are converted into butyrate, which plays a crucial role in maintaining gut health through a process known as cross-feeding among the various gut microbiota [6]. An increase in *Bifidobacterium* is widely recognized as a key indicator of gut health, as it exerts multifaceted health-promoting functions, primarily through the production of its metabolites [15-17]. Although generally mild, excessive intake of NDOs (approximately 40–50 g/day) is known to induce intestinal symptoms, such as abdominal bloating, discomfort, and diarrhea. Similar adverse effects can also occur at lower doses (2.5–10 g/day). Therefore, establishing safe, practical daily intake levels is critical for sustained consumption of NDOs. Human clinical trials have shown that the daily intake of 2.5 g FOS [18] and 1 g GOS [19] significantly increases the population of *Bifidobacterium* in the gut microbiota recently. Recent studies have further explored the feasibility of low-dose applications, with *ex vivo* studies showing microbial proliferation of *Bifidobacterium* with as little as 380 mg of GOS [20]. However, evidence from human intervention trials using such low doses is limited. Compared to other NDOs, lactulose has shown promising prebiotic effects even at lower doses. Previous studies have shown that daily intake of 650 mg [21] or 1 g [22] of lactulose significantly increases fecal *Bifidobacterium* counts, suggesting its potential efficacy at low doses. These studies employed single-arm designs and, without control groups or randomization, increased the risk of measurement bias. In addition, the methodologies used to assess gut microbiota, such as culture-based techniques and quantitative PCR, are limited in their ability composition. Recent findings suggest that responsiveness to prebiotics may vary across gut enterotypes [23]. However, information on lactulose interventions is scarce.

Investigating the minimum effective dose of lactulose using updated analytical techniques in a randomized trial is essential to develop safe and sustainable functional foods applicable across populations. In this study, we hypothesized that lactulose may modulate the gut microbiota at 500 mg/day, a dose lower than previously reported. We conducted a double-blind crossover trial employing 16S rRNA gene sequencing to assess changes in the relative abundance of *Bifidobacterium*, with an exploratory analysis of enterotype-specific effects.

MATERIALS AND METHODS

Research ethics: This trial was approved by the Kobuna Orthopedic Clinic Ethics Review Committee on September 26, 2024 (Approval Number: MK-2409-03). Trial information was registered with the UMIN-CTR (https://www.umin.ac.jp/ctr/) before obtaining consent from participants on October 17 (UMIN000055862). The study was conducted in accordance with the Declaration of Helsinki (Fortaleza Revision, 2013) and the Ethical Guidelines for Medical and Biological Research Involving Human Subjects (Notice No. 1 of 2021, partially amended on March 27, 2023, issued jointly by the Ministry of Education, Culture, Sports, Science and Technology; the Ministry of Health, Labor and Welfare; and the Ministry of Economy, Trade and Industry). Before conducting the trial, the purpose, procedures, and participants' rights were fully explained, and informed consent was obtained. The trial protocol was not modified after consent was obtained.

Study Design: This study was conducted at the Nihonbashi Cardiology Clinic (Tokyo, Japan) between October 2024 and June 2025. To minimize the substantial inter-individual variability in gut microbiota composition, we employed a randomized, double-blind, placebo-controlled crossover design. The study comprised a one-week pre-observation period, followed by a 28-day first-intake period, a 14-day washout phase, and a subsequent 28-day second-intake period. Applicants who met the inclusion criteria and did not meet any of the exclusion

criteria and who were recruited through the recruitment website were enrolled.

The inclusion criteria were as follows: Healthy males and females aged 18–64 years at the time of consent; participants who fully understood the purpose and content of this study and agreed to participate by providing written informed consent.

The exclusion criteria were as follows: participants were excluded if they had any of the following.

(1) Treatment for serious cancer, respiratory, hepatic, renal, cardiac, lung, gastrointestinal, blood, endocrine, or metabolic diseases, or those with a serious history of these diseases; (2) History of surgery on the digestive tract (excluding minor procedures such as adenoidectomy or appendectomy); (3) Gastrointestinal disorders such as irritable bowel syndrome or inflammatory bowel disease; (4) Regular use of medication that affects the intestinal environment; (5) Regular consumption of foods with function claims, foods for specified health uses, or supplements affecting the intestinal environment (e.g., including those containing probiotics or prebiotics); (6) Serious drug or food allergies, or a history of such allergies; (7) Milk allergy; (8) Lactose intolerance; (9) Pregnant or breastfeeding women, or women expecting to become pregnant during this study; (10) Alcohol intake equivalent to more than 60 g of pure alcohol per day; (11) Smoking habit; (12) Participation in other clinical trials within 3 months of consent or planning to participate in other clinical trials during this study; (13) Participants deemed ineligible by the principal investigator.

Intervention: The investigational products, lactulose and placebo, were manufactured by Morinaga Milk Industry and provided in capsule form. The investigational product, lactulose, was formulated to contain 500 mg of lactulose (MLC-97 lactulose crystal anhydrate powder, >97% Morinaga Milk Industry, Tokyo, Japan) per daily dose of 3 capsules. In the placebo arm, lactulose was replaced with glucose. Capsules made of hydroxypropyl methylcellulose, designed to disintegrate in the stomach, were used. The investigational products were stored at room temperature and consumed as three capsules per day.

Independent assignment managers labeled the investigational products to maintain blinding of participants and research staff in accordance with a double-blind study manner. In addition to confirming that taste, odor, appearance, color, and packaging were indistinguishable between lactulose and placebo before and after the study.

Randomization: Participants were randomly assigned to either Group A (lactulose first) or Group B (placebo first). An independent allocation manager created a randomization table using a computer-generated block randomization method (block size = 4), stratified by sex. Participants, with an allocation ratio of 1:1. Investigational products were coded and managed in a blinded manner, and participants were assigned according to the randomization table. The correspondence table linking block size, number of investigational products, and allocation group was concealed until study completion.

Efficacy evaluation: The primary outcome was the relative abundance of *Bifidobacterium* in the gut microbiota. Exploratory analyses evaluated alpha and beta diversity of the gut microbiota following lactulose intervention.

16S rRNA gene analysis: Stool samples were collected from participants before and on day 28 of each intake period using the FS-0016 kit (TechnoSuruga Laboratory, Shizuoka, Japan), which contains guanidine thiocyanate as the preservation solution. Bacterial DNA extraction, 16S rRNA sequencing, data processing, and taxonomic assignment were performed as previously described (Ejima et al., 2024). The stool samples in the preservation

solution were stored at -80 °C until DNA extraction. Stool samples were mechanically disrupted using glass beads, and DNA was extracted using an automated system (Kurabo, Osaka, Japan). The Illumina NextSeq 1000 platform (NextSeq 1000/2000 P1 Reagent Kit, 600 cycles; Illumina, San Diego, CA, USA) was used for paired-end sequencing of the V3–V4 region of the bacterial 16S rRNA gene. Sequencing data were analyzed using QIIME2 (version 2022.8) and taxonomically assigned based on the Greengenes2 database (version 2022.10).

Diary and diet survey: Participants kept a daily survey diary to record changes in physical condition, medication use, investigational product consumption, bowel habits, and dietary intake. During the study, participants were prohibited from consuming any food, supplements, or health products (including Foods for Specified Health Uses, Foods with Function Claims, and Nutritional Functional Foods) containing lactic acid bacteria, bifidobacteria, oligosaccharides, or dietary fiber. Cases where participants visited a medical facility, took medication, or consumed any prohibited food products owing to unavoidable circumstances were recorded in detail, including the name, amount, and reason for use or visit, in their participant diary. The diary entries were used to assess participants' compliance with the study requirements. The lower value between the diary records and the actual returned capsules was used to calculate the intake rate. Dietary habits were assessed using the Brief-type Self-Administered Diet History Questionnaire (BDHQ) before the first intervention and at the end of the second intervention.

Safety assessment: Adverse events were identified in the survey diaries, and their severity was assessed by the principal investigator according to the NCI-CTCAE version 5.0 (Common Terminology Criteria for Adverse Events, Japanese translation, JCOG edition). The principal investigator (consumption medical doctor) determined whether adverse events were associated with the use of the investigational products. The safety analysis set (SAF) included participants who received at least one intervention after allocation.

Sample Size: Sample size was calculated using G*Power 3.1 (Heinrich-Heine-Universität, Düsseldorf, Germany). Unpublished data from a previous study showed that a 1- and 2-week lactulose intervention in participants with ≤20% *Bifidobacterium* led to a ~8% increase. The required number of participants was calculated based on these data. Given that the lactulose dose was reduced to 500 mg, we conservatively estimated a 3% difference in the mean *Bifidobacterium* abundance, with a 10% standard deviation. The required number of participants to compare the means between the two groups at a two-sided significance level of 5% with 80% power was 90, with a target of 100 to account for an expected ~10% dropout rate.

Statistical analysis: Statistical analyses were conducted by an independent statistical analyst. The primary analysis was performed on the per-protocol set (PPS), excluding participants with <80% compliance, prohibited food intake, gastrointestinal surgery, or use of antibiotics, probiotics, laxatives, or antidiarrheal agents. To confirm the robustness of the results, an exploratory analysis was conducted on the complete analysis set (FAS), which included participants with at least one post-intervention efficacy data point. Missing data were handled as missing and were not imputed.

Gut microbiota composition data were analyzed after applying a centered log-ratio (CLR) transformation to the count data. The CLR transformation was applied to bacterial taxa detected in at least 10% of participants, using a detection threshold of 1. For count values of zero, a value of 0.5 was substituted.

The primary outcome analysis relative abundance of *Bifidobacterium* in the gut microbiota as the dependent variable. The investigational product group

and intake period were treated as fixed effects, and participants were treated as random effects to estimate the effect of the investigational products. The same model was applied to exploratory analyses of alpha diversity indices, including Chao1, Pielou's evenness, Faith's phylogenetic diversity, observed features, and the Shannon index. Carryover effects were analyzed using a model that included an interaction between the group and period for each investigational product, in addition to the fixed effects of the main analysis model.

Beta diversity was assessed using Bray–Curtis distances calculated from 16S rRNA amplicon sequence variant (ASV) data. Principal coordinate analysis (PCoA) was performed to visualize the microbial community structure. Enterotype classification was performed using Ward's method based on Bray–Curtis distances, and the optimal number of clusters was determined using the Calinski–Harabasz index, Davies–Bouldin index, and silhouette score. The statistical significance of the differences between enterotype clusters was evaluated using permutational multivariate analysis of variance (PERMANOVA).

For safety analysis, McNemar's test was used to compare the incidence rates of adverse events between the lactulose and placebo groups. Comparisons between and within groups were conducted using Welch's t-test, paired t-test, Mann–Whitney U test, and Fisher's exact test, as appropriate. A two-sided significance level of 5% was applied, unless otherwise stated.

All statistical analyses were performed using R version 4.5.0. The packages were obtained from CRAN (https://cran.r-project.org/) and Bioconductor (https://www.bioconductor.org/). The Tidyverse package (v2.0.0) was used for data visualization, transformation, and manipulation. The Compositions package (v2.0-8) was used for the CLR transformation of the gut microbiota count data, and lmerTest (v3.1-3) was used to construct the mixed linear models. The vegan package (v2.6-6.1) was used to calculate Bray–Curtis distances for beta diversity. The cluster package (v2.1.8.1) was used to calculate silhouette coefficients, and clusterCrit (v1.3.0) was used to calculate the Calinski–Harabasz and Davies–Bouldin indices. The differential abundance of other bacterial taxa in the gut microbiota was comprehensively analyzed using the ALDEx2 package (v1.32.0).

RESULTS

Participants' enrollment: The participant flow is shown in Figure 1. Participant enrollment was conducted from November 30 to December 1, and informed consent was obtained from all 437 participants. Of these, 100 participants who met the inclusion criteria but did not meet the exclusion criteria were selected and randomly assigned to groups A (receiving lactulose first) and B (receiving placebo first). The principal investigator, a licensed medical doctor, confirmed that all the selected participants were healthy. After randomization, one participant withdrew before the intervention began due to treatment for an illness. After the intervention began, 2 participants withdrew from the trial for personal reasons. The FAS population, which had efficacy data for at least one outcome, comprised 99 participants. The protocol-compliant participants, excluding those who violated protocol requirements, such as consuming prohibited foods (including foods containing probiotics or prebiotics), taking prohibited concomitant medications during the trial period, or failing to follow the protocol-defined method for the investigational product, comprised 78 participants and were defined as the PPS analysis population. The SAF analysis population comprised 99 participants who had consumed the study food at least once. The final follow-up was conducted on April 25, 2025.

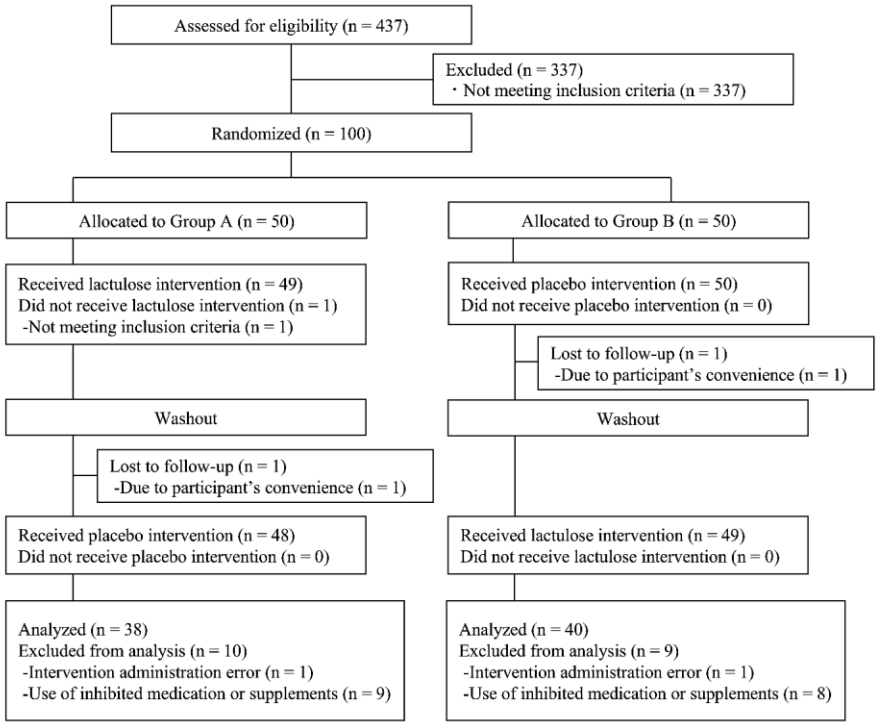


Figure 1. Participant flow diagram.

The diagram illustrates the process of enrolling, randomization, and analyzing participants. Of the 437 individuals screened, 100 were enrolled and randomized into groups A (lactulose first) and B (placebo first). The analysis sets included the full analysis set (FAS, n = 99), per-protocol set (PPS, n = 78), and safety analysis set (SAF, n = 99).

Participant's background: Table 1 presents the participants' backgrounds in the ITT and PPS analyses. There were no differences in baseline characteristics between the groups in either the ITT or PPS populations. Among the participants in the PPS group, adherence to the investigational product intake was maintained at a minimum of 96.4%. No significant differences in intake compliance were observed between groups A and B during either the first (p = 0.99) or second intervention periods (p = 0.34).

Table 1. Baseline information of participants.

	All participants				**ITT population**							**PPS population**						
					Group A			Group B			*p*-		Group A			Group B			*p*-
	(*N* = 100)				(n = 50)			(n = 50)			value		(n = 38)			(n = 40)			value
Sex (Male: Female)	62	:	38		31	:	19	31	:	19	1.00[1]		24	:	14	24	:	16	0.82[1]
Age (years)	47.2	±	10.4		46.8	±	9.8	47.6	±	11.0	0.71[2]		46.9	±	10.3	48.0	±	10.8	0.64[2]
Height (cm)	167.4	±	7.5		167.1	±	7.8	167.6	±	7.2	0.76[2]		166.8	±	7.5	166.9	±	6.7	0.94[2]
Weight (kg)	61.6	±	7.5		61.2	±	8.3	62.0	±	6.6	0.60[2]		60.5	±	8.1	61.1	±	6.3	0.73[2]
BMI (kg/m^2)	22.0	±	1.6		21.9	±	1.7	22.1	±	1.5	0.54[2]		21.7	±	1.9	21.9	±	1.5	0.59[2]
Relative abundance of *Bifidobacterium* (%)	6.8	±	4.0		6.7	±	4.1	6.9	±	4.0	0.89[2]		7.0	±	3.9	6.9	±	4.1	0.87[2]

Values are expressed as mean ± standard deviation. No significant differences were observed between groups A and B at baseline. [1]Fisher's exact test, [2]Welch's t-test.

Dietary Survey: No significant differences in dietary habits were observed between the groups before and after the investigational product intake, as assessed by the BDHQ survey (Table 2).

Table 2. Dietary intake assessed by BDHQ

	Before the first intervention							At the end of the second intervention						
	Group A (n = 38)			Group B (n = 40)			*p*-value[1]	Group A (n = 38)			Group B (n = 40)			*p*-value[1]
Energy (kcal/day)	1,508	±	591	1,546	±	594	0.78	1,480	±	516	1,441	±	546	0.75
Mass (g/day)	1,755	±	695	1,738	±	668	0.91	1,576	±	536	1,536	±	593	0.76
Water (g/day)	1,431	±	591	1,403	±	553	0.83	1,261	±	454	1,228	±	497	0.76
Protein (g/day)	56	±	25	56	±	24	0.99	57	±	23	55	±	20	0.71
Fat (g/day)	45	±	20	44	±	18	0.82	45	±	19	46	±	16	0.87
Carbohydrate (g/day)	201	±	93	215	±	97	0.53	191	±	72	190	±	98	0.94
Soluble dietary fiber (g/day)	2.0	±	1.0	2.0	±	1.0	0.97	1.8	±	0.7	1.9	±	0.9	0.49
Insoluble dietary fiber (g/day)	6.1	±	2.6	6.2	±	2.7	0.88	5.6	±	1.9	5.7	±	2.1	0.83
Total dietary fiber (g/day)	8.5	±	3.6	8.5	±	3.8	0.98	7.6	±	2.6	7.8	±	3.1	0.79

Daily nutrient intake before and after the intervention was assessed using the Brief-type self-administered Diet History Questionnaire (BDHQ). No significant differences were observed between groups. Values are expressed as mean ± standard deviation. [1]Welch's t-test

Effect of lactulose intervention on the abundance of *Bifidobacterium* in the gut microbiota: Figure 2 shows intergroup differences in *Bifidobacterium* abundance, the primary outcome of this study. A significant increase in the relative abundance of *Bifidobacterium* was observed following lactulose intervention compared with placebo intervention in PPS ($p = 0.003$), as analyzed using a linear mixed model based on CLR-transformed values. To verify the robustness of these findings, we performed an additional analysis using the FAS dataset, which produced consistent results ($p = 0.003$). The between-investigational products difference in the relative abundance of *Bifidobacterium* was 2.1% (95% CI: 0.6 to 3.6) in the PPS dataset. No significant carryover effect was detected in this crossover study ($p = 0.78$). The relative abundance of *Bifidobacterium* significantly increased from pre- to post-intervention with lactulose ($p = 0.04$; Figure 3). No significant intergroup differences in alpha diversity were observed between the lactulose and placebo groups (Table 3). Analyses using the ALDEx2 pipeline revealed no substantial changes in the composition of other bacterial taxa within the gut microbiota (Supplemental Table 1).

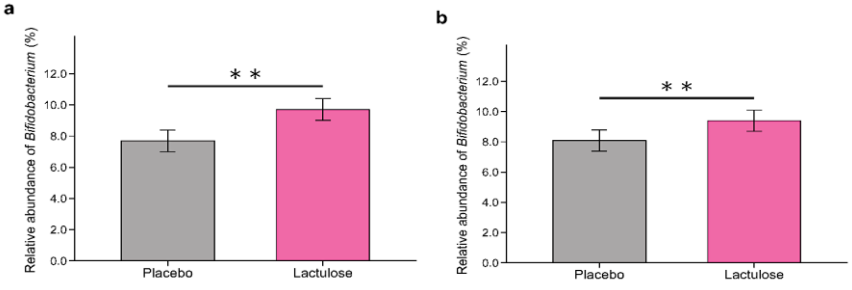


Figure 2. Prebiotic effect of lactulose on *Bifidobacterium* in gut microbiota.

Comparison of *Bifidobacterium* abundance between the lactulose (pink) and placebo (gray) groups in (a) PPS (n = 78, Placebo: 7.69 ± 0.75, Lactulose: 9.75 ± 0.75) and (b) FAS populations (n = 99, Placebo: 8.10 ± 0.70, Lactulose: 9.43 ± 0.70). Statistical analysis was performed using a linear mixed model based on centered log-ratio-transformed values, with the investigational product intake group and intake timing as fixed effects and participants as a random effect. Values represent least-square means, and error bars indicate standard errors. PPS: per-protocol set, FAS: complete analysis set, $^{**}p < 0.01$

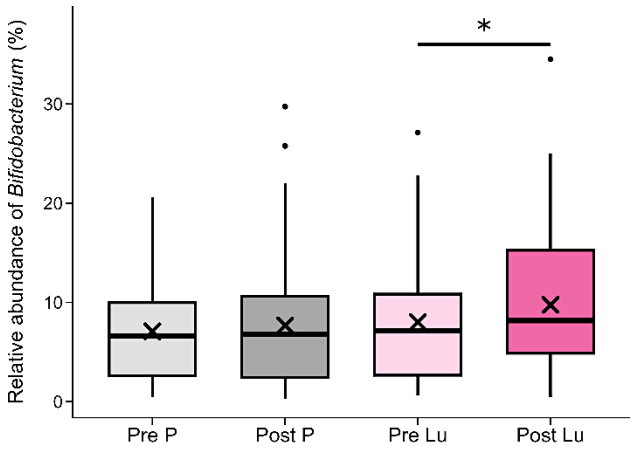


Figure 3. Change in relative abundance of *Bifidobacterium* before and after lactulose intake.

Boxplot showing a significant increase in the relative abundance of *Bifidobacterium* following a 28-day lactulose intervention at 500 mg/day in the PPS population (n = 78; **p* = 0.04). The upper and lower edges of the box represent the first and third quartiles, respectively, while the central line indicates the median. Whiskers extend to the minimum and maximum values within 1.5 × interquartile range, and individual points represent outliers beyond this range. Pre: Before intervention, Post: After intervention, P: Placebo intervention, L: Lactulose intervention

Table 3. Alpha diversity indices of gut microbiota.

	n	Placebo			Lactulose			Difference[1] (95% CI[2])	*p*-value[3]
Chao1	78	324.9	±	9.7	333.6	±	9.7	8.6 (- -10.2, 27.5)	0.37
Pielou's Evenness	78	0.7	±	0.004	0.7	±	0.004	-8.0×10^{-4} (-0.009, 0.008)	0.85
Faith's Phylogenetic Diversity	78	32.0	±	1.2	32.3	±	1.2	0.3 (-2.0, 2.6)	0.81
Observed Features	78	303.2	±	8.5	313.4	±	8.5	10.3 (-5.7, 26.2)	0.21
Shannon Index	78	5.9	±	0.05	5.9	±	0.05	0.03 (-0.06, 0.11)	0.55

Alpha diversity metrics (Chao1, Pielou's Evenness, Faith's Phylogenetic Diversity, Observed Features, Shannon Index) were compared between the lactulose and placebo. No significant differences were observed. Values are expressed as least square means ± standard errors. [1]Lactulose minus placebo, [2]Confidence Interval, [3]Analysis using linear mixed model

Enterotype classification and diversity shifts following lactulose intervention: To confirm the detailed changes in the gut microbiota, we conducted an exploratory analysis focusing on the microbiota composition before and after lactulose intake. Bray-Curtis distances were calculated based on 16S ASV data, and beta diversity was assessed using PCoA. The gut microbiota of the participants prior to lactulose intake was broadly classified into three clusters using hierarchical clustering (Figure 4a). Cluster 1 represented an enterotype characterized by a microbiota rich in *Prevotella, Bacteroides, Faecalibacterium*, and *Bifidobacterium*. Cluster 2 was characterized by a gut microbiota with lower *Prevotella* compared to Cluster 1 but rich in *Bifidobacterium*, *Bacteroides*, *Faecalibacterium,* and *Phocaeicola*. In contrast, cluster 3 showed a low *Bifidobacterium* abundance and a high abundance of *Fusobacterium*, *Phocaeicola*, and *Ruminococcus.* A comparison of alpha diversity (Chao1, Observed Features) across clusters revealed that clusters 1 and 2 maintained alpha diversity following lactulose intervention, whereas cluster 3 showed a trend toward increased alpha diversity following lactulose intervention (Figure 4b, c). Shifts in gut microbiota composition following lactulose intervention are visualized in Figure 4d.

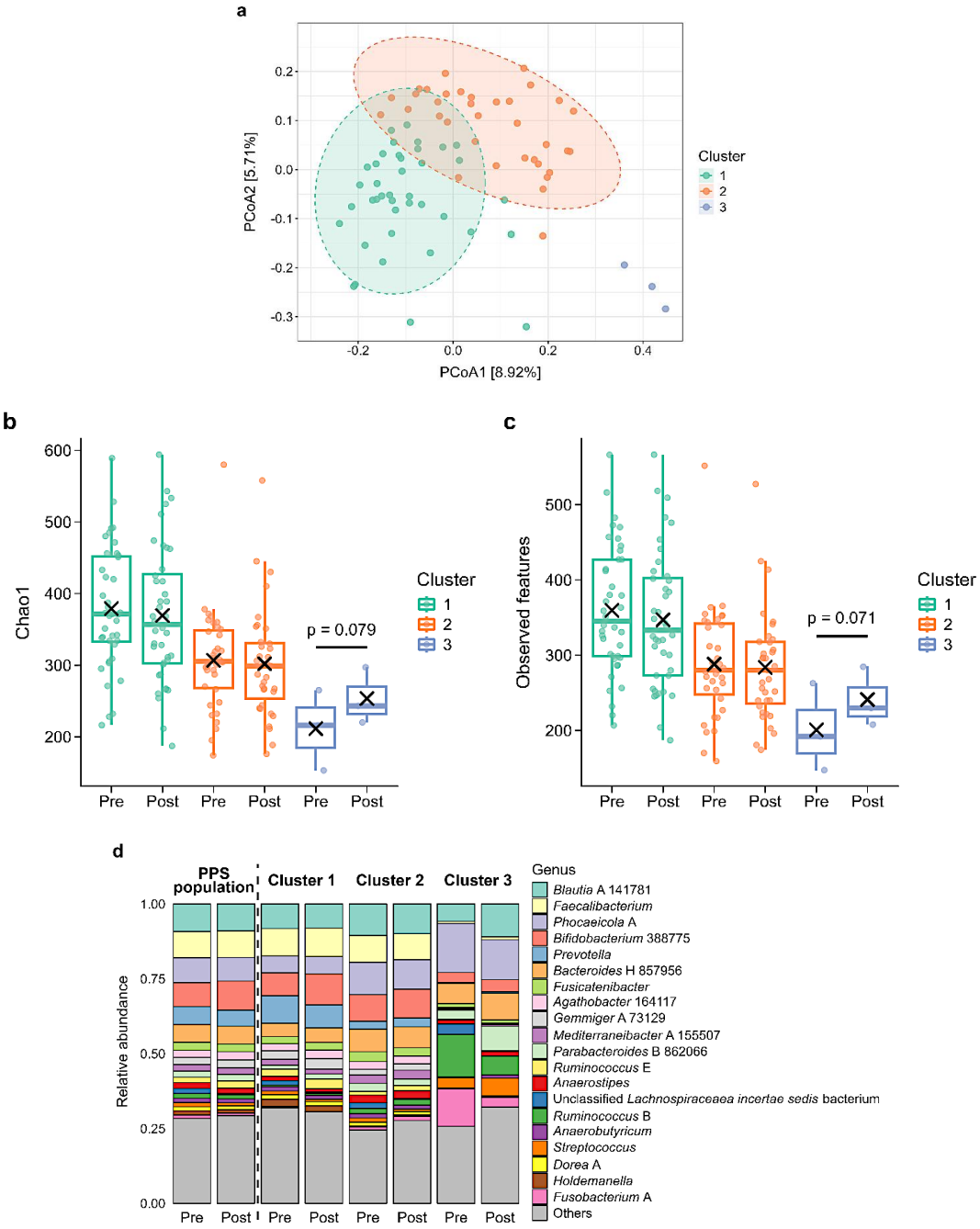


Figure 4. Impact of lactulose on gut microbiota composition and alpha diversity across clusters based on pre-intervention beta diversity. (a)Principal coordinate analysis (PCoA) based on Bray-Curtis distances of 16S rRNA amplicon sequence variants (ASVs) prior to lactulose intake revealed three distinct enterotype clusters: Cluster 1 (n = 40, green circles), Cluster 2 (n = 35, orange circles), and Cluster 3 (n = 3, blue circles). The three enterotypes differed significantly (PERMANOVA, $p = 0.001$). (b,c)Comparison of the Chao1 index (b) and Observed Features index (c) across clusters, indicating a trend toward increased alpha diversity in Cluster 3 following lactulose intervention (paired t-test, Chao1: $p = 0.079$, Observed Features: $p = 0.071$). Each data point is plotted individually as a dot. The box represents the interquartile range (IQR), with the central line indicating the median and the mean marked with a cross. Whiskers extend to the minimum and maximum values within 1.5 × IQR, and values beyond this range are shown as outliers. (d)Stacked bar plots showing the relative abundance of the 20 major types of bacterial genera across the three clusters identified in (a) Cluster 1 was characterized by a higher relative abundance of *Prevotella*, *Faecalibacterium*, *Blautia*, *Bifidobacterium*, and *Phocaeicola*. Cluster 2 showed greater abundances of *Phocaeicola*, *Blautia*, *Faecalibacterium*, *Bifidobacterium*, and *Bacteroides*. Cluster 3 was distinguished by the predominance of *Phocaeicola* and *Ruminococcus*, followed by *Fusobacterium*, *Bacteroides*, and *Blautia*. Pre: Before lactulose intervention, Post: After lactulose intervention

Safety assessment: One case was reported among 99 participants during lactulose intake, while 7 cases occurred among 99 participants during the placebo intake period. The incidence of adverse events (persons/person) was not significantly different between the lactulose and placebo groups (p = 0.22). All adverse events were transient, mild, or moderate (e.g., mild headache). The principal investigator determined that none of the adverse events was causally related to the investigational products. According to the participants' diaries, no cases of diarrhea or abdominal bloating were reported during the lactulose intake period. Furthermore, the participants maintained their usual bowel habits throughout the trial period, with no significant increase in bowel movement frequency.

DISCUSSION

This study investigated the effects of a 500 mg lactulose intervention, the lowest dose reported to date, on the gut microbiota. The results showed that even a low dose of lactulose increased the relative proportion of *Bifidobacterium* in the gut microbiota by approximately 2.1%. Previous randomized controlled trials [24,25] using higher doses of 2 or 4 g reported an increase of roughly 7–8%, suggesting a dose-dependent relationship in the prebiotic effects of NDOs [18,22]. This aligns with the expected dose-response trends. Notably, similar results were observed in both the PPS and the FAS, and no significant differences in dietary intake were observed before and after the intervention. These findings support the robustness of the trial outcomes and suggest that even minimal lactulose intake can beneficially modulate gut microbiota without confounding dietary influences.

Dietary interventions, including prebiotic interventions, are considered important for maintaining a healthy gut microbiota [26]. Furthermore, the relative abundance of *Bifidobacterium* has been reported to negatively correlate with serum uric acid levels and positively correlate with HDL-cholesterol [27]. It is important for gut microbiota diversity and robustness [28]. The changes in the gut microbiota observed in this trial may be significant in supporting a sustained *Bifidobacterium*-rich environment, which is associated with various health benefits.

Safety is a key consideration for sustained lactulose intake to maintain *Bifidobacterium* levels. Lactulose is also used as a pharmaceutical and is recognized as an oligosaccharide with few adverse effects and a favorable safety profile [29]. In this study as well, no adverse events attributable to lactulose were observed. Furthermore, there were no reports of diarrhea onset or abdominal bloating, nor was there an excessive increase in bowel movement frequency. Therefore, 500 mg of lactulose was considered consistent with previous reports.

From a practical perspective, incorporating lactulose into various food formats may help sustain its intake. Lactulose has a sweet taste and is commonly used in products such as beverages, yogurt, and gummies [30]. Based on the present findings, incorporating low-dose lactulose (500 mg) into alternative formats, such as capsules or products that do not rely on sweetness, could be a feasible option.

Recent studies have reported that establishing a *Bifidobacterium*-rich gut microbiota during weaning is important for long-term health [31]. Although this trial involved healthy adults, conducting low-dose continuous lactulose intervention trials in such target populations could yield important insights into long-term dietary strategies for maintaining gut health. In the present study, lactulose intake did not significantly alter alpha diversity, which is consistent with previous reports [24,25]. A study investigating the relationship between FOS intake and alpha diversity reported that alpha diversity gradually increased over 90 days of continuous intake [32]. Long-term lactulose intake trials could provide further insights.

In participants with a low alpha diversity enterotype, a state often referred to as dysbiosis, where

Fusobacterium, a genus associated with ulcerative colitis, is abundant, low-dose lactulose intervention showed a trend toward restoration of alpha diversity. Although the number of participants in this cluster was small, making definitive conclusions difficult, future interventions targeting populations with low alpha diversity may help elucidate the mechanisms underlying microbiota modulation by lactulose, potentially contributing to personalized approaches to gut health interventions.

This study had several limitations. The trial was conducted in Japan, where it has been reported that many Japanese individuals harbor *Bifidobacterium* [33]. Data from the American Gut Project (AGP), one of the largest datasets on the human microbiome, also indicate that the relative abundance of *Bifidobacterium* is significant [28]. While the evidence may be extrapolated, obtaining evidence from outside Japan is desirable. Furthermore, to minimize the participant burden, blood tests and measurements of fecal metabolites were not planned for this study. Supplementing these data would likely enable a more detailed investigation of the relationship between lactulose and the gut microbiota. Future studies combining gut microbiota-related outcomes with clinical indicators could help clarify this relationship for potential health benefits.

This study is the first to demonstrate that the lowest lactulose dose ever reported can significantly increase the abundance of *Bifidobacterium* in healthy adults, even at levels far below conventional bifidogenic doses. This research provides novel evidence that small amounts of lactulose can selectively modulate the composition of the gut microbiota without inducing laxative effects. The findings advance our understanding of lactulose by highlighting its prebiotic potential in everyday dietary contexts.

Lactulose is a safe, well-established ingredient that can be easily incorporated into routine diets, making it a practical approach for supporting gut health. Consuming ultra-low-dose lactulose may serve as a practical and safe functional food ingredient to support long-term gut health, and continued research on its incorporation into functional food matrices will be important. These results support the potential use of lactulose-based functional foods in public health and preventive nutrition programs to promote microbiome resilience.

CONCLUSIONS

Lactulose, known as the bifidus factor, demonstrated its effect even at an ultra-low dose of 500 mg in healthy adults. In addition, this study suggests that lactulose may support the restoration of alpha diversity. No adverse events attributable to the investigational products were observed, indicating that 500 mg of lactulose can be safely and continuously consumed. Thus, lactulose is a nondigestible oligosaccharide that contributes to health by improving the gut environment.

Abbreviations: UMIN-CTR: University Hospital Medical Information Network Clinical Trials Registry, FOS: Fructooligosaccharides, GOS: Galactooligosaccharides, PCR: Polymerase Chain Reaction, BDHQ: Brief-type Self-Administered Diet History Questionnaire, NCI-CTCAE: National Cancer Institute Common Terminology Criteria for Adverse Events, JCOG: Japan Clinical Oncology Group, SAF: Safety analysis set, PPS: Per Protocol Set, FAS: Full analysis set, CLR: Centered log-ratio, ASV: Amplicon sequence variant, PERMANOVA: Permutational multivariate analysis of variance, CRAN: Comprehensive R Archive Network, CI: Confidence Interval, IQR: Interquartile Range, HDL: High-density lipoprotein, AGP: American Gut Project

Competing Interests: All authors are employed by Morinaga Milk Industry Co., Ltd.

Author Contributions: conceptualization: R.S., methodology: R.S., S.M., and M.N., formal analysis: R.S. and S.M., investigation: R.S. and S.M., data curation: R.S., S.M., and M.N., writing—original draft preparation: R.S.,

writing—review and editing: M.N. and T. M., visualization: R.S. and S.M., supervision: M.N. and T. M., project administration: M.N. and T. M. All authors have read and agreed to the published version of the manuscript.

ACKNOWLEDGMENTS: We gratefully acknowledge the contributions of Dr. Yoshitaka Iwama, a licensed medical doctor and the principal investigator.

FUNDING: This work was supported by the authors, Morinaga Milk Industry Co., Ltd. The company, an affiliated institution of the authors, provided all research funds.

REFERENCES

1. Wu G., Xu T., Zhao N., Lam Y.Y., Ding X., Wei D., *et al*. A core microbiome signature as an indicator of health. *Cell*. 2024;187(23):6550-6565.e11.
   DOI: https://doi.org/10.1016/j.cell.2024.09.019
2. Manske S. Gastrointestinal health: A foundation for whole-body wellness. *Altern Ther Health Med*. Jul 2024;30(7):6-10.
3. Van Hul M., Cani P.D., Petitfils C., De Vos W.M., Tilg H., El-Omar E.M. What defines a healthy gut microbiome? *Gut*. 2024;73(11):1893-1908.
   DOI: https://doi.org/10.1136/gutjnl-2024-333378
4. Rook O., Zwart H. Awareness of human microbiome may promote a healthier lifestyle and more positive environmental attitudes. *Communications Medicine*. 2025;5(1):39.
   DOI: https://doi.org/10.1038/s43856-025-00747-4
5. Alqahtani M.S. The gut microbiota–metabolic axis: Emerging insights from human and experimental studies on type 2 diabetes mellitus—a narrative review. *Medicina*. 2025;61(11):2017.
   DOI: https://doi.org/10.3390/medicina61112017
6. Kumari A., K G.R., Sudhakaran V.A., Warrier A.S., Singh N.K. Unveiling the health benefits of prebiotics: A comprehensive review. *Indian J Microbiol*. Jun 2024;64(2):376-388.
   DOI: https://doi.org/10.1007/s12088-024-01235-4
7. Rithi A.T., Mitra A., Banerjee A., Ilanchoorian D., Marotta F., Radhakrishnan A.K. Effect of prebiotics, probiotics, and synbiotics on gut microbiome in diabetes among coastal communities. Review. *Functional Food Science*. 2024;4(1):11-28.
   DOI: https://doi.org/10.31989/ffs.v4i1.1271
8. Rondinella D., Raoul P.C., Valeriani E., Venturini I., Cintoni M., Severino A., *et al*. The detrimental impact of ultra-processed foods on the human gut microbiome and gut barrier. *Nutrients*. 2025;17(5):859.
   DOI: https://doi.org/10.3390/nu17050859
9. Daunizeau C., Franck M., Boutin A., Ruel M., Poliakova N., Ayotte P., *et al*. The gut microbiota of indigenous populations in the context of dietary westernization: A systematic review and meta-analysis. *Front Nutr*. 2025; 12:1652598.
   DOI: https://doi.org/10.3389/fnut.2025.1652598
10. Apalowo O., Adegoye G., Obuotor T. Exploring the functional roles of sustainable dietary patterns on gut microbiota composition and optimal health. Review. *Bioactive Compounds in Health and Disease*. 2024;7(2):110-130.
    DOI: https://doi.org/10.31989/7i2.1279
11. Smolinska S., Popescu F.-D., Zemelka-Wiacek M. A review of the influence of prebiotics, probiotics, synbiotics, and postbiotics on the human gut microbiome and intestinal integrity. *Journal of Clinical Medicine*. 2025;14(11):3673.
    DOI: https://doi.org/10.3390/jcm14113673
12. Yoo S., Jung S.-C., Kwak K., Kim J.-S. The role of prebiotics in modulating gut microbiota: Implications for human health. *International Journal of Molecular Sciences*. 2024;25(9):4834.
    DOI: https://doi.org/10.3390/ijms25094834
13. Lee D.H., Seong H., Chang D., Gupta V.K., Kim J., Cheon S., *et al*. Evaluating the prebiotic effect of oligosaccharides on gut microbiome wellness using in vitro fecal fermentation. *npj Science of Food*. 2023;7(1):18.
    DOI: https://doi.org/10.1038/s41538-023-00195-1
14. Pedrosa L.d.F., de Vos P., Fabi J.P. From structure to function: How prebiotic diversity shapes gut integrity and immune balance. *Nutrients*. 2024;16(24):4286.
    DOI: https://doi.org/10.3390/nu16244286
15. Gavzy S.J., Kensiski A., Lee Z.L., Mongodin E.F., Ma B., Bromberg J.S. *Bifidobacterium* mechanisms of immune modulation and tolerance. *Gut Microbes*. 2023;15(2):2291164.
    DOI: https://doi.org/10.1080/19490976.2023.2291164
16. Fabiana B., Leonardo M., Christian M., Gabriele Andrea L., Chiara T., Giulia L., *et al*. Compendium of *bifidobacterium*-based probiotics: Characteristics and therapeutic impact on human diseases. *Microbiome Research Reports*. 2024;4(1):2.
    DOI: https://doi.org/10.20517/mrr.2024.52

17. Zhao H., Zhou J., Yuan L., Sun Z., Liu Y., Zhao X., *et al*. Exploring the alleviating effects of bifidobacterium metabolite lactic acid on non-alcoholic steatohepatitis through the gut-liver axis. *Front Microbiol*. 2024; 15:1518150. DOI: https://doi.org/10.3389/fmicb.2024.1518150
18. Bouhnik Y., Raskine L., Simoneau G., Paineau D., Bornet F. The capacity of short-chain fructo-oligosaccharides to stimulate faecal bifidobacteria: A dose-response relationship study in healthy humans. *Nutr J*. 2006; 5:8. DOI: https://doi.org/10.1186/1475-2891-5-8
19. Tamai S., Nakamura Y., Ozawa O., Yamauchi K. Effects of galactooligosaccharides intake on human fecal flora and metabolites. *Journal of Applied Glycoscience*. 1994;41(3):333-338. DOI: https://doi.org/10.11541/jag1994.41.333
20. Harthoorn L.F., Heyse J., Baudot A., van Hengel I.A.J., Van den Abbeele P. Galacto-oligosaccharides exert bifidogenic effects at capsule-compatible ultra-low doses. *Metabolites*. 2025;15(8). DOI: https://doi.org/10.3390/metabo15080530
21. Tomoda T., Nakano Y., Kageyama T. Effect of yogurt and yogurt supplemented with *Bifidobacterium* and/or lactulose in healthy persons: A comparative study. *Bifidobacteria and Microflora*. 1991;10(2):123-130. DOI: https://doi.org/10.12938/bifidus1982.10.2_123
22. Sakai Y., Seki N., Hamano H., Ochi H., Abe F., Shimizu F., *et al*. A study of the prebiotic effect of lactulose at low dosages in healthy Japanese women. *Biosci Microbiota Food Health*. 2019;38(2):69-72. DOI: https://doi.org/10.12938/bmfh.18-013
23. Kok C.R., Rose D., Hutkins R. Predicting personalized responses to dietary fiber interventions: Opportunities for modulation of the gut microbiome to improve health. *Annu Rev Food Sci Technol*. 2023; 14:157-182. DOI: https://doi.org/10.1146/annurev-food-060721-015516
24. Sakai Y., Ochi H., Tanaka M. Lactulose ingestion induces a rapid increase in genus *Bifidobacterium* in healthy Japanese: A randomised, double-blind, placebo-controlled crossover trial. *Microorganisms*. 2022;10(9). DOI: https://doi.org/10.3390/microorganisms10091719
25. Sakai Y., Seki N., Hamano K., Ochi H., Abe F., Masuda K., *et al*. Prebiotic effect of two grams of lactulose in healthy japanese women: A randomised, double-blind, placebo-controlled crossover trial. *Benef Microbes*. 2019;10(6):629-639. DOI: https://doi.org/10.3920/bm2018.0174
26. Gyriki D., Nikolaidis C.G., Bezirtzoglou E., Voidarou C., Stavropoulou E., Tsigalou C. The gut microbiota and aging: Interactions, implications, and interventions. *Front Aging*. 2025; 6:1452917. DOI: https://doi.org/10.3389/fragi.2025.1452917
27. Gong H., Gao H., Ren Q., He J. The abundance of Bifidobacterium in relation to visceral obesity and serum uric acid. *Sci Rep*. 2022;12(1):13073. DOI: https://doi.org/10.1038/s41598-022-17417-3
28. Feng Y., Duan Y., Xu Z., Lyu N., Liu F., Liang S., *et al*. An examination of data from the american gut project reveals that the dominance of the genus *bifidobacterium* is associated with the diversity and robustness of the gut microbiota. *Microbiologyopen*. 2019;8(12): e939. DOI: https://doi.org/10.1002/mbo3.939
29. Dabak M.R., Güler K. Comparative evaluation of lactulose and bisacodyl in the management of chronic constipation: Efficacy, safety, and patient preferences. *Bagcilar Medical Bulletin*. 2023;8(4):335-340. DOI: https://doi.org/10.4274/BMB.galenos.2023.2023-08-073
30. Vičič V., Pandel Mikuš R., Ferjančič B. Review of history and mechanisms of action of lactulose (4-o-β-d-galactopyranosyl-β-d-fructofuranose): Present and future applications in food. *J Food Sci Technol*. 2024;61(11):2036-2045. DOI: https://doi.org/10.1007/s13197-024-05997-z
31. Shimizu Y., Yokoi Y., Ohira S., Izumi H., Kawakami S., Ihara M., *et al*. Modulation of bifidobacterium by hd5 during weaning is associated with high abundance in later life. *Commun Med (Lond)*. 2025;5(1):250. DOI: https://doi.org/10.1038/s43856-025-00977-6
32. Tandon D., Haque M.M., Gote M., Jain M., Bhaduri A., Dubey A.K., *et al*. A prospective randomized, double-blind, placebo-controlled, dose-response relationship study to investigate efficacy of fructo-oligosaccharides (fos) on human gut microflora. *Sci Rep*. 2019;9(1):5473. DOI: https://doi.org/10.1038/s41598-019-41837-3
33. Nakano A., Kawata D., Ueno H.M., Tatara Y., Ishida M., Tamada Y., *et al*. Association between dairy consumption, gut microbiome composition, and lipid biomarkers in Japanese adults: A cross-sectional analysis from the Iwaki Health Promotion Project. *International Dairy Journal*. 2026; 173:106445.DOI: https://doi.org/https://doi.org/10.1016/j.idairyj.2025.106445