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RESEARCH ARTICLE

Effects of fermented milk containing *Lacticaseibacillus paracasei* strain Shirota on constipation and gut microbiota: A randomised pilot study in Vietnam

V. Hoang Van[1], *T. Dong Van*[2], *N. Nguyen Bao*[2], *U. Nguyen Quynh*[1], *C. Nguyen Thi Linh*[1], *C. Dao Xuan*[2] *and T. Akiyama*[3]*

[1]Institute of Microbiology and Biotechnology, Vietnam National University, Hanoi, No. 144 Xuan Thuy street, Cau Giay district, Hanoi city, Vietnam; [2]Bach Mai Hospital, No. 78 Giai Phong street, Dong Da district, Hanoi city, Vietnam; [3]Yakult Central Institute, 5-11 Izumi, Kunitachi-shi, Tokyo 186-8650, Japan; *takuya-akiyama@yakult.co.jp

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Abstract

Fermented milk containing *Lacticaseibacillus paracasei* strain Shirota (LcS) has been shown to improve stool consistency in various countries; however, its effect on hard or lumpy stools (HLS) in the Vietnamese population remains unclear. We investigated the effects of LcS-fermented milk on constipated Vietnamese adults with a high prevalence of HLS. In a single-centre, open-label trial, 51 participants with HLS were randomised to receive one bottle per day of fermented milk containing ≥6.5×10^9 cfu of LcS for 4 weeks (probiotic group) or no intervention (control group), followed by a 2-week washout. The primary endpoint was the proportion of participants with HLS (Bristol Stool Form Scale score of 1 or 2) in ≥25% of bowel movements over 4 weeks. Secondary endpoints included total stool frequency, HLS frequency, ideal stool form, and defecation-related symptoms – all recorded in daily diaries. The Chinese Constipation Questionnaire (CCQ) and gut microbiota composition (16S rRNA sequencing) were assessed every 2 weeks. During the intervention, the probiotic group had a significantly lower proportion of participants with HLS in ≥25% of bowel movements compared to controls (odds ratio: 0.00; 95% confidence interval: 0.00-0.16; $P = 0.005$). The probiotic group also showed increased total stool frequency ($P = 0.001$), reduced HLS frequency ($P = 0.035$), and fewer participants with CCQ scores ≥5 indicating constipation ($P < 0.001$) at 4 weeks. Gut microbiota beta-diversity differed between groups after 2 weeks ($P = 0.031$), with reductions in *Peptococcaceae*, *Clostridium_methylpentosum_group*, and *Clostridia* (unclassified at the order level), followed by increases in *Lachnospiraceae_UCG-004* at 4 weeks and *Lachnospiraceae_ND3007_group* post-follow-up ($P < 0.050$), suggesting microbial changes linked to constipation improvement. No serious adverse events related to the intervention were observed. These findings support daily LcS-fermented milk as a dietary strategy in Vietnam to manage constipation via gut microbiota modulation.
The study was registered at ClinicalTrials.gov (ID: NCT05982743).

Keywords

fermented foods – *Lacticaseibacillus paracasei* strain Shirota – probiotics – digestive health – hard stools

1 Introduction

Constipation is a common symptom-based gastrointestinal disorder characterised by infrequent bowel movements, often accompanied by pain and a sense of incomplete evacuation, affecting 2.6 to 26.9% of the adult population worldwide (Forootan *et al.*, 2018; Schmidt and Santos, 2014). Acute constipation can lead to intestinal obstruction, potentially requiring surgical intervention, whereas the chronic form significantly impairs patients' quality of life and places a substantial burden on the healthcare system (Belsey *et al.*, 2010; Benninga *et al.*, 2005). The production of hard or lumpy stools (HLS) is strongly associated with the development of constipation and is considered a core symptom (Patimah *et al.*, 2017). HLS is frequently observed in individuals with chronic constipation and in healthy individuals. Therefore, reducing the incidence of HLS may benefit a broad range of individuals by reducing their overall risk of constipation.

Among various treatment options, probiotics have emerged as a promising intervention for managing constipation. Recent systematic reviews and meta-analyses have shown that probiotics can significantly improve bowel movement frequency and stool consistency in adults with constipation, although the magnitude of the effect varies depending on the strain and formulation (Ding *et al.*, 2024; Garzon Mora and Jaramillo, 2024). *Lactobacillus casei* strain Shirota (reclassified as *Lacticaseibacillus paracasei* strain Shirota in April 2020) (LcS) is a well-characterised probiotic, first isolated and cultured by Dr Minoru Shirota in 1930. LcS reaches the intestines alive and confers various health benefits to the host (Qi *et al.*, 2020; Yan *et al.*, 2022; Zheng *et al.*, 2020). Several studies have investigated the effects of fermented milk beverages containing LcS (Yakult®) on constipation symptoms, including HLS. Randomised controlled trials conducted in constipated populations in Europe (Koebnick *et al.*, 2003; Sakai *et al.*, 2011; Tilley *et al.*, 2014; Tsujibe *et al.*, 2024), the United States (Cook *et al.*, 2025), and Japan (Matsumoto *et al.*, 2006) have demonstrated that daily intake of LcS-containing fermented milk alleviates gastrointestinal symptoms and reduces the prevalence of hard stools. In one such study, Matsumoto *et al.* observed an increase in beneficial microbes (e.g. *Bifidobacterium*) along with elevated concentrations of organic acids, suggesting a favourable shift in the intestinal environment (Matsumoto *et al.*, 2006). Additionally, Chen *et al.* examined the effects of LcS-containing fermented milk in Chinese patients with functional constipation, stratified by baseline stool consistency (Chen *et al.*, 2019). Notably, in individuals with hard stools (the HS group), 4 weeks of daily LcS supplementation significantly alleviated constipation symptoms and increased stool frequency. These clinical improvements were accompanied by distinct alterations in the gut microbiota, including increased abundances of *Pseudobutyrivibrio* and *Roseburia*, and elevated concentrations of organic acids. These findings suggest that the anti-constipation effects of LcS, particularly in individuals with hard stools, may be mediated through the modulation of the intestinal microbiota and its metabolic environment. However, the specific patterns of modulation may vary across populations.

The promising findings about the anti-constipation effect of LcS-containing fermented milk have not been evaluated in the Vietnamese population. To address this gap, the present pilot study was conducted as a single-centre randomised controlled trial to investigate the efficacy of this beverage in healthy Vietnamese adults with HLS. Additionally, we evaluated whether the observed clinical outcomes were associated with gut microbiome shifts.

2 Materials and methods

Ethical considerations

This study was reviewed and approved by the IRB of Bach Mai Hospital (approval ID: 2138/QD-BVBM, dated 2 August 2021) and conducted in accordance with the principles of the Declaration of Helsinki, the International Council for Harmonisation-Good Clinical Practices (ICH-GCP E6), and Vietnamese regulatory laws governing biomedical research involving human participants. The study was registered at ClinicalTrials.gov (ID: NCT05982743).

Study design and setting

This single-centre, open-label, randomised controlled trial was conducted at Bach Mai Hospital and Vietnam National University (Hanoi, Vietnam), from April 2022 to March 2023. Participants with HLS were identified from individuals attending routine health check-ups at the Outpatient Department of Bach Mai Hospital, based on the Bristol Stool Form Scale (BSFS) score. Block randomisation stratified by sex was performed using randomly permuted blocks of 2 or 4 to allocate participants to either the probiotic group or the control group in a 1:1 ratio through a centralised, web-based randomisation system. The system automatically generated the randomisation number and treatment assignment in

real time, ensuring blinded group allocation. The probiotic group consumed one bottle of LcS-containing fermented milk daily for 4 weeks, and the control group received no interventions. Subsequently, both groups underwent a 2-week washout period without interventions. CRO Smart Research (https://www.smartresearch.com.vn/) supported the study by drafting the study documents, generating and concealing the randomisation sequence, managing the site, monitoring data, and performing biostatistics.

Eligibility criteria

The inclusion criteria were: age between 18 and 60 years, awareness of frequently producing HLS (BSFS score of 1 or 2), production of HLS in ≥25% of bowel movements during a 2-week screening period and provision of written informed consent.

The exclusion criteria were primary organic disease of the colon or pelvic floor or use of medication causing constipation, presence of significant concomitant diseases including neurological or metabolic disorders, known allergy to milk ingredients, pregnancy or breastfeeding, inability to refrain from or anticipated use of medications including diuretics, laxatives, prescription and non-prescription drugs, vitamins, and herbal supplements, except for paracetamol, oral contraceptives, or hormonal replacement therapy from 2 weeks before screening until study completion, inability to refrain from or anticipated use of probiotics, prebiotics, or yogurts during the same period, participation in another investigational study within 2 months before this study and drug or alcohol abuse.

Investigational product

LcS-fermented milk (Yakult®), each 65 ml, containing at least 6.5×10^9 cfu of LcS at the end of shelf life, was supplied by Yakult Vietnam Co., Ltd. (Ho Chi Minh City, Vietnam). The viable microbial count was guaranteed by the manufacturer using the same quality specifications applied to the commercially available product. The investigated product (IP) was stored at 2-10 °C. Although the manufacturer's recommended storage temperature in Vietnam is 5-10 °C, a broader range was predefined to account for potential operational challenges in maintaining a narrow temperature band. Continuous temperature monitoring with a data logger ensured compliance with this range. Shipments were delivered biweekly, with expiry dates managed to ensure at least 21 days of remaining shelf life upon arrival at the study site. Participants consumed one bottle daily for 4 weeks, preferably after breakfast, reflecting real-world consumer behaviour. Adherence was defined as consumption of at least 80% of the IP. Participants could withdraw at any time.

Study endpoints

The primary efficacy endpoint was the proportion of participants producing HLS in ≥25% of bowel movements. Secondary efficacy endpoints included total stool frequency; frequency of HLS; ideal stool form (BSFS score 4); and defecation-related symptoms, including straining during evacuation, sensation of remaining stool in the rectum, sensation of anorectal blockage, and use of manual manoeuvres, and the Chinese Constipation Questionnaire (CCQ) score. A cut-off score of ≥5 on the CCQ, with higher scores indicating functional constipation, was established to distinguish healthy participants from patients with functional constipation, with 91% sensitivity and 91% specificity (Chan *et al.*, 2005). Gut microbiota measures were assessed using faecal samples, including alpha-diversity, beta-diversity, and relative abundance of *Bifidobacterium* and other bacterial taxa. Adverse events (AEs) were recorded and followed up for safety throughout the study period.

Data collection

Data collection schedules are presented in Figure 1 and Supplementary Table S1. Demographic and clinical data were collected during the study visits. Stool consistency (BSFS) and bowel habits were recorded in a daily diary per bowel movement. Participants provided stool samples for stool microbiota analysis and completed the CCQ every 2 weeks after the start of the intervention.

Analysis of stool microbiota

Stool microbiota were analysed using next-generation sequencing on samples collected at the end of a 2-week baseline period (Week 0), on Weeks 2 and 4 after the intervention, and following a subsequent 2-week washout period (Week 6). Genomic DNA was extracted from stool samples using the QIAamp Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany), following a previously published protocol, with slight modifications (Matsuki *et al.*, 2016). The V1-V2 regions of the 16S rRNA gene were amplified using the forward primer 5′-AGAGTTTGATCMTGGCTCAG-3′ and the reverse primer 5′-TGCTGCCTCCCGTAGGAGT-3′ (Kim *et al.*, 2013) in PCR reactions with a total volume of 15 µl, comprising Phusion® High-Fidelity PCR Master Mix (New England Biolabs, Ipswich, MA, USA), 2 µM of each primer, and 10 ng of template DNA. The thermal cycling protocol included an initial denaturation at 96 °C for 1 min, fol-

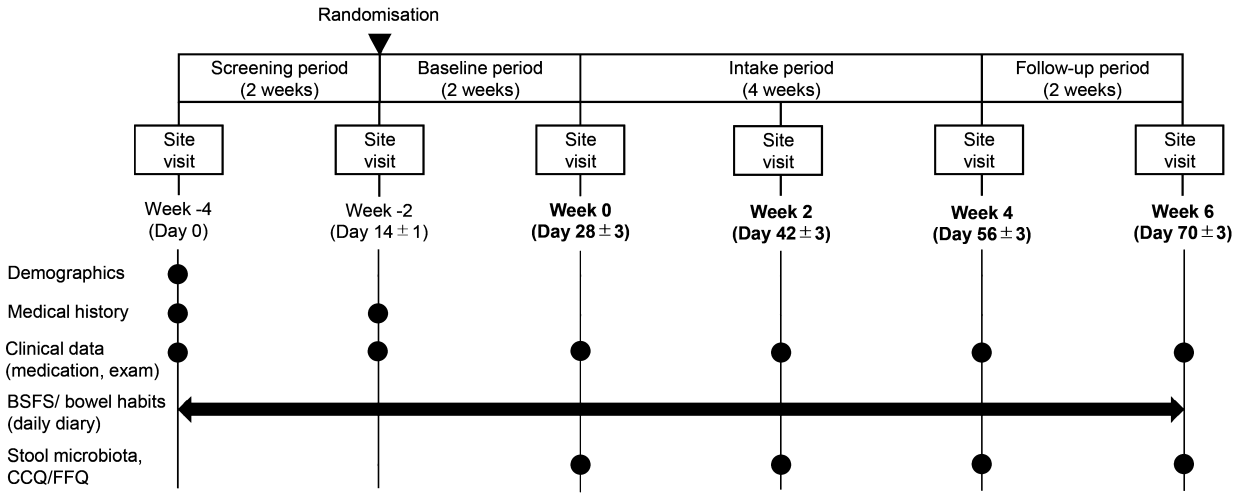

FIGURE 1 Data collection schedule.

lowed by 30 cycles of denaturation at 96 °C for 10 s, annealing at 50 °C for 30 s, and extension at 72 °C for 30 s, with a final extension step at 72 °C for 5 min. PCR products were quantified and qualified by mixing with an equal volume of 1X loading buffer containing SYBR Green, followed by electrophoresis on a 2% agarose gel. The PCR products were then combined in equidensity ratios and purified using a Qiagen Gel Extraction Kit (Qiagen, Hilden, Germany). Sequencing libraries were generated using the TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, San Diego, CA, USA) following the manufacturer's instructions, with index codes added. Library quantity and quality were assessed using the Qubit® 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) and the Agilent 2100 Bioanalyzer system (Agilent Technologies, Santa Clara, CA, USA). Libraries were then sequenced on an Illumina NovaSeq platform, producing 250 bp paired-end reads.

Microbiota analysis was performed based on 16S rRNA gene V1-V2 sequencing using the open-source software QIIME 2 (Version Qiime2-amplicon-2024.2) (Bolyen *et al.*, 2019). The resulting sequences were assigned to amplicon sequence variants (ASVs) using the USEARCH algorithm (Edgar, 2010), with a 99% identity threshold. Taxonomy for each representative ASV was assigned using an RDP-naïve Bayesian classifier with a minimum bootstrap threshold of 50% (Wang *et al.*, 2007). A single representative from each OTU was aligned using the MUSCLE alignment tool (Edgar, 2004), and a phylogenetic tree was constructed using FastTree (Price *et al.*, 2009). Alpha-diversity (observed ASVs, Chao1 index, Shannon index, and Faith's phylogenetic diversity) and beta-diversity (Bray-Curtis, Jaccard, unweighted UniFrac, and weighted UniFrac) were estimated for 57,571 randomly selected sequences (the minimum sample depth across samples) to account for differences in sampling effort.

Changes to trial protocol

Although dietary information was originally planned to be collected using a Food Frequency Questionnaire (FFQ), valid data could not be obtained due to issues during the data-collection process. As a result, dietary intake could not be analysed as initially intended.

Sample size

The sample size calculation was based on the primary endpoint – the proportion of individuals producing HLS in ≥25% of bowel movements. The expected proportion in the control arm (not consuming the IP) was 85%, versus 36.8% in the probiotic arm. This expectation was based on data from a study conducted in Belgium that applied similar eligibility criteria and used fermented milk containing the same dose of LcS (Sakai *et al.*, 2011). Accordingly, a sample size of 22 participants per group was required. This study aimed to recruit 50 participants to account for potential losses during follow-up. Assuming a screening failure rate of approximately 50%, 100 individuals were screened.

Statistical methods

Efficacy analyses followed the intention-to-treat principle and included all randomised individuals with available efficacy data (Full Analysis Set). Statistical analyses were performed using R (version 4.3.1), unless otherwise specified. Between-group comparisons were performed using Fisher's exact test for categorical variables and the Mann-Whitney U test for continuous variables. Mixed-effects logistic regression with a random intercept, adjusting for baseline values and sex,

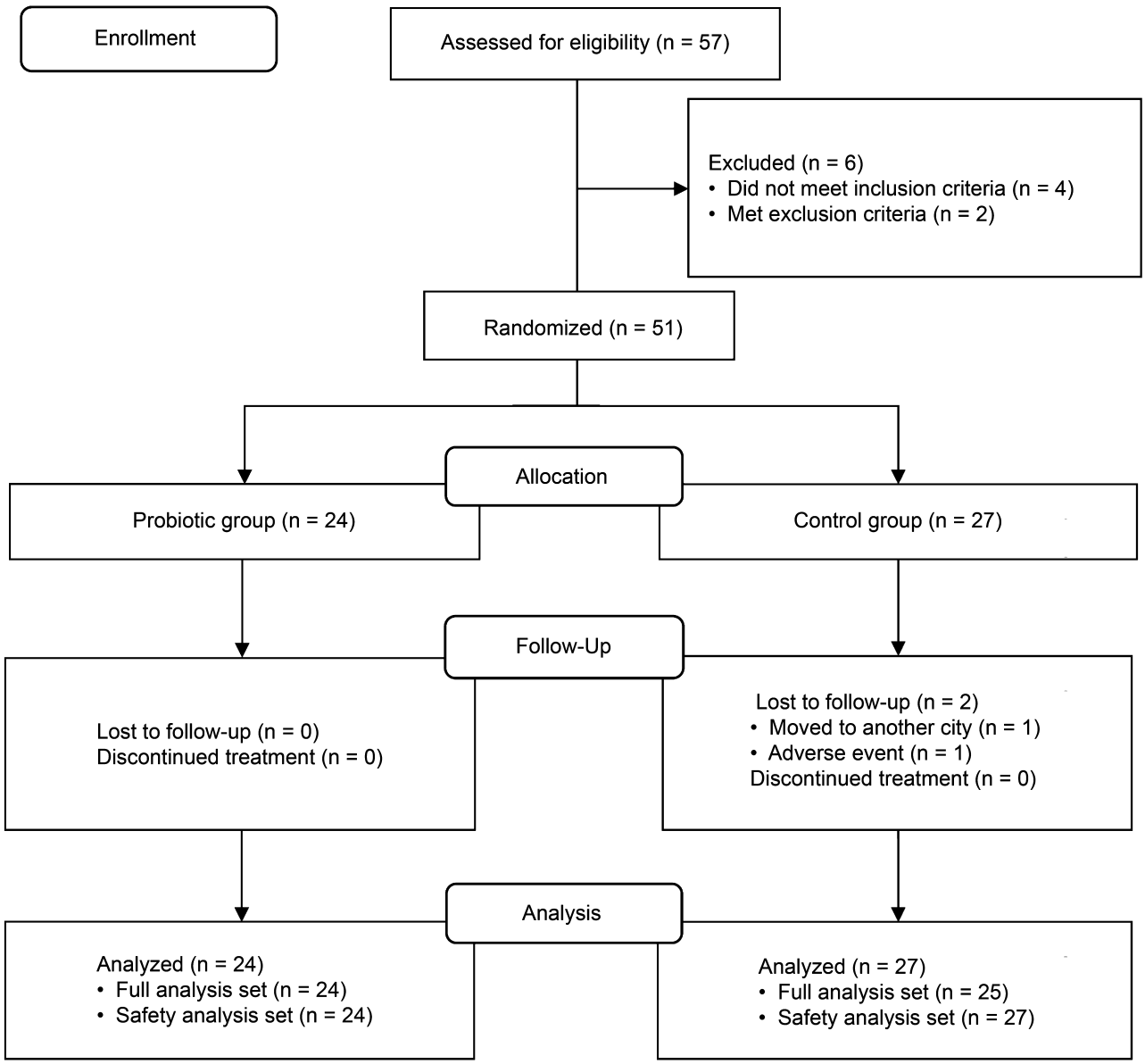

FIGURE 2 CONSORT flow diagram.

was used to estimate the overall treatment effect on the risk of producing HLS in ≥25% of bowel movements and exceeding the CCQ score ≥5 throughout the intervention. For microbiota outcomes, differential abundance analyses at the phylum, class, order, family, and genus levels were performed at each time point using ANCOM-BC, applying the default settings of the ancombc function with Holm-adjusted *P*-values (Lin and Peddada, 2020). Alpha- and beta-diversity metrics were compared between the groups using the Wilcoxon-Mann-Whitney U test and PERMANOVA on the QIIME 2 platform. *P*-values less than 0.05 were considered statistically significant.

3 Results

Patient disposition, demographic, and baseline characteristics

A total of 51 eligible participants were randomised in a 1:1 ratio to the probiotic (n = 24) and control (n = 27) groups. The flow of participants throughout the study, from enrolment to analysis, is shown in Figure 2. Demographic and baseline characteristics are presented in Table 1. Two participants were excluded from the efficacy analysis because of missing efficacy data, resulting in 49 participants in the Full Analysis Set.

All 24 randomised individuals in the probiotic arm were considered treatment compliant, having con-

TABLE 1 Demographics and baseline characteristics[1]

Characteristics	Probiotic (n = 24)	Control (n = 25)	Total (n = 49)
Female/male, n (%)	19 (79.2)/ 5 (20.8)	20 (80.0)/ 5 (20.0)	39 (79.6)/ 10 (20.4)
Age, years, mean (SD)	33.3 (11.5)	34.5 (13.4)	33.9 (12.4)
Body weight, kg, mean (SD)	54.1 (8.2)	53.0 (7.8)	53.6 (7.9)
Height, cm, mean (SD)	161.0 (8.6)	160.0 (7.5)	160.0 (8.0)
BMI, kg/m², mean (SD)	20.9 (2.5)	20.6 (2.2)	20.8 (2.3)
SBP, mmHg, mean (SD)	108.0 (10.6)	107.0 (6.6)	107.0 (8.7)
DBP, mmHg, mean (SD)	68.8 (9.0)	69.2 (7.7)	69.0 (8.3)
Laxative use, n (%)	0 (0)	0 (0)	0 (0)
Antibiotic use, n (%)	0 (0)	0 (0)	0 (0)
PPI use, n (%)	0 (0)	0 (0)	0 (0)
Medical history, n (%)	1 (4.2)	1 (4.0)	2 (4.1)
Fibroids	1 (4.2)	0 (0)	1 (2.0)
Haemorrhoids	0 (0)	1 (4.0)	1 (2.0)

[1] BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure; PPI = proton pump inhibitor; SD = standard deviation.

TABLE 2 Proportion of participants who produced hard or lumpy stools (HLS) in ≥25% of bowel movements

HLS ≥	Intake period								Follow-up period		
25%,	Week 0		Week 2			Week 4			Week 6		
n (%)	Probiotic	Control	Probiotic	Control	P[1]	Probiotic	Control	P[1]	Probiotic	Control	P[1]
Yes	20 (83.3)	23 (92.0)	13 (54.2)	22 (88.0)	**0.012**	11 (45.8)	22 (88.0)	**0.002**	12 (50.0)	22 (88.0)	**0.005**
No	4 (16.7)	2 (8.0)	11 (45.8)	3 (12.0)		13 (54.2)	3 (12.0)		12 (50.0)	3 (12.0)	

[1] *P*-values were estimated using Fisher's exact test.

sumed at least 80% of the dispensed product (Supplementary Table S2).

Analysis of efficacy – primary endpoint

The proportion of individuals with HLS (BSFS score of 1 or 2) in ≥25% of bowel movements from Week 0 to Week 6 is shown in Table 2. Overall, this proportion decreased in the probiotic group but remained stable in the control group, resulting in significant differences between the groups at Weeks 2, 4, and 6 (P = 0.012, P = 0.002, and P = 0.005, respectively; Table 2). The mixed-effects logistic regression further revealed that daily probiotic consumption significantly reduced the odds of experiencing HLS in ≥25% of bowel movements, with an odds ratio of 0.00 (95% confidence interval [CI]: 0.00-0.16) during the intervention period (P = 0.005) (Table 3).

Secondary endpoint (clinical outcomes)

Changes in defaecation status based on stool diary data are shown in Table 4 and Supplementary Table S3. A significant increase in total stool frequency was observed in the probiotic group compared with the control group after 4 weeks of probiotic consumption (P = 0.001), whereas the frequency of HLS significantly decreased (P = 0.035). Notably, the increase in total stool frequency persisted following a 2-week washout period, during which LcS-fermented milk was not administered to the probiotic group (P = 0.001) (Supplementary Figure S1). No significant effects on stool frequency were observed for ideal stool form, straining, residual stool sensation, anorectal blockage, or manual manoeuvres.

Changes in constipation-related symptom scores (based on the CCQ) from Week 0 to Week 6 are presented in Supplementary Table S4 and Supplementary Figure S2. In the probiotic group, the proportion of individuals with a CCQ score ≥5, indicative of constipation (Chan *et al.*, 2005), declined from 100% at baseline to 45.8% at the end of the intervention period (Week 4), and then rose slightly to 62.5% after the follow-up period (Week 6). In contrast, the control group remained relatively stable (88–96%). Significant

TABLE 3 Mixed-effects logistic regression parameters for hard or lumpy stools (HLS) in ≥25% of bowel movements

Predictors	HLS in ≥25% of bowel movements		
	Odds Ratios	Confidence interval	*P*-value
Intercept	42339.67	89.96 – 19927541.56	**0.001**
Sex [Male vs Female]	0.39	0.01 – 20.37	0.644
Week [Week 2 vs Week 0]	0.02	0.00 – 0.64	**0.026**
Week [Week 4 vs Week 0]	0.01	0.00 – 0.40	**0.014**
Group [Probiotic vs Control]	0.00	0.00 – 0.16	**0.005**

between-group differences were evident at Weeks 4 ($P < 0.001$) and Week 6 ($P = 0.018$). Mixed-effects logistic regression further confirmed that daily probiotic consumption significantly reduced the odds of constipation during the intervention period (odds ratio, 0.20; 95% CI: 0.06–0.65) (Supplementary Table S5). Trends were consistent across total and sub-item scores, with the most pronounced differences observed at Week 4.

Secondary outcome (gut microbiota)

The baseline bacterial composition at the phylum level is shown in Figure 3. Participants' gut microbiota was dominated by *Firmicutes* and *Bacteroidetes*, followed by *Proteobacteria* and *Actinobacteria* (including *Bifidobacterium*). This composition aligns with previous findings in healthy, non-constipated Vietnamese individuals (Pereira-Dias *et al.*, 2021).

Alpha-diversity metrics showed no significant differences between the groups at any time point (Supplementary Table S6). Significant differences in beta-diversity were observed at 2 weeks post-intervention, based on Bray–Curtis dissimilarity ($P = 0.009$) and Jaccard diversity metrics ($P = 0.016$) (Supplementary Table S7). Differential abundance analysis using ANCOM-BC indicated no significant differences in the abundance of *Bifidobacterium* between the groups (Table 5, Supplementary Figure S3, Supplementary Tables S8-S12). In contrast, several taxa were affected by probiotic consumption. At 2 weeks post-intervention, *Peptococcaceae*, *Clostridium_methylpentosum_group*, and *Clostridia* (unclassified at the order level) were significantly decreased in the probiotic group compared to the control. These changes diminished later and were replaced by an increase in *Lachnospiraceae_UCG-004* at 4 weeks post-intervention and in *Lachnospiraceae_ND3007_group* at the end of the follow-up period.

Summary of adverse events

Four AEs were reported in four participants across both study arms. In the probiotic group, two AEs (eye pain and headache) occurred in two participants (8.3%), whereas in the control group, two AEs (bleeding haemorrhoids and leg joint pain) occurred in two participants (7.4%) (Supplementary Tables S13 and S14). Bleeding haemorrhoids in the control group were classified as a serious AE (SAE), resulting in study withdrawal. Except for the SAE, all AEs were mild, unrelated to the IP or study procedures, and did not require medical intervention (Supplementary Tables S13 and S14). All AEs were resolved by the end of the study period.

4 Discussion and conclusions

The results of this open-label, randomised controlled trial conducted among constipated adults in Vietnam demonstrated that daily consumption of fermented milk containing LcS significantly reduced the proportion of participants experiencing HLS in ≥25% of bowel movements after 2 and 4 weeks of intervention. The proportion remained largely unchanged in the control group (88%) but decreased to 45.8% in the LcS group after 4 weeks. These findings align with previous studies that investigated the effects of LcS-containing fermented milk on individuals with constipation, particularly those conducted in comparable settings in Belgium (Sakai *et al.*, 2011) and the United States (Cook *et al.*, 2025). In the Belgian study, 3 weeks of LcS consumption reduced the proportion of participants with ≥25% HLS to 36.8% in the treatment group, compared to 85% in the control group. Similarly, in a US study, the proportion decreased to 28% in the treatment group versus 64% in the control group after 4 weeks. Additionally, despite differences in age groups, a controlled trial in Vietnamese children demonstrated that a 12-week intake of fermented milk containing LcS significantly reduced the incidence of constipation and helped prevent acute respiratory infections compared to the control group (Mai *et al.*, 2021). These findings suggest that the efficacy of LcS-containing fermented milk can be generalised across different populations and geographical regions.

TABLE 4 Changes in defaecation status (based on stool diary) from Week 0 to Week 6.[1]

Stool frequency	Intake period								Follow-up period		
	Week 0		Week 2			Week 4			Week 6		
	Probiotic (n = 24)	Control (n = 25)	Probiotic (n = 24)	Control (n = 25)	*P*-value	Probiotic (n = 24)	Control (n = 25)	*P*-value	Probiotic (n = 24)	Control (n = 25)	*P*-value
Total, median [IQR]	5.0 [4.0, 6.3]	5.0 [3.0, 6.0]	6.0 [4.8, 8.0]	5.0 [4.0, 6.0]	0.075	6.5 [5.0, 7.3]	4.0 [4.0, 6.0]	**0.001**	6.0 [5.0, 8.0]	4.0 [3.0, 5.0]	**0.001**
HLS, median [IQR]	3.0 [2.0, 4.0]	3.5 [2.0, 5.0]	3.0 [1.0, 4.5]	4.0 [3.5, 5.0]	0.141	2.0 [1.0, 4.0]	3.5 [2.0, 4.3]	**0.035**	2.5 [1.8, 4.0]	3.0 [2.5, 4.5]	0.115

[1] *P*-values were estimated using the Mann-Whitney U test; Bold values are significant. IQR = interquartile range.

In this study, we also observed a notable reduction in clinically meaningful constipation-related symptoms (defined as CCQ score ≥5) in participants consuming fermented milk containing LcS. The proportion of participants reporting such symptoms decreased substantially in the LcS group – from 100% at baseline to 45.8% after 4 weeks of consumption – whereas this proportion increased in the control group from 88 to 96% over the same period. Similar improvements were reported in previous clinical trials. For instance, in the aforementioned study in the United States, daily intake of LcS-containing fermented milk led to improvements in Patient Assessment of Constipation Quality of Life (PAC-QOL) scores (Cook *et al.*, 2025). In a placebo-controlled randomised trial among Belgian women during the puerperium period, significant improvements were reported in both overall Patient Assessment of Constipation Symptom scores and the satisfaction subscale of the PAC-QOL in the LcS group compared to placebo (Sakai *et al.*, 2015). Finally, a double-blind, placebo-controlled trial in Germany among individuals with functional constipation demonstrated that consumption of LcS-containing fermented milk alleviated constipation-related symptoms by reducing the prevalence of hard stools (Koebnick *et al.*, 2003). Although the specific symptom profile may vary depending on population characteristics and study context, the collective evidence from these trials supports the reliability and generalisability of our findings.

The gut microbiome plays multifaceted roles in the maintenance and restoration of human health. Our study revealed the impact of LcS-fermented milk on the beta-diversity of the gut microbiota after 2 weeks, followed by changes in specific taxa in the subsequent study period. The three bacterial groups associated with the beta-diversity shift at 2 weeks post-intervention – *Peptococcaceae*, *Clostridium_methylpentosum*, and *Clostridia* (unclassified at the order level) – have been linked to an unfavourable gut environment for constipation. *Clostridium_methylpentosum_group* and *Clostridia* (unclassified at the order level) have been shown to positively correlate with inflammatory markers tumour necrosis factor-α, interleukin-1β, and prostaglandin E2 in the colon of loperamide-induced functional constipation mice (Gao *et al.*, 2023). Additionally, an increased abundance of these bacterial groups is significantly linked to motilin suppression and somatostatin elevation, both of which are key gastrointestinal hormones that together result in decreased intestinal motility and the progression of constipation within this model (Gao *et al.*, 2023). *Peptococcaceae* is

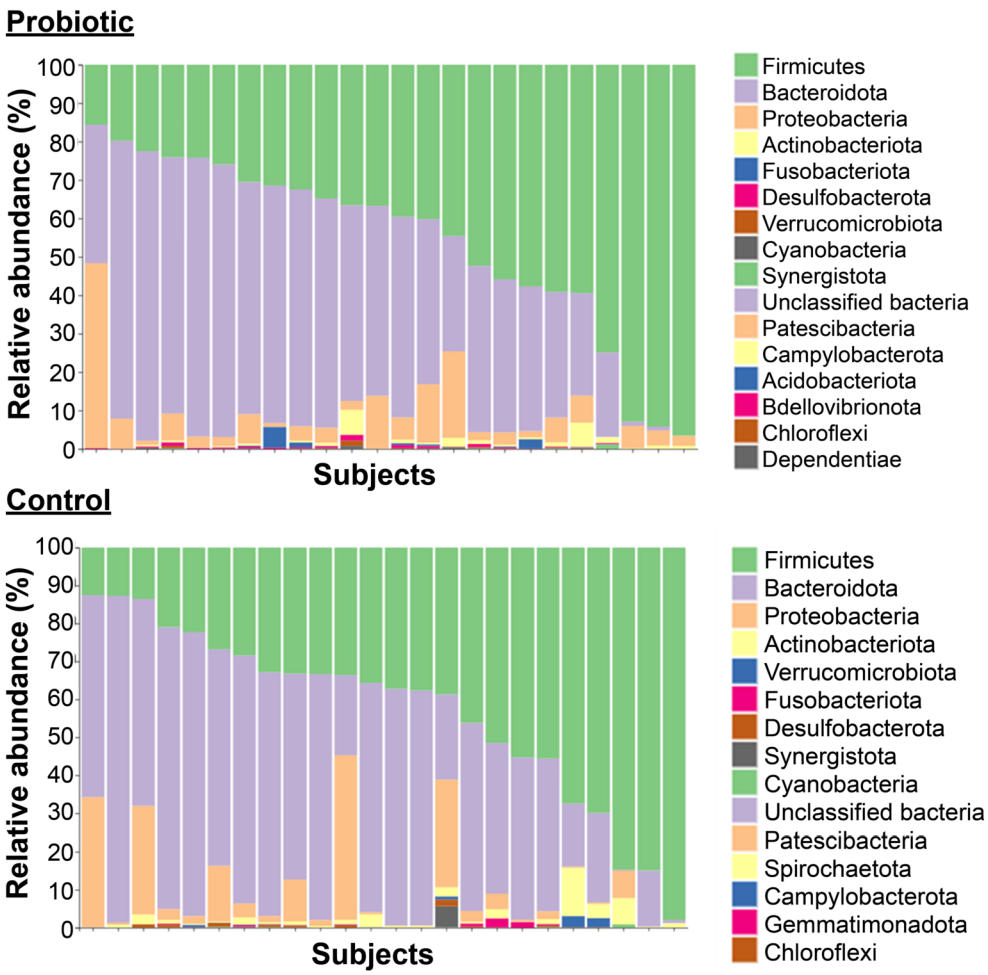

FIGURE 3 Baseline gut microbiota composition at the phylum level. The stacked bar chart shows relative abundances of bacterial compositions at the phylum level at baseline in the faecal microbiome of individuals suffering from hard stools.

associated with slower gastrointestinal motility, as non-dietary induction of gastrointestinal transit leads to a decreased abundance of this bacterium (Kashyap *et al.*, 2013). The reduction of these taxa by LcS-fermented milk may provide objective support for our study's findings that probiotic drinks decrease the prevalence of HLS and improve overall constipation. This microbial community change in the probiotic group was followed by an orderly increase in *Lachnospiraceae_UCG-004* and *Lachnospiraceae_ND3007 group* at 4 weeks post-intervention and at the end of the follow-up period, respectively. These two bacterial groups are key contributors to short-chain fatty acid production and are significantly depleted in constipated individuals (Gao *et al.*, 2022; Johnson-Martínez *et al.*, 2024). Their abundance is also reduced in patients with Parkinson's disease, where constipation is prevalent (Drobny *et al.*, 2021; Nishiwaki *et al.*, 2020; Wallen *et al.*, 2020). A distinct effect of LcS-containing fermented milk on *Lachnospiraceae_UCG-004* was previously reported in an intervention study involving patients with constipation who met the Rome III criteria (Chen *et al.*, 2019). In that study, the consumption of a probiotic beverage led to an increase in *Lachnospiraceae_UCG-004* among individuals with harder stools (BSFS score <3), whereas a decrease was observed in those with softer stools (BSFS score 4–5). The reproducibility of this finding, particularly in Southeast Asian populations, suggests a consistent and potentially region-specific modulatory effect of LcS-fermented milk on this genus.

In contrast to this reproducible finding, we did not observe an increase in the abundance of *Bifidobacterium* after daily consumption of LcS-containing fermented milk, as reported in previous studies conducted in Japan (Matsumoto *et al.*, 2006; Nagata *et al.*, 2011). This discrepancy is likely multifactorial. First, population-level differences in gut microbial ecology may contribute. International metagenomic surveys indicate that the Japanese gut microbiome is enriched in Actinobacteria, particularly *Bifidobacterium*, whereas Vietnamese adults harbour distinct community structures with different dominant taxa (Nishijima *et al.*,

TABLE 5 Summary of differential abundance analysis using ANCOM-BC[1]

Taxon	Time point	Log fold change (SE)	*P*-value (Holm)
Bifidobacterium	Week 2	0.47 (0.64)	1.000
	Week 4	0.78 (0.70)	1.000
	Follow-up	0.15 (0.71)	1.000
Lachnospiraceae_UCG-004	Week 2	0.77 (0.53)	1.000
	Week 4	1.81 (0.45)	**0.014**
	Follow-up	1.59 (0.52)	0.548
Lachnospiraceae_ND3007_group	Week 2	–0.26 (0.46)	1.000
	Week 4	1.06 (0.44)	1.000
	Follow-up	1.63 (0.44)	**0.039**
Peptococcaceae	Week 2	–1.42 (0.34)	**0.003**
	Week 4	0.00 (0.43)	1.000
	Follow-up	–0.23 (0.42)	1.000
Clostridium_methylpentosum_group	Week 2	–1.62 (0.42)	**0.010**
	Week 4	–0.30 (0.44)	1.000
	Follow-up	0.54 (0.42)	1.000
Clostridia (unclassified at the order rank)	Week 2	–1.21 (0.33)	**0.020**
	Week 4	–0.20 (0.30)	1.000
	Follow-up	–0.20 (0.34)	1.000

[1] SE = standard error.

2016). In our cohort, the median relative abundance of *Bifidobacterium* remained below 0.025% throughout the study – orders of magnitude lower than the 2–3% reported in healthy Japanese individuals (Watanabe *et al.*, 2021) and the 4–5% observed in constipation-prone individuals (Matsumoto *et al.*, 2006). Under such *Bifidobacterium*-poor conditions, the same probiotic intervention may not translate into a detectable expansion of this niche. Moreover, the extremely low baseline abundance reduces the dynamic range for detecting changes with 16S rRNA gene–based profiling and, together with the sample size, may have limited statistical power. The modest, non-significant upward trend observed during the intervention may therefore reflect a small effect that could become detectable in larger studies or in populations with higher baseline *Bifidobacterium* levels. Second, dietary patterns and host genetic background may further modulate the bifidogenic response to LcS. *Bifidobacterium* species preferentially utilise complex glycans, including milk-derived carbohydrates (Xiao *et al.*, 2024). In Japan, dairy consumption is relatively common and has been positively associated with *Bifidobacterium* abundance, potentially reflecting the availability of undigested lactose as a substrate in the colon (Kato *et al.*, 2018). In contrast, traditional Vietnamese diets have historically contained little dairy and rely more heavily on rice and plant-based carbohydrates (Van *et al.*, 2022). These differences in carbohydrate sources and lactose availability may alter microbial resource utilisation and cross-feeding interactions, potentially limiting the extent to which LcS intake promotes *Bifidobacterium* expansion in this population.

Collectively, LcS-fermented milk induced a favourable shift in the microbiota of constipated adults with a high prevalence of HLS in Vietnam, supporting improvements in clinical outcomes. However, the specific mechanisms linking these microbial changes to constipation remain unclear. Evidence from animal models suggests that LcS supplementation promotes intestinal homeostasis via multiple pathways to alleviate gastrointestinal motility disorders (Cheng *et al.*, 2023). For example, in constipated mice, LcS was suggested to accelerate small intestinal transit and increase faecal pellet output, accompanied by elevated levels of pipecolinic acid in the gut (Ou *et al.*, 2019). Furthermore, in a randomised, double-blind, placebo-controlled trial, LcS intake significantly improved colonic transit in individuals with slow-transit constipation (Krammer *et al.*, 2011). These findings underscore the need for further investigation of the complex interactions between LcS, the host gut

environment, and functional outcomes to better elucidate the mechanisms underlying its beneficial effects on constipation.

One limitation of this study is its open-label design. As the study endpoints relied on self-reported measures (BSFS, Stool Diary, and CCQ), participants' awareness of the intervention may have influenced their response, potentially introducing bias. To address this limitation, stool samples were collected from all participants, and the gut microbiota composition was analysed. The observed favourable modulation of constipation-associated microbiota in the probiotic group provides objective support for the efficacy of the intervention and strengthens the clinical findings. Another limitation relates to dietary assessment. Although dietary intake was originally intended to be evaluated using an FFQ, technical issues during data collection prevented the acquisition of valid dietary information. Consequently, we were unable to explore potential interactions between habitual diet and the intervention. Nevertheless, given the randomised controlled design of the study, any unmeasured dietary variation is expected to have been evenly distributed between groups. Therefore, while this limitation constrains secondary exploratory analyses, it is unlikely to compromise the internal validity of the between-group comparisons or alter the conclusions regarding the effect of LcS consumption.

In conclusion, daily consumption of fermented milk containing LcS over 4 weeks significantly reduced the incidence of HLS in constipated adults in Vietnam compared with no intervention. Constipation-related symptoms improved concurrently. These clinical benefits were accompanied by favourable shifts in the gut microbiota, supporting the biological plausibility of the observed effects. Although the physiological significance of individual microbial changes requires further investigation, the overall microbiota modulation observed in this study is consistent with the clinically meaningful improvement in constipation-related symptoms. These results suggest that daily intake of LcS-containing fermented milk is a beneficial and well-tolerated approach for managing constipation in this population, with no new safety concerns identified.

Supplementary materials

Data is available on https://doi.org/10.1163/18762891-bja00116 under Supplementary Materials.

Table S1. Schedule for data collection.

Table S2. Investigational product compliance.

Table S3. Stool frequency and associated symptoms.

Table S4. Change in constipation-related symptom scores (based on the CCQ) from Week 0 to Week 6.

Table S5. Mixed-effects logistic regression parameters for a total CCQ score of ≥5.

Table S6. Changes in alpha-diversity metrics.

Table S7. Changes in beta-diversity metrics.

Table S8. Microbiota log-fold change versus control at the phylum level.

Table S9. Microbiota log-fold change versus control at the class level.

Table S10. Microbiota log-fold change versus control at the order level.

Table S11. Microbiota log-fold change versus control at the family level.

Table S12. Microbiota log-fold change versus control at the genus level.

Table S13. Summary of overall adverse events, severity, outcome, causality, and actions taken in the investigational product – Safety Analysis Set.

Table S14. Summary of adverse events by terminology.

Figure S1. Stool frequency every 2 weeks, from Week 0 to Week 6.

Figure S2. Changes in CCQ scores every 2 weeks from Week 0 to Week 6.

Figure S3. Changes in the relative abundance of *Bifidobacterium* and bacterial groups identified by ANCOM-BC.

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Authors' contribution

Conceptualisation, VHV and TA; data curation, VHV, TDV, NNB, UNQ, and CNTL; formal analysis, VHV, UNQ, CNTL, and TA; investigation, VHV, TDV, NNB, UNQ, and CNTL; methodology, VHV, UNQ, CNTL, and TA; project administration, TDV, NNB, CDX, and TA; resources, VHV, CDX, and TA; supervision, TA; validation, VHV and TA;

visualisation, VHV, UNQ, CNTL, and TA; writing – original draft, VHV and TA; writing – review and editing, VHV, TDV, NNB, UNQ, CNTL, CDX, and TA.

Conflict of interest

Takuya Akiyama is affiliated with Yakult Honsha Co., Ltd. – the sponsor of this study. The investigational product was manufactured and distributed in Vietnam by its subsidiary. The article reflects the views of the authors and not necessarily those of the funder. All other authors declare no potential conflicts of interest.

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