LWT - Food Science and Technology 216 (2025) 117291

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Effects of galactooligosaccharides (GOS) on the gut microbiota in lactose intolerant individuals

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ARTICLE INFO

Keywords:
Prebiotics
Lactose intolerance
Gut microbiota
Galactooligosaccharides

ABSTRACT

Lactose intolerance (LI), which affects approximately 75% of the global population, leads to symptoms such as abdominal pain, bloating and diarrhea. The primary management strategy for LI involves the avoidance of lactose-containing products. Although there is no cure for LI of genetic origin, the administration of prebiotics, including the consumption of galactooligosaccharides (GOS) and, potentially, the regular, low-level intake of lactose, may modify the gut microbiota to aid in symptom management. We conducted a six-week, single-blind trial on 14 participants who self-reported LI to examine the effects of GOS vs. low level lactose in the context of a normal diet on the gut microbiota. Fecal samples, gastrointestinal symptom reports and dairy consumption data were collected throughout the study. No significant changes in alpha and beta microbial diversity were observed within the GOS and lactose groups individually. There were significant increases in Actinobacteria ($P < 0.0001$) and Firmicutes ($P = 0.0006$) phyla in the GOS group. Additionally, a considerable elevation in amount of the genus *Bifidobacteria* ($P < 0.0001$) and a reduction in self-reported diarrhea incidents ($P = 0.0085$) were observed in the GOS group. The findings suggest that GOS enhances the presence of commensal *Bifidobacteria* and potentially alleviates LI symptoms.

1. Introduction

Digestion of the milk sugar lactose requires intestinal mucosal production of the enzyme lactase-phlorizin hydrolase, commonly known as lactase. Lactase cleaves the β-1-4 bond between glucose and galactose in lactose to release its monosaccharide components, which is required for its intestinal absorption (Misselwitz, Butter, Verbeke, & Fox, 2019). Intestinal lactase production initiates by the second month of gestation, increases throughout gestation, peaks at birth and continues to be produced until around the time of weaning (Deng, Misselwitz, Dai, & Fox, 2015; Swallow, 2003). In most people, lactase activity begins to decrease around the time of weaning (Brüssow, 2013). This decreased lactase activity post-weaning is referred to as lactase non-persistence (LNP) and results in the incomplete digestion of lactose (Usai-Satta, Scarpa, Oppia, & Cabras, 2012). About 75% of the global population have LNP (Silanikove, Leitner, & Merin, 2015). About 25% of humans have genetic variations that allow maintaining a high level of lactase activity into adulthood, enabling continued lactose consumption without symptoms, a trait known as lactase persistence (LP) (Fassio, Facioni, & Guagnini, 2018).

Upon consuming lactose-containing foods, people with LNP can experience lactose intolerance (LI) (Angima, Qu, Park, & Dallas, 2024). The unabsorbed lactose then passes into the colon, where it increases osmotic pressure, leading to absorption of water through the intestinal wall, resulting in diarrhea (Mattar, de Campos Mazo, & Carrilho, 2012; Fassio et al., 2018). The unabsorbed lactose can be fermented by bacteria that can work in concert to produce volatile by-products like short-chain fatty acids (SCFAs) and gases - carbon dioxide, hydrogen and methane (Rowland et al., 2018). Gas production can result in flatulence, bloating and abdominal discomfort (Fassio et al., 2018; Robles & Priefer, 2020).

Management strategies for LI include the avoidance of lactose-containing foods or taking supplemental lactase with lactose-containing foods (Deng et al., 2015).

Recently, prebiotic-based strategies have been investigated for their potential to improve lactose intolerance symptoms by modulating the

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https://doi.org/10.1016/j.lwt.2024.117291
Received 6 August 2024; Received in revised form 15 December 2024; Accepted 27 December 2024
Available online 2 January 2025

colonic microbiota (Azcarate-Peril et al., 2017; Chey et al., 2020; Savaiano, Ritter, Klaenhammer, James, Longcore, & Chandler, 2013). Most dietary prebiotics are carbohydrates that cannot be degraded by endogenous enzymes of the host but are hydrolyzed and fermented by intestinal microorganisms (Swennen, Courtin, & Delcour, 2006). Oligosaccharides, such as fructooligosaccharides (FOS), galactooligosaccharides (GOS) and inulin, which are found in onions, garlic, bananas and chicory root, are popular dietary prebiotics. GOS can be synthesized on a commercial scale from lactose using the enzymatic transgalactosylation activity of β-galactosidases (Sako, Matsumoto, & Tanaka, 1999). Recent advances in industrial applications of GOS highlight the use of non-conventional yeast strains such as *Sporobolomyces* and *Sporidiobolus* for GOS production (Kot, Kieliszek, Piwowarek, Błażejak, & Mussagy, 2021). These yeasts offer novel opportunities for large-scale and cost-effective production, broadening the accessibility of GOS for clinical and consumer use. GOS can modulate the large intestinal microbiota by providing a food for commensal *Bifidobacteria* with a preferential growth advantage (Davis, Martinez, Walter, & Hutkins et al., 2010; Ladirat et al., 2014). *Bifidobacteria* produces an array of different transport proteins that can bring GOS and lactose into the cell (Andersen et al., 2011; Böger, van Leeuwen, Lammerts, van Bueren, & Dijkhuizen, 2019; Schwab & Gänzle, 2011), as well as β-1-4 hydrolases, which degrade GOS and lactose to lactate, acetate and ethanol without producing gases. Studies have indicated that GOS consumption can increase the proportion of *Bifidobacteria* in the large intestine in humans with LNP and may decrease the severity of LI symptoms (Azcarate-Peril et al., 2017; Chey et al., 2020; Savaiano, Ritter, Klaenhammer, James, Longcore, & Chandler, 2013).

The main purpose of this study is to explore alternative management strategies that can improve the lives of those with LI by allowing more flexibility in their diet. This study investigates the effect of supplemental GOS compared with low-level lactose in the context of a normal diet on the gut microbiota and changes in gastrointestinal symptoms in self-reported lactose intolerant individuals.

2. Materials and methods

2.1. Study design

The experimental design was a completely randomized clinical trial, which consisted of a one-week screening period followed by a 3-week supplementation period and a 3-week washout period. Institutional Review Board (IRB) approval was obtained through Oregon State University (OSU) (IRB Number IRB-2021-1044), and written informed consent was obtained from all participants.

The primary endpoint of this study was the change in the gut microbiota composition of lactose-intolerant individuals after a 3-week supplementation period with GOS compared to a control group consuming low-level lactose. Participants were instructed to consume either 5.2 g of GOS powder (treatment) or 1 g of lactose (control) in the morning and evening (twice daily). Participants dissolved the provided powders in 250 mL of water and consumed them before or after meals (see Table 1). The Biotis™ GOS-O powder was donated by Friesland Campina (Amersfoort, Netherlands) and was comprised of 73.7% GOS, 20.9% lactose, 5.3% monosaccharides (galactose and glucose) and 1.0% ash. Therefore, 5.2 g of GOS powder provided 3.8 g of GOS and 1.1 g of lactose. To match the lactose content in the GOS supplement, 1 g of lactose, which was donated by Glanbia Nutritionals (Twin Falls, ID, USA) was used for the control. Throughout the study, participants were asked to make no other changes to their baseline diets.

Table 1
Intervention groups.

Group	Assigned interventions
GOS n = 7	Supplement: 5.2 g of prebagged powdered GOS, 2*x*/day, oral (dissolved in 250 mL of water); 3 weeks supplementation, 3 weeks washout
Control n = 7	Supplement:1 g of prebagged lactose powder, 2*x*/day, oral (dissolved in 250 mL of water); 3 weeks supplementation, 3 weeks washout

Low-lactose consumption was chosen as the control instead of a lactase supplement to reflect a real-world dietary management strategy for LI. This approach helps evaluate the effects of GOS on gut microbiota and gastrointestinal symptoms under normal dietary conditions. Using a lactase supplement as a control would not provide insights into the typical dietary effects of GOS. Low-lactose consumption is a common method for managing LI, making the findings more applicable to everyday practices. This strategy allows for assessing GOS as a potential dietary option that could complement or replace enzyme supplementation, broadening the dietary management options for individuals with LI.

2.2. Sample size calculation

This pilot study aimed to explore the effects of GOS on the gut microbiota in lactose-intolerant individuals. As an exploratory study, a formal power analysis was not performed. Instead, 14 participants were recruited to gather preliminary data on the potential effects and feasibility of GOS supplementation. These initial results will help estimate effect sizes and variability for future, larger-scale studies.

2.3. Human subject trial

The participant recruitment process and the randomized trial design, aimed at assessing the effects of GOS on LI, are outlined through the CONSORT diagram depicted in Fig. 1. Subjects were recruited from across the OSU campus via flyers, social media and email campaigns. Potential candidates completed an online screening questionnaire to establish eligibility. Inclusion criteria were age over 18, self-reported LI (with or without formal diagnosis), daily bowel movements, no known gastrointestinal diseases, no major gastrointestinal surgeries, no regular use of laxatives or antacids and no use of antibiotics or pre/probiotic supplements for one month prior to the study. Post-screening, eligible participants were randomized into GOS or the lactose control group using a random number generator.

Fourteen adults (3 males and 11 females) aged 19–55 years (mean: 29 years; SD: 11 years) were enrolled (Table 2). Participants were asked to maintain their normal diet but avoid any lactase supplement, pre/probiotic supplements and antibiotics. Participants logged their dairy consumption weekly with a food frequency questionnaire. Two lactose control participants (one female and one male left the study early (one after week 3 and the other after week 4) due to personal reason.

2.4. Gastrointestinal symptoms

For the duration of the study, participants recorded gastrointestinal symptoms every other day in an online questionnaire (Supplementary Fig. 1). The questionnaire was specifically designed for this study to capture incidence and severity of LI-related symptoms (abdominal pain, bloating, burping, constipation, diarrhea, discomfort during defecation, flatulence, heartburn, incomplete defecation, nausea and urgency to defecate). The online questionnaire was modeled after the gastrointestinal symptom rating score (GSRS) for irritable bowel syndrome (IBS). Symptoms were rated on a 7-point Likert scale: no discomfort at all (0), minor discomfort (1), mild discomfort (2), moderate discomfort (3), moderately severe discomfort (4), severe discomfort (5) and very severe discomfort (6). Participants recorded their stool type for each bowel movement using the Bristol Stool Chart (Blake, Raker, & Whelan, 2016), and they were asked to assign one stool type per bowel movement in the online survey.

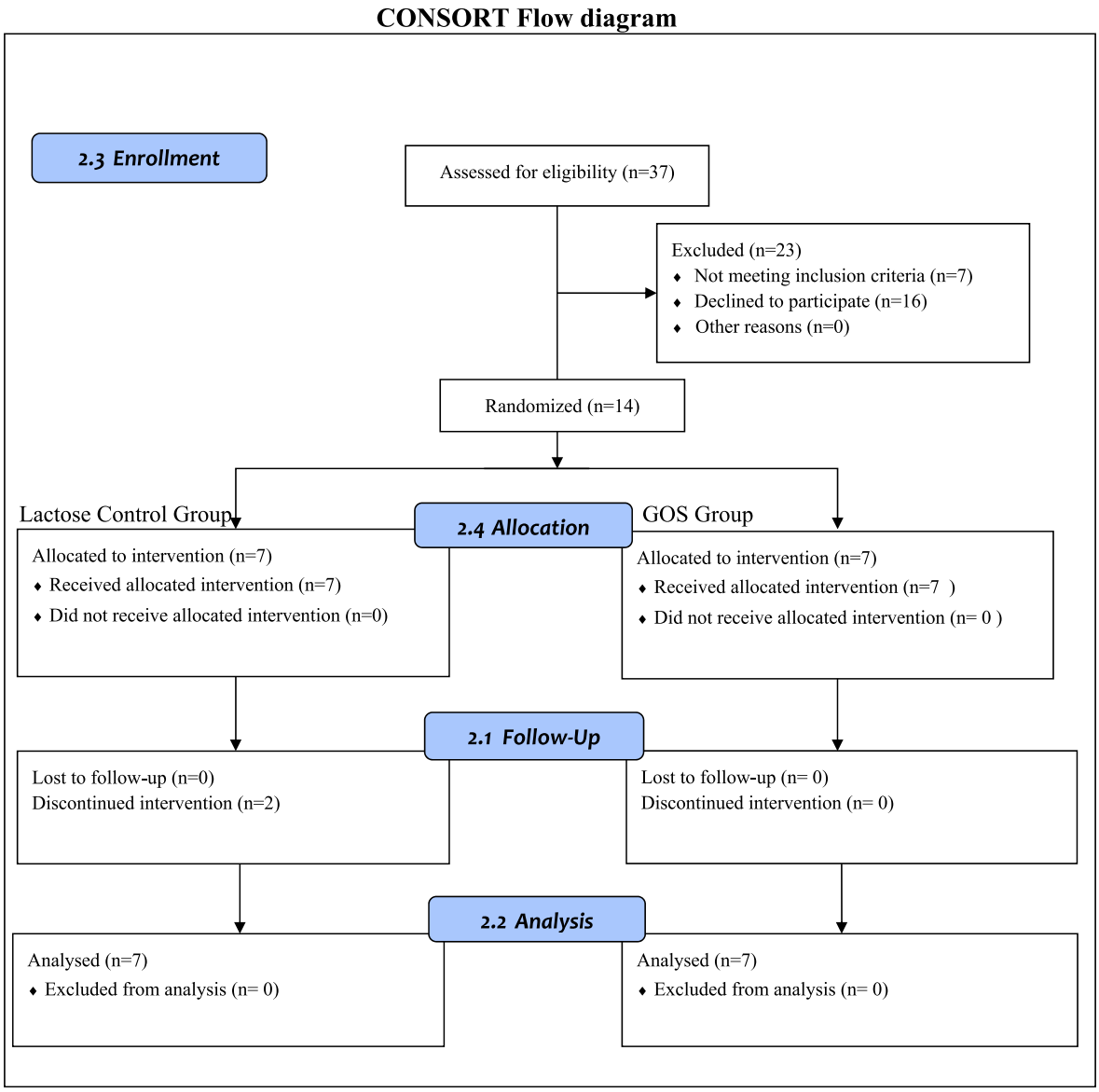

Fig. 1. CONSORT flow diagram of recruitment, allocation, follow-up and analysis.

Table 2
Baseline characteristics for participants (mean ± STD). F: female and M: male.

Characteristics	Control (n = 5)	GOS (n = 7)
Age (years)	20 ± 11	29 ± 11
Gender (self-identified)	4 F and 1 M	6 F and 1 M

2.5. Lactase activity measurement

Lactase activity was measured using the breath hydrogen test. Participants fasted overnight and provided a baseline breath sample. They then consumed a lactose-containing solution, and breath samples were collected at regular intervals for analysis of hydrogen levels. Increased hydrogen levels indicated reduced lactase activity, confirming lactose malabsorption.

2.6. Microbiome

Fecal samples were systematically collected on a weekly basis by the study subjects using the Easy Sampler® Stool Collection kit (ALPCO®, New Hampshire, USA). After collection, the samples were promptly transported to the laboratory with room temperature and stored at −80 °C for later analysis. The DNA was extracted using a QIAamp Powerfecal Pro Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The DNA concentration was quantified with a Qubit 4 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA), and the extracted DNA was diluted to achieve to 10 ng/μL for the library preparation.

The PCR assay targeted the V4 region of the 16S rRNA gene was conducted for 35 cycles using 10 ng of DNA, 10 μL *Pfx* AccuPrime Master Mix (Thermo Fisher Scientific), 1 μL each of 500 nM forward and reverse primer and water up to 20 μL. Each cycle consisted of 20 s at 95 °C, 15 s at 55 °C, and 1 min at 72 °C. After the last cycle, the temperature was held at 72 °C for 5 min before being dropped to 4 °C until samples were removed from the thermocycler. Agarose gel electrophoresis was run to confirm amplification. The SequalPrep™ normalization kit from Applied Biosystems™ (Thermo Fisher Scientific) was used to normalize the PCR product of each sample. The normalized samples were then pooled, and the constructed library was sent to the Oregon State University Center for Quantitative Life Sciences (CQLS) for sequencing using an Illumina MiSeq platform (500 cycles, 2 × 250 bp).

2.7. Microbiome data analysis

Raw sequencing data were downloaded from Illumina BaseSpace (https://basespase.illumina.com). Amplicon sequencing variants (ASVs) were identified using DADA2 version 1.14.1 according to the DADA2 tutorial for 1.16 (https://benjjneb.github.io/dada2/tutorial.html). Taxa were assigned using the SILVA database version 138. A phyloseq object was made and rarefied to the minimum sequencing depth with the set. Seed = 3. Rarefied data was used to calculate measures of alpha (Shannon or Simpson index) and beta diversity. Prior to rarefaction or normalization, mitochondrial and chloroplast sequences were removed from the data.

Using phyloseq, the resulting object was filtered to include only samples with more than 5000 reads and ASVs were agglomerated at the genus level to reduce the number of unannotated ASVs. Highly rare genera, defined as those observed fewer than three times in at least 20% of the samples and with a relative abundance of less than 0.001%, were removed to filter out noise from the dataset.

2.8. Dietary data for dairy foods

Participants reported their dairy consumption through a weekly online Food Frequency Questionnaire (FFQ), specifically designed for this study (as shown in Supplementary Fig. 2). The FFQ, which had not undergone prior validation, included a checklist for participants to report their weekly consumption frequency of specific dairy products, along with an option to record other dairy products they consumed. Data collection began in week 2 and continued through week 7. To analyze the data, week 2 was used as the baseline to establish participants' typical dairy consumption habits, followed by weeks 3 and 4, which were designated as the supplementation period to monitor the effects of the intervention. Finally, weeks 5, 6, and 7 constituted the washout phase, allowing researchers to observe any residual effects of the intervention on dairy consumption patterns.

2.9. Statistical analyses

Statistical analyses were conducted using SAS (version 9.4; SAS Institute Inc, Cary, NC, USA) and R (versions 3.6.1 and 4.0; https://www.R-project.org/). An intention-to-treat approach was employed, including data from all participants from entry until their exit from the study. PROC MIXED in SAS facilitated the repeated-measures-in-time analysis to evaluate the effect of GOS on the weekly relative abundance of fecal microbiota at the phyla and genus levels, alongside measures of microbial diversity and gastrointestinal symptoms. The model included fixed effects for treatment (GOS or lactose control), study week and their interaction. Due to the limited participant number (n = 14), it was not feasible to adjust for gender or age. A first-order auto-regressive variance-covariance structure was applied to account for repeated measures within participants over time, with the Kenward-Rogers approximation used to adjust the degrees of freedom for *P*-values.

To assess the short-term treatment effect, linear contrasts compared the average of weeks 2–4 against the baseline (week 1) using the ESTIMATE statement. Similarly, for long-term effects, the average of weeks five to seven was contrasted against week one. Additionally, weekly microbial beta diversity was visualized using non-metric multidimensional scaling (NMDS), and groups were compared employing permutational multivariate analysis of variance (PERMANOVA) based on distance matrices, utilizing the Adonis function in R's vegan package.

Analysis of gastrointestinal function as recorded through the Bristol Stool Chart was conducted by categorizing numeric scores into symptoms: constipation (0–2), normal (3–4) and diarrhea (5–7). Pearson's chi-square test of homogeneity determined the presence of any significant changes in stool types between the GOS and lactose control groups across the three study periods (baseline, supplementation and washout).

Dairy consumption data was collected from participants who reported the frequency of their dairy intake on a weekly basis. The responses were quantified as follows: 0 for 'did not consume', 1 for 'consumed once', 2 for 'consumed 2–4 times a week', 4 for 'consumed once a day', and 8 for 'consumed 2 or more times a day', creating a possible range of 0–56 for each week. Using ANOVA in R, we analyzed this data to evaluate whether the time variable-week or period-had any significant effect on dairy consumption. Additionally, we compared the GOS and control treatment groups to evaluate if the type of treatment led to any differences in dairy food consumption.

3. Results

3.1. Participant demographics and compliance

The study was completed with a total of 12 participants. Two lactose control participants left the study early (one after week 3 and the other after week 4) due to personal reasons, but their data were included in the analysis up until the time they left. The participant group was primarily female, with only two male participants. Apart from two participants aged 47 and 54 years, all were between the ages of 19 and 35. All but one participant (Shannon 2.59, Simpson 0.857) had a relatively high baseline alpha diversity (Shannon 2.65–3.73, Simpson 0.864–0.960). Given these variations, it was not possible to group data according to age, gender or microbial diversity.

3.2. Microbiome sequence processing

Microbiome sequencing of the V4 region of the 16S rRNA gene yielded a total of 9,627,144 raw reads, averaging 67,323 reads per sample. Phyloseq filtering (described in Methods) resulted in a total of 9,245,894 reads, with an average of 64,656 reads per sample. Collector's curves suggested a minimum sequencing depth of 20,767 (set.seed = 3), leading to a phyloseq object being rarefied to this depth. The range of reads per sample included in the analysis, post-filtering and denoising with chimeras removed, varied from 22,103 to 186,244.

3.3. Microbial diversity

In both groups (GOS and lactose control), measures of alpha diversity had no significant differences over the course of the study. The Shannon index, comparing baseline to supplementation, baseline to washout period, trended towards an increase in the GOS group, but no significant differences were observed (Fig. 2A) and no changes between the baseline and treatment. Similarly, the Simpson index showed an increase between the baseline and washout periods for the GOS group, though this was not significant (Fig. 2B).

The beta diversity analysis revealed modest but statistically significant clustering by study period ($R^2 = 0.05397$, $P = 0.002$; Fig, 3A) and by study group ($R^2 = 0.02911$, $P = 0.007$; Fig. 3B). These low R^2 values suggest that while differences in microbial community composition are associated with the study period and study group, they account for a relatively small proportion of the overall observed variance. The significance of these findings is tempered by the constrained sample size, which may affect the power of the analysis.

3.4. Taxonomic analysis

For the analysis of relative abundance changes across the study, only phyla present in most participants were considered. The four major phyla analyzed were Actinobacteria, Firmicutes, Bacteroidetes and Proteobacteria. A significant increase in Actinobacteria was observed in the GOS group between baseline and supplementation period ($P < 0.0001$; Fig. 4A). However, there were no significant differences observed for Actinobacteria in the lactose control group across the study. The phylum Bacteroidetes significantly decreased in relative

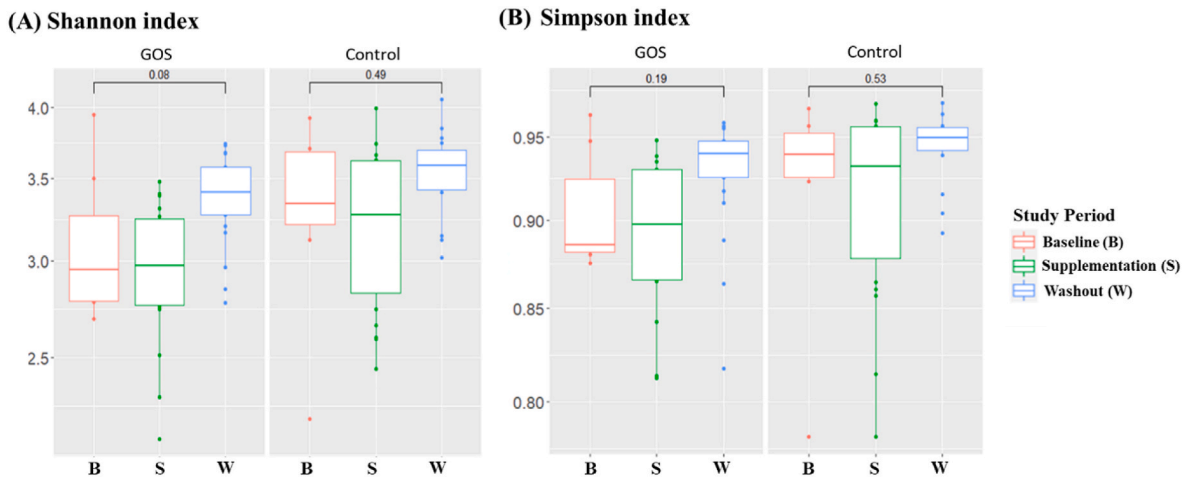

Fig. 2. Box plots displaying Shannon (a) and Simpson (b) diversity indices for the GOS and lactose control groups during baseline (B), supplementation (S) and washout (W) periods. Median, interquartile range, and outliers are showed, with whiskers marking the data range.

abundance in the GOS group from baseline to the supplementation period ($P = 0.0006$; baseline, 53.95 ± 9.15; treat, 16.14 ± 6.51). No significant differences in the relative abundance of Bacteroidetes were observed in the lactose control group, though, the relative abundance tended to decrease ($P = 0.2432$; baseline, 41.11 ± 9.15; treat, 28.95 ± 6.25; Fig. 4A). A significant increase in Firmicutes was observed in the GOS group from baseline to the supplementation period ($P = 0.0417$; baseline, 40.00 ± 7.64; treat, 58.48 ± 5.22), but no significant differences were seen in the lactose group (Baseline, 48.69 ± 7.64; treat, 58.23 ± 4.96; Fig. 4A). However, the data does not indicate a corresponding change in the abundance of the genus Lactobacillus. The increase in Firmicutes is likely attributed to other genera within this phylum. For Proteobacteria, no significant changes were observed in the GOS group, although there was a tendency towards a decrease from baseline to supplementation ($P > 0.7352$; baseline, 1.28 ± 0.95; treat, 0.41 ± 0.68). In the lactose control group, a significant increase was observed when comparing supplementation and washout period ($P = 0.0307$; treat, 1.48 ± 0.65; washout, 3.80 ± 0.75; Fig. 4A).

At the genus level, three taxa significantly increased from baseline to the supplementation period in the GOS group: *Bifidobacterium* ($P < 0.0001$; baseline, 3.09 ± 3.37; treat, 19.40 ± 2.41), *Blautia* ($P = 0.0007$; baseline, 3.30 ± 3.70; treat, 18.09 ± 2.60) and *Fusucatenibacter* ($P = 0.0078$; baseline, 0.48 ± 0.86; treat, 3.12 ± 0.62; Fig. 4B). This effect diminished post-supplementation. No significant differences in these genera were observed in the control group. *Allstipes* significantly decreased from the baseline to the supplementation period in both groups (GOS ($P = 0.0118$; baseline, 6.10 ± 1.48; treat, 2.61 ± 1.27) and control ($P = 0.0278$; baseline, 5.11 ± 1.48; treat, 2.09 ± 1.27)). *Coprococcus_3* also significantly decreased ($P = 0.0418$; baseline, 0.50 ± 0.21; treat, 0.24 ± 0.23) in the GOS group between these periods. Butyrate-producing genera, including *Faecalibacterium* ($P = 0.5673$; baseline, 6.23 ± 1.71; treat, 7.37 ± 1.13), *Anaerostipes* ($P = 0.09$; baseline, 0.73 ± 1.14; treat, 3.76 ± 0.83) and *Agathobacter* ($P = 0.371$; baseline, 0.81 ± 1.66; treat, 2.35 ± 1.31), showed an increasing trend in the GOS group from baseline to supplementation, though not significantly. Probiotic genera *Dorea* ($P = 0.0655$; baseline, 1.11 ± 0.89; treat, 2.67 ± 0.75) tended to increase from the baseline to supplementation in both the GOS and lactose control groups; however, the effect was insignificant. Due to the low presence of *Lactobacillus* in the participant samples, no comparisons were made for this genus.

3.5. Gastrointestinal symptoms and Bristol Stool Chart

The GOS group exhibited a significant decrease in the reported rating for diarrhea from the baseline to the supplementation period ($P = 0.0085$). The mean reported score for diarrhea at baseline was 1.2857 ± 0.4682, which can be translated as "minor discomfort" as per the rating score on the questionnaire. In the supplementation period, the reported mean for diarrhea in the GOS group was 0.1905 ± 0.4048, which can be translated as no discomfort (Table 3).

There was a significant increase from baseline to supplementation in the reported mean for bloating in the GOS group ($P = 0.0181$). The mean reported rating for bloating was 1.2857 ± 0.4128, which can be translated as "minor discomfort" at the baseline time point. In the supplementation period, the mean of the reported rating for bloating was 2.1905 ± 0.3494, which can be translated as "mild discomfort." There were no significant changes in the other symptoms. However, symptoms such as flatulence ($P = 0.0916$) and abdominal pain ($P = 0.1565$) that may result from microbial gas production that tended to increase from baseline to treatment in GOS group. In the control group, the only significant difference noted was for burping, which was reported to decrease from baseline to the supplementation period ($P = 0.0326$) and the washout period ($P = 0.0121$; Table 3).

There were no significant changes in the proportion of each stool type (constipated, diarrhea and normal) across study period (baseline, supplementation and washout phases) within the GOS group (Fig. 5, $P = 0.4283$ based on the Pearson chi-squared test of homogeneity). However, the test's assumptions were not fully satisfied due to the limited sample size. There were no significant changes in the proportion of each stool type (constipation, diarrhea and normal) across the study periods (baseline, supplementation and washout phases) within the control group (Fig. 5, $P = 0.6733$ based on the Pearson chi-squared test of homogeneity). This finding suggests that the control treatment did not significantly affect the distribution of stool types over the different phases of the study.

3.6. Dairy consumption

In our examination of dairy consumption (Table 4), ANOVA analyses showed no significant effects across weeks or periods within the GOS and control treatment groups. The dairy intake remained consistent over time, with P values of 0.776 for GOS and 0.962 for control on a weekly basis, and 0.408 for GOS and 0.874 for control across periods. Though a direct comparison between GOS and the low lactose control suggested a trend towards higher consumption of dairy in the GOS group (P value of 0.0937), as there is no effect of time, this finding indicates that the GOS group started with slightly higher dairy consumption and is not an effect of treatment. Overall, our results suggest steady dairy consumption regardless of time or treatment type.

Despite consistent dairy consumption throughout the study, as

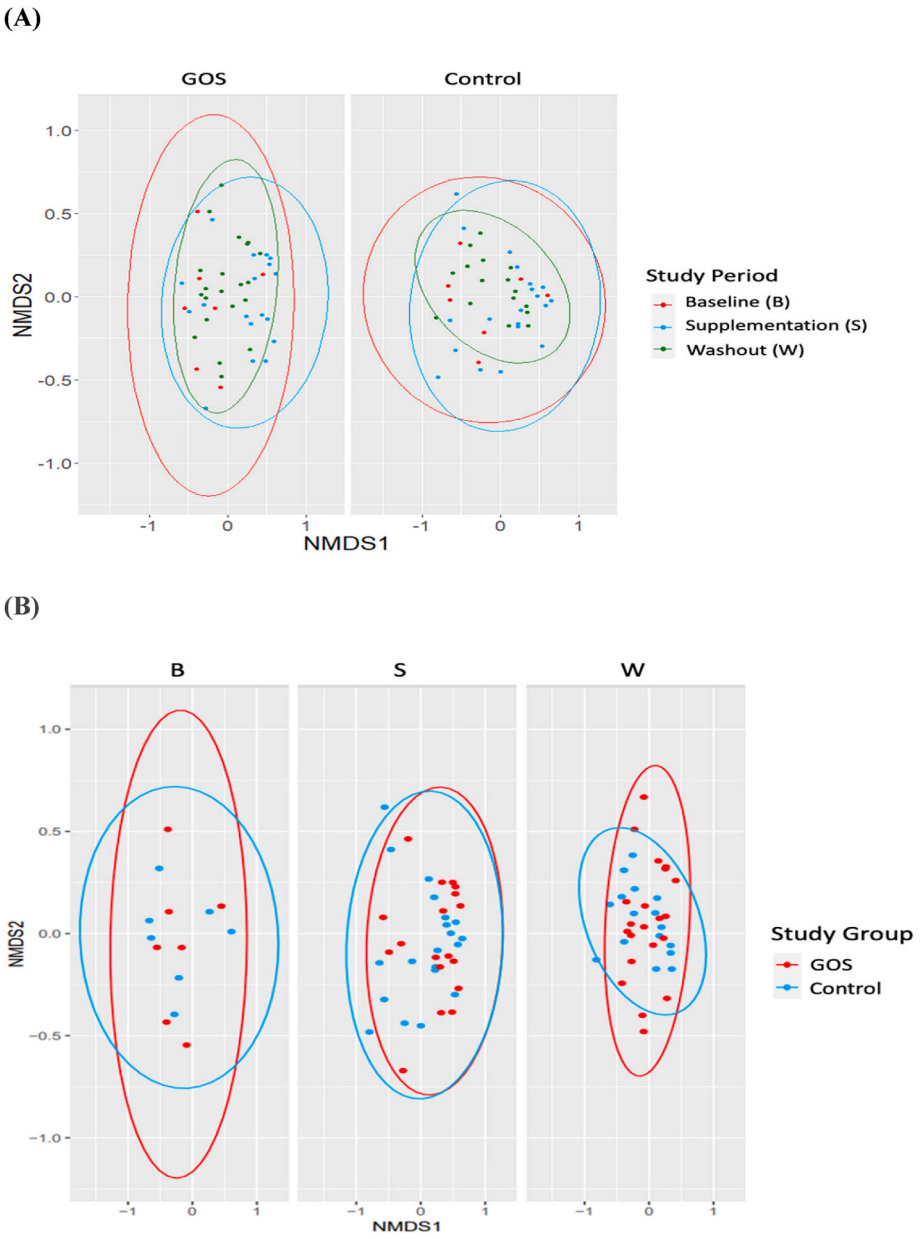

Fig. 3. NMDS for the Bray-Curtis dissimilarity with confidence ellipses. **(A)** Grouping by study period, B (baseline), S (supplementation), and W (washout) periods; **(B)** Grouping by treatment.

evidenced by stable weekly and period-specific intake within both treatment groups, participants in the GOS group reported a significant decrease in diarrhea symptoms. Even with this consistent lactose exposure, the GOS group showed a significant reduction in diarrhea symptoms, indicating the potential effectiveness of GOS.

4. Discussion

The objective of this study was to explore how GOS compared with a low lactose control group influences the gut microbiota of individuals with lactose intolerance. As higher microbial diversity is associated with a balanced microbiota that supports overall gut function and health, we examined the effect of these treatments on microbial diversity.

To assess these components of microbial diversity, metrics such Shannon and Simpson indices account for both richness and evenness, offering a comprehensive view of the ecosystem's balance. The Shannon index is influenced heavily by richness, whereas the Simpson index places greater emphasis on evenness (Johnson & Burnet, 2016).

The NMDS and PERMANOVA analyses, informed by Adonis testing in R's vegan package, revealed statistically significant, yet minimal, clustering by study period and group ($R^2 = 0.05397$, $P = 0.002$; $R^2 = 0.02911$, $P = 0.007$). These low R^2 values indicate that any microbial composition shifts associated with the study period and GOS or lactose supplementation were very modest.

Significant changes in specific bacterial taxa were identified from baseline to the supplementation period, primarily in the GOS group. The commensal gut microbiota comprises numerous species across six major phyla: Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Fusobacteria, and Verrucomicrobia. Among these, Firmicutes and Bacteroidetes are the most prevalent, collectively representing 90% of the gut microbiota. During the supplementation period, increases in the relative abundance of Actinobacteria and Firmicutes were observed in the GOS group. Notably, Bifidobacterium and Lactobacillus, both of which are known for their ability to metabolize GOS, showed significant increases. These findings align with previous studies that demonstrated the selective utilization of GOS by these bacteria due to their unique

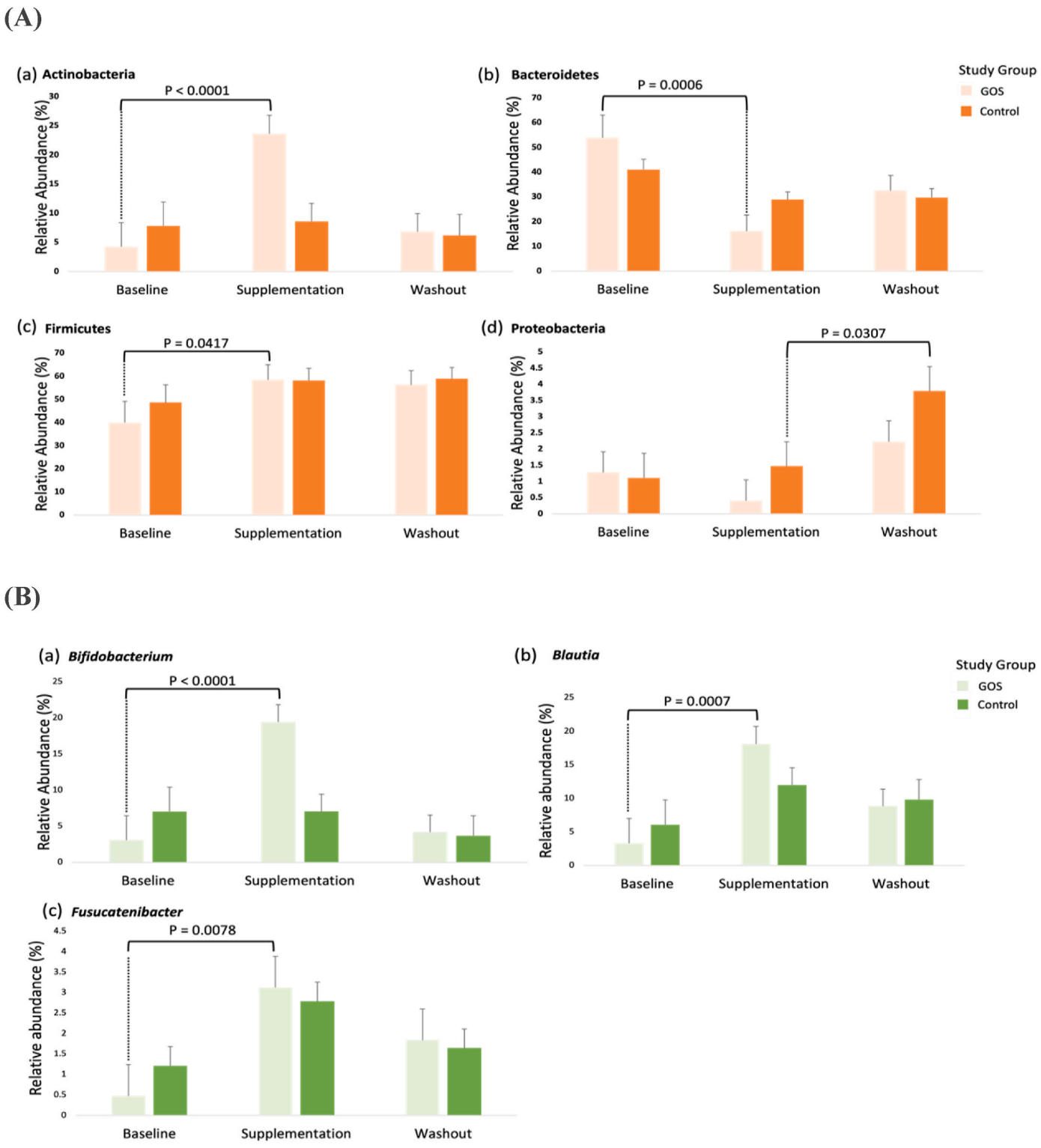

Fig. 4. (A) The relative abundance (%) of the four major bacterial phyla for the GOS group (light orange) and control (dark orange) group across baseline, supplementation and washout periods. **(B)** Bacterial genera relative abundance (%) in GOS (light green) and control (dark green) groups across baseline, supplementation and washout periods. Bar heights represent means, with error bars showing standard deviation.

enzymatic capabilities, such as β-galactosidase activity (Davis, Martínez, Walter, Goin, & Hutkins, 2011; Holscher et al., 2015). While this study highlights the beneficial increase in *Bifidobacterium* and *Lactobacillus*, future research should focus on species-level analyses to better understand the broader impact of GOS. Such studies should include exploring the reduction of pathogenic bacteria and other nuanced changes within the microbiota to provide a more comprehensive understanding of its response to GOS supplementation. Despite accounting for a minor proportion of the gut bacteria, Actinobacteria play an essential role in regulating gut homeostasis (Binda, Lopetuso, Rizzatti, Gibibo, Cennamo, & Gasbarrini et al., 2018). Actinobacteria are Gram-positive and anaerobic bacteria that include *Bifidobacteria, Propionibacteria,*

Table 3
Gastrointestinal symptom score means.

Group	Symptoms	Baseline	Supplementation	Washout	Baseline vs. Treatment (*P* values)	Baseline vs. Washout (*P* values)
GOS	Diarrhea	1.2857	0.1905	0.2381	0.0085**	0.0671
	Bloating	1.2857	2.1905	1.1429	0.0181*	0.7785
	Abdominal pain	0.8571	1.5741	0.7143	0.1565	0.8114
	Flatulence	1.1429	1.8095	1.1429	0.0916	1
	Burping	0.8571	1.5741	0.7143	0.3541	0.1341
	Urgency to defecate	0.7143	0.4762	0.2857	0.5962	0.5088
	Discomfort during defecation	0.1429	0.4762	0.7143	0.4737	0.3495
	Incomplete bowel movement	0.5714	1	0.9524	0.3393	0.4802
	Constipation	0.1429	0.7619	0.8095	0.081	0.1428
	Heartburn	0.2857	0.5238	0.2857	0.5052	1
	Nausea	0.2857	0.2381	0.2381	0.8879	0.9119
	Sum	7.5714	9.8095	6.8095	0.3595	0.8115
Control	Diarrhea	0.8571	1.2381	0.7205	0.3507	0.8173
	Bloating	1.7143	1.9048	1.9048	0.6129	0.3494
	Abdominal pain	1	1.1905	1.1217	0.7038	0.8464
	Flatulence	2.4286	2.1429	1.7759	0.4664	0.2557
	Burping	1.4286	0.7619	0.4033	0.0326*	0.0121*
	Urgency to defecate	1	1.7143	1.8426	0.1145	0.2165
	Discomfort during defecation	1.5714	2.1429	1.507	0.2208	0.9196
	Incomplete bowel movement	0.8571	1.4762	0.9388	0.1688	0.8851
	Constipation	0.4286	0.7619	0.2409	0.3442	0.6914
	Heartburn	1	1.0476	0.7651	0.8938	0.6533
	Nausea	0.5714	1.0952	0.5517	0.124	0.9652
	Sum	12.8571	15.4762	10.8862	0.284	0.5565

*$0.05 > P > 0.01$, **$0.01 > P > 0.001$, ***$P < 0.001$.

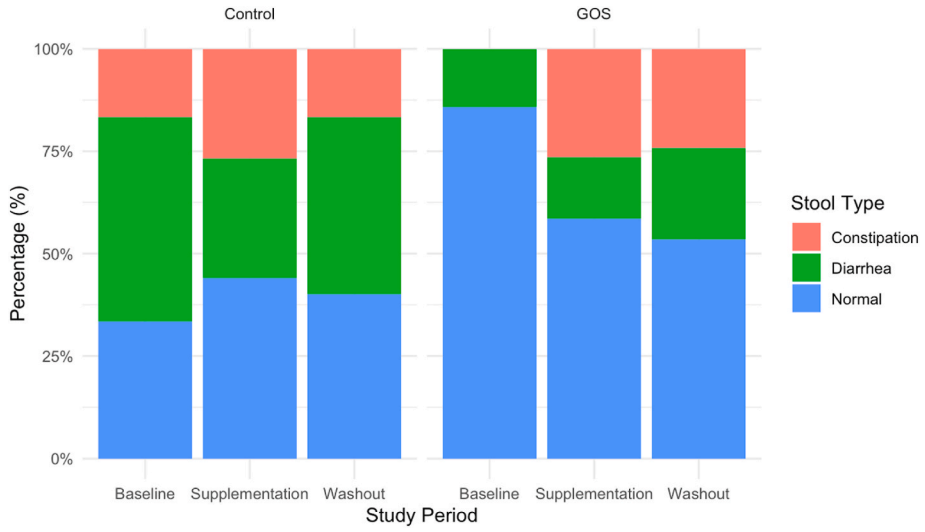

Fig. 5. Comparative stool type proportions from Bristol Stool Scale: (a) Control group across baseline, supplementation and washout periods. (b) GOS group across group across baseline, supplementation and washout periods. Stool types: constipation (red), diarrhea (green) and normal (blue).

Table 4
Weekly and periodic dairy intake with average, minimum and maximum values for GOS and control groups.

Group	Period	Week	Average	Min	Max
GOS	Baseline	2	12.13	1	47
	Supplementation	3	9.12	1	21
		4	6.00	3	11
	Washout	5	9.57	4	24
		6	8.12	5	14
		7	9.95	2	21
Control	Baseline	2	5.60	0	11
	Supplementation	3	5.67	2	11
		4	7.33	0	14
	Washout	5	6.50	3	13
		6	7.80	4	16
		7	6.00	4	8

Corynebacteria and *Streptomyces*, which are involved in the fermentation of large polysaccharides and oligosaccharides. *Bifidobacteria* also contribute to immunological function by stimulating the generation of regulatory T-cells (at least in a mice model), which moderate immune-inflammatory and autoimmune responses (O'Mahony et al., 2008). However, this increase in relevant abundance declined once the supplementation ceased.

Like Actinobacteria, Firmicutes increased during the supplementation period and declined once the supplementation ceased. The phylum Firmicutes is comprised of Gram-positive bacteria that are primarily from the genera *Bacillus, Clostridium, Enterococcus, Lactobacillus, Faecalibacterium* and *Ruminococcus* (Stojanov, S., Berlec, A., & Štrukelj, 2020). Firmicutes typically make up 40–65% of the colon or fecal microbiota (Huang et al., 2018). This phylum has been associated with increases in production of SCFAs, primarily butyrate (Venegas et al., 2019a). SCFAs (acetate, propionate and butyrate) are the major metabolites produced

in the gut by bacterial fermentation using dietary fibers and resistant starch (Silva, Bernardi, & Frozza, 2020). Previous studies have demonstrated that SCFAs are an essential factor in improving inflammatory bowel disease (Zhang et al., 2022) and are an important fuel source for colonic epithelial cells (Venegas et al., 2019a). Previous studies showed that there was a significant increase in *Faecalibacterium* and *Lactobacillus* in response to GOS supplementation (Azcarate-Peril et al., 2017; Lordan, Thapa, Ross, & Cotter, 2020).

The study also observed a decrease in *Bacteroidetes* in response to GOS consumption, consistent with findings from previous research (Liu et al., 2017). This reduction in *Bacteroidetes* may be explained by a competitive exclusion dynamic, potentially driven by the increased abundance of Firmicutes, as noted in prior studies on GOS supplementation (Liu et al., 2017). Additionally, variations in responses could stem from inter-individual differences in baseline microbiota composition and dietary habits (Toscano et al., 2017). Notably, GOS appears to have a selective effect, as it promotes the growth of specific bacterial lineages, such as *Bifidobacteria*, without consistently increasing other groups (Davis, Martínez, Walter, & Hutkins, 2010). This specificity highlights the potential of GOS as a tool for selectively modulating gut microbiota.

A significant increase was observed in the relative abundance of the genus *Blautia* in the GOS group. *Blautia* is a dominant genus in the gut microbiota, is considered a probiotic for maintaining the environmental balance in the intestine and preventing inflammation.

(Liu et al., 2021). In a study by Ozato et al. (2019), *Blautia* was demonstrated to be the only genus whose abundance showed a negative significant relationship with higher visceral fat area in a Japanese population. Therefore, the observed increase in *Blautia* following GOS supplementation could be interpreted as a beneficial change, given the genus's association with health promotion.

Additionally, there was a significant increase in the abundance of *Fusucatenibacter* in the GOS group during the supplementation period. Research on *Fusucatenibacter*'s impact on gut microbiota and its physiological functions in the host is limited. However, some studies have noted a significant decrease in *Fusucatenibacter* among patients with ulcerative colitis (Gryaznova et al., 2021), suggesting a potential role in gut health. Further research is necessary to fully understand the effects of *Fusucatenibacter* on host health.

At the genus level, an increase in *Bifidobacteria* was observed with GOS supplementation. However, these changes were transient, subsiding after the end of the supplementation period. The bifidogenic effect of GOS is well-documented in several studies (Bouhnik et al., 1997; Depeint, Tzortzis, Vulevic, I'Anson, & Gibson, 2008; Davis et al., 2010). This research aimed to test the hypothesis that GOS supplementation would increase lactose-metabolizing bacteria, thereby facilitating the fermentation of lactose reaching the colon in lactose-intolerant individuals. Indeed, previous studies have identified a negative correlation between increases in *Bifidobacteria* and gastrointestinal symptoms after GOS consumption (Azcarate-Peril et al., 2017; Chey et al., 2020).

Bifidobacteria express a higher activity of β-galactosidase than do many other commensal colonic microbiota members. These enzymes are capable of hydrolyzing lactose into its monosaccharide components, suggesting a mechanism whereby a higher relative abundance of *Bifidobacteria* could lead to rapid colonic fermentation of lactose. This process potentially mitigates osmotic diarrhea, a common symptom of lactose intolerance (Hertzler & Savaiano, 1996).

Bifidobacteria, which lack certain enzymes necessary for carbohydrate metabolism, such as aldolase and glucose-6-phosphate NADP + oxidoreductase, do not utilize the glycolysis pathway. Instead, they utilize a distinctive metabolic route termed the 'bifid shunt,' which involves the enzyme phosphoketolase to metabolize fructose-6-phosphate. This alternative pathway enables *Bifidobacteria* to generate a higher ATP yield from carbohydrate metabolism - 2.5 ATP - compared to the 2 ATP yielded by the Embden-Meyerhof-Parnas glycolytic pathway (Parche et al., 2006).

Furthermore, acetate and butyrate are produced as metabolites through this pathway. Volatile SCFAs such as acetate and butyrate, key products of bacterial carbohydrate fermentation, are believed to have significant physiological effects, including anti-inflammatory properties and satiety-promotion (Rivière, Selak, Lantin, Leroy, & De Vuyst, 2016). Lactate is also utilized by bacteria like *Eubacterium halii* and *Anaerostipes caccae* to produce butyrate as a major fermentation product (Duncan, Louis, & Flint, 2004).

Consumption of GOS may alleviate symptoms of LI through a potential mechanism where increased populations of lactose-metabolizing bacteria, such as *Bifidobacteria*, rapidly ferment lactose. This study's findings are consistent with those of Chey et al. (2020) and Azcarate-Peril et al. (2017), which suggest that GOS supplementation alleviates lactose intolerance symptoms by enhancing the growth of *Bifidobacteria*. These bacteria rapidly ferment lactose, thereby reducing osmotic diarrhea. This swift fermentation process minimizes the risk of osmotic diarrhea. Moreover, these bacteria may produce less hydrogen, which could lead to a reduction in symptoms such as flatulence, abdominal pain and bloating (Hertzler & Savaiano, 1996). A diverse array of gut microbes can produce β-galactosidase to release glucose and galactose from lactose and then further ferment these monosaccharides (Firrman et al., 2023). However, this fermentation by many bacteria can result in gases like hydrogen, carbon dioxide and methane, contributing to common lactose intolerance symptoms (Misselwitz et al., 2019). Strains of *Lactobacillus* and *Bifidobacterium* also produce β-galactosidase and metabolize lactose very efficiently (Fassio et al., 2018; Li et al., 2012). These strains stand out because they do not produce gas during the fermentation of lactose, potentially reducing bloating and flatulence (McKay, Holbrook, & Eastwood, 1982).

Participants consuming GOS exhibited a decrease in the occurrence of diarrhea compared to baseline and while those participants consuming lactose (control group) exhibited a non-significant increase in the occurrence of diarrhea compared to baseline, marking a significant difference in diarrhea symptoms between the two groups. The decrease in diarrhea from baseline to the supplementation period in the GOS group is surprising, as the GOS powder fed to the LI participants did contain 1.1 g of lactose. Studies suggest that people with LI can tolerate around 12 g of lactose prior to symptom development (Misselwitz et al., 2019). Bloating was increased significantly during the supplementation period, and the other gas-related symptoms tended to increase during the GOS supplementation period while not in the control group. The increase in gas production may be attributed to the fermentation activities of gut bacteria such as Bacteroides and Clostridium, which are recognized for their hydrogen production (Rowland et al., 2018). Although bloating increased, it was not considered severe, indicating that the GOS dosage was generally well-tolerated by the study participants.

Previous research has indicated a bifidogenic effect of GOS at doses of approximately 5 g per day and no further increases in *Bifidobacteria* abundance when the dose was raised to 10 g per day (Davis et al., 2010). The primary known adverse effect of GOS is osmotic diarrhea, caused by increased osmotic pressure due to high concentrations of GOS in the colon. This effect was not observed in the current study, and no participants withdrew due to adverse effects, indicating good tolerance to the GOS dosage used. However, a dose of around 5 g per day might have resulted in less microbial gas production while still maintaining a bifidogenic effect.

The proportion of "normal stool" was higher in the GOS group than the control group, aligning with the gastrointestinal symptom results, which showed a significant decrease in diarrhea in the GOS group when comparing baseline to supplementation. Across the study periods in the GOS group, no significant differences in the Bristol stool chart-rated stool types were evident. This result contrasts with the noted significant decrease in self-reported diarrhea based on the questionnaire. The discrepancy in these results might stem from the subjective nature of the data collected.

Although a statistical increase in bloating rating was observed during

the supplementation period, there may not be a clinically significant increase in this symptom. Throughout the study, ANOVA analyses of dairy consumption indicate that participants in both the GOS and control treatment groups maintained a consistent intake of dairy. This sustained consumption, evidenced by p-values reflecting no significant weekly or period-based variations, suggests that participants continued to ingest dairy-and by extension, lactose-during the study. Despite this continued exposure to lactose, the GOS group experienced a significant reduction in diarrhea symptoms, as opposed to the control group, highlighting the potential benefits of GOS in managing lactose-related gastrointestinal discomfort.

However, this study has certain limitations that warrant consideration. A major limitation is the absence of a placebo-control group, which makes it difficult to exclude the influence of external factors on the observed effects. Incorporating a control group in future studies would help ensure that the results are solely attributable to GOS supplementation. Additionally, the short duration of the study limits the ability to assess the long-term effects of GOS supplementation. Extended follow-up periods in future research are necessary to determine whether the observed changes in gut microbiota and symptoms persist over time and to monitor for any potential adverse effects.

Future studies should also aim to evaluate the efficacy of GOS in larger, geographically diverse populations to account for individual variability in response. Exploring the potential benefits of combining GOS with other prebiotics or probiotics could further enhance its therapeutic impact. Future work could also include additional analyses to better understand changes induced by GOS, including functional analyses such as metatranscriptomics and metabolomics. These approaches could elucidate the molecular pathways driving the observed microbiota changes, offering a clearer understanding of GOS's role in gut microbiota modulation.

5. Conclusions

We found that GOS supplementation led to changes in microbial composition in subjects with lactose intolerance. We observed both increases in potentially beneficial organisms and decreases in some potentially harmful microorganisms, pointing to a net positive alteration in the gut microbiome due to GOS supplementation. Notably, these microbial changes did not persist after supplementation ended. GOS supplementation in the context of a diet with dairy led to a decrease in self-reported diarrhea. Otherwise, the GOS dose used was generally well-tolerated in this group of subjects with lactose intolerance, with bloating being the only significant increase noted during supplementation. In conclusion, GOS supplementation resulted in an increased abundance of commensal *Bifidobacteria*, potentially reducing symptoms of lactose intolerance. Further in-depth investigation is required to conclusively determine the role of GOS in managing lactose intolerance.

CRediT authorship contribution statement

Gloria Angima: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **Yunyao Qu:** Writing – review & editing, Validation, Data curation. **Eiseul Kim:** Software, Validation, Writing – review & editing. **Gerd Bobe:** Validation, Methodology, Formal analysis. **David C. Dallas:** Writing – review & editing, Supervision, Software, Resources, Funding acquisition, Conceptualization. **Si Hong Park:** Writing – review & editing, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors have no declaration of interest.

Acknowledgments

This work was supported by funding from BUILD Dairy, the Oregon Dairy Nutrition Council and the USDA Multistate Workgroup W4002.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2024.117291.

Data availability

Data will be made available on request.

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