Effect of a Nutritionally Balanced Diet Comprising Whole Grains and Vegetables Alone or in Combination with Probiotic Supplementation on the Gut Microbiota

Young Joo Oh[1,2], Kisun Nam[1], Yanghee Kim[1], Seo Yeon Lee[1], Hyung Sook Kim[3], Jung Il Kang[1], Sang Yun Lee[1], and Keum Taek Hwang[2]

[1]Pulmuone Co., Ltd., Seoul 06367, Korea
[2]Department of Food and Nutrition, and Research Institute of Human Ecology, Seoul National University, Seoul 08826, Korea
[3]Department of Food and Nutrition, The University of Suwon, Gyeonggi 18323, Korea

ABSTRACT: Dysbiosis is a microbial imbalance, which often causes diseases and can be triggered by diet. Here, we determined the effect of a nutritionally balanced diet rich in vegetables and whole grains alone and/or in combination with probiotics on the gut microbiota of healthy adults. We conducted a parallel-group randomized trial enrolling 63 healthy participants who were administered either a balanced diet (B-diet group), a probiotic capsule containing *Lactobacillus plantarum* PMO 08 (probiotics group), or a balanced diet plus probiotic capsule (synbiotics group) once daily for 2 weeks. The gut microbiota of each participant was analyzed via 16S ribosomal RNA MiSeq-based sequencing. Gastrointestinal symptoms and defecation habits were evaluated using questionnaires. The B-diet group showed significantly reduced Firmicutes-to-Bacteroidetes ratio ($P<0.05$) and abundances of the genera *Blautia* ($P<0.01$), *Dorea* ($P<0.05$), and *Lachnoclostridium* ($P<0.05$). Furthermore, the abundance of *Bacteroides* increased ($P<0.05$) compared to baseline levels. In the synbiotics group, *Lactobacillus* abundance increased significantly ($P<0.05$) and defecation difficulty decreased ($P<0.05$), confirming a synergistic effect of combined intake. All groups showed a significant reduction in the abundance of Clostridiaceae ($P<0.001$) and alleviation of bloating symptoms ($P<0.05$). Moreover, the relative abundance of *Faecalibacterium* significantly increased in the probiotics group ($P<0.05$). Therefore, the individual or combined intake of a nutritionally balanced diet and *L. plantarum* PMO 08 beneficially modifies the gut microbiota with the potential to alleviate gastrointestinal symptoms and improve defecation habits.

Keywords: Firmicutes-to-Bacteroidetes ratio, gut health, gut microbiota, *Lactobacillus plantarum* PMO 08, nutritionally balanced diet

INTRODUCTION

The intestinal ecosystem is closely related to human health. Intestinal dysbiosis is associated with various diseases, such as inflammatory bowel disease (IBD), obesity, non-alcoholic liver disease, and high blood pressure (Singh et al., 2017). The composition of the gut microbiota is dependent on internal factors, such as sex, race, and age, and is affected by external environmental factors, such as antibiotics, drugs, stress, and diet. Diet is a source of nutrients for microorganisms and is considered the most important factor influencing the gut microbiota (Shin et al., 2019). Arumugam et al. (2011) introduced the following three enterotypes of the human gut microbiome according to dietary habits: the *Bacteroides* enterotype in individuals with a high intake of protein and animal fats; the *Prevotella* enterotype in individuals with a high intake of fiber; and the *Ruminococcus* enterotype, which is similar to the *Bacteroides* enterotype but characterized by fast sugar absorption and a greater likelihood of developing obesity. According to a previous study that analyzed the gut microbiota of immigrants from Thailand to the USA, changes in diet alter the ecological environment of the intestines. In particular, *Prevotella* may replace *Bacteroides*, which gradually decreases the ability to digest dietary fiber (Vangay et al., 2018). Since such an intestinal ecosystem can have a strong influence on human physiology by interacting with metabolites or symbi-

otic microorganisms produced by other microorganisms, the abundance of different species in the gut microbiota can be used to predict disease and as an indicator for improvement (Hills et al., 2019). Approximately 90% of Gram-negative Bacteroidetes and Gram-positive Firmicutes reside in the intestines, with a higher proportion of Firmicutes species associated with various diseases, such as obesity, type 2 diabetes, and IBD (Magne et al., 2020). As the human gut microbiome can change with dietary habits, recent research has focused on investigating the relationship between dietary ingredients and functional foods and the gut microbiota (De Angelis et al., 2019).

Modern society is exposed to the "Western-style diet", which is characterized by excessive intake of energy-rich foods, such as fat, cholesterol, and animal protein. This type of diet causes inflammatory conditions, and sustained inflammatory reactions can lead to various diseases, such as obesity, metabolic syndrome, and colon cancer (Cândido et al., 2018). Specifically, chronic inflammation is caused by lipopolysaccharides, a component of the cell wall of Gram-negative bacteria that is closely related to dietary factors, such as animal fat intake (Schoeler and Caesar, 2019). Therefore, the composition of the gut microbiota has been well studied based on investigations of dietary control for preventing chronic diseases (Chae, 2016; Barrett et al., 2018; Garcia-Mantrana et al., 2018; Losasso et al., 2018). Compared to the regular diet, the Mediterranean diet, which comprises olive oil, whole grains, nuts, vegetables, and fruits, among other foods, increases the levels of *Bacteroides*, *Prevotella*, *Bifidobacterium*, and *Lactobacillus* and decreases the level of *Clostridium*. Intake of polyphenols, dietary fiber, plant proteins, and polysaccharides from plant foods induces changes in the gut microbiota (Garcia-Mantrana et al., 2018). An analysis of the gut microbiota based on the dietary patterns of 101 adults in Italy revealed that vegans and vegetarians have higher counts of Bacteroidetes-related operational taxonomic units than omnivores (Losasso et al., 2018). Furthermore, an association analysis of relative bacterial abundance with very low-density lipoprotein (VLDL) in pregnant women revealed that Lachnospiraceae is significantly higher in those consuming a vegetarian diet, and showed a negative correlation between Lachnospira and both VLDL cholesterol and triglyceride levels (Barrett et al., 2018). In addition, a previous study evaluated the effect of a Korean Dietary Approaches to Stop Hypertension (DASH) diet on metabolic syndrome (low-salt diet consisting of grains, vegetables, and fruits) for 10 days. The Korean DASH diet was positively correlated with a reduction in the Firmicutes-to-Bacteroidetes ratio, insulin, and homeostatic model assessment of insulin resistance (Chae, 2016).

Based on recent findings of probiotic function, studies are being conducted on the potential of probiotics for the treatment and prevention of chronic diseases (Shokryazdan et al., 2017). Probiotics are defined as living microorganisms that have a beneficial effect on the host when consumed in adequate amounts. Many of their beneficial effects, such as normalization of intestinal microbiota, competitive exclusion of pathogens, regulation of the intestinal transit, and modulation of the immune response, have been reported (FAO/WHO, 2001; Lee et al., 2018). This study included *Lactobacillus plantarum* PMO 08, which is isolated from kimchi and has an excellent bile salt hydrolase activity (Yeo et al., 2000). *In vitro* studies have confirmed that *L. plantarum* PMO 08 has a high acid, salt, and bile resistance; meets the requirements of a probiotic; has a high intestinal viability; and is very adhesive (Jang et al., 2011a; Oh et al., 2020). Furthermore, *L. plantarum* PMO 08 demonstrates anti-cholesterol properties by regulating the expression of *SREBP-2* mRNA, which is involved in cholesterol metabolism, in a high-fat-induced obese mouse model (Oh et al., 2019). In addition, *L. plantarum* PMO 08 suppresses pruritus in a concentration-dependent manner in a mouse model of histamine- or compound 48/80-induced hypersensitivity (Jang et al., 2011b). Although the biomodulatory effect of *L. plantarum* PMO 08 intake has been confirmed in preclinical studies, its effect on the composition of the gut microbiota has not yet been determined.

This study aimed to evaluate the effect of a nutritionally balanced diet consisting of whole grains and vegetables alone or in combination with probiotics on the composition of gut microbiota in healthy adults. Participants were provided with one nutritionally balanced meal per day in the form of a lunch meal box for 2 weeks, and the abundance of major intestinal microorganisms was compared before and after the intervention. Furthermore, *L. plantarum* PMO 08 was provided in the form of a capsule as a probiotics. In addition, the synergistic effects of a synbiotic diet consisting of a nutritionally balanced meal and a probiotic supplement were observed.

MATERIALS AND METHODS

Subjects and experiment design

This study enrolled healthy adult volunteers aged 19∼65 years from October 8, 2019 to October 22, 2019. All participants provided written informed consent. Participants were randomly assigned to one of three groups and administered either a nutritionally balanced meal (B-diet group), a probiotic capsule (probiotics group), or a nutritionally balanced meal with a probiotic capsule (symbiotic group) once per day for 2 weeks following a 2-week washout period. Feces were collected before and after intake of the experimental diet, and all participants completed questionnaires related to defecation and dietary
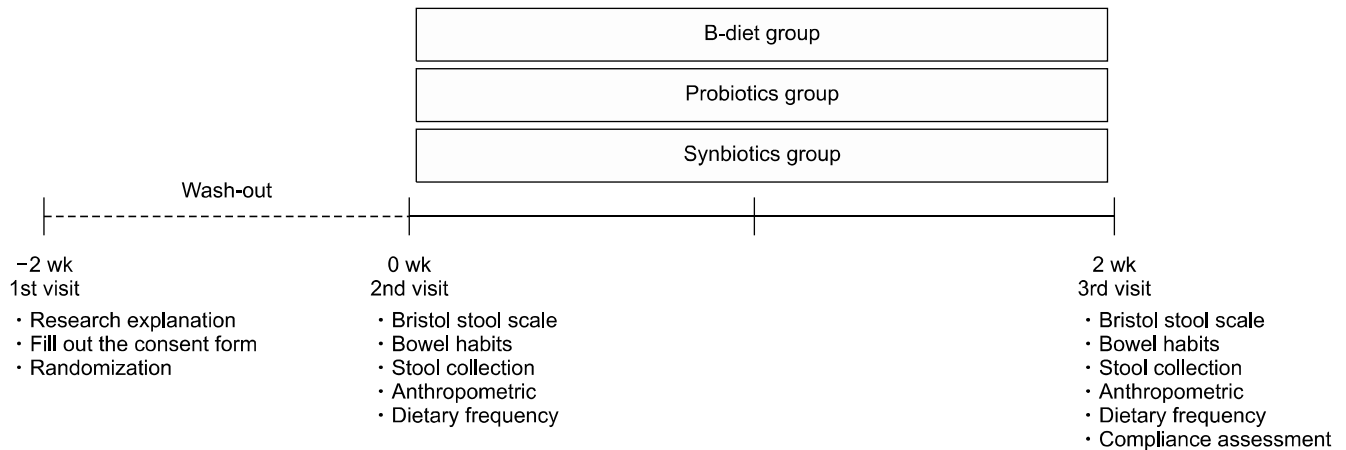
Fig. 1. Schematic outline of the study protocol for the B-diet, probiotics, and synbiotics groups.

habits. Anthropometric biomarkers such as the body weight (kg), body fat percentage (%), and body mass index (BMI; m/kg^2) were measured at the beginning and end of the intervention periods using a Body Composition Analyzer (InBody 230; InBody Co., Ltd., Seoul, Korea). The overall clinical study design is shown in Fig. 1. This study was conducted with approval of the Institutional Review Board of Suwon University (approval number: 1910-045-01).

Experimental diet

The nutritionally balanced diet used in the study was a modified version of the 211 diet, a balanced meal plan developed by Pulmuone (Seoul, Korea). The 211 diet emphasizes selection of protein foods with low saturated fats, vegetables, and whole grains to prevent excessive carbohydrate consumption (Ha et al., 2020). Nutritionally balanced meals are rich in whole grains (e.g., oats, barley, quinoa, lentil, and chickpeas), vegetables (e.g., broccoli, carrot, cabbage, tomato, and mushroom), nuts and beans, and have a low content of red meat. The nutritionally balanced diet was high in fiber and low in saturated fats, and was provided in commercialized lunch boxes produced by the Pulmuone Green Juice Co., Ltd. (Chungbuk, Korea). On weekdays, a refrigerated lunch box containing a nutritionally balanced diet was delivered each day, and two frozen lunch boxes were provided on Friday for the weekend. Table 1 shows the nutritional content of each product and a list of the food ingredients. The probiotic capsule (400 mg) containing 10 billion colony forming unit (CFU) of *L. plantarum* PMO 08 was provided by Pulmuone. Participants in the probiotics and synbiotics groups were given the same probiotic capsule, whereas those in the B-diet group were provided with hard capsules of the same formulation as a probiotic supplement filled with maltodextrin instead of probiotics. The capsules were bottled and packaged in sets of 14. Participants were instructed to consume one capsule with wa-

Table 1. Nutrient content of the balanced diet[1]

	Weekday (Mon~Fri)	Weekend (Sat~Sun)
Energy (kcal)	561	592
Carbohydrate (g)	88 (63%)[2]	84 (57%)
Protein (g)	23 (16%)	28 (19%)
Fat (g)	13 (21%)	16 (24%)
Dietary fiber (g)	14	4

[1]Consists of the following: whole grains (oats, sorghum, adlay, quinoa, and brown rice), beans (kidney beans, black beans, lentils, lima beans, chickpeas, pea, and corn), vegetables (eggplant, potato, sweet potato, red pepper, sweet pumpkin, carrot, wild parsley, romaine lettuce, garlic, Japanese apricot, white radish, broccoli, black olive, king oyster mushroom, celery, asparagus, cabbage, lettuce, button mushroom, onion, lotus root, cucumber, red onion, red cabbage, cheongyang chili, red pepper, green pepper, cauliflower, tomato, paprika, zucchini, and fragrant mushroom).
[2]Percent of calories from each nutrient.

ter once per day. Compliance with intake of a healthy balanced diet with probiotic supplement was investigated using a questionnaire, and participants were instructed to maintain their usual dietary habits until completion of the study.

Assessment of defecation habits

Defecation habits were evaluated using the Bristol Stool Scale (Lewis and Heaton, 1997) and constipation diagnosis questionnaires (Longstreth et al., 2006), which include stool frequency and regularity, gut transit time, feeling of incomplete evacuation, difficulty in defecation (the act of pushing and squeezing), and bloating, assessed on a four-point scale. Average scores before and after the intervention period were compared.

Analysis of gut microbiota composition

Fecal samples before and after the intervention period were collected using a stool collection kit (Noble Biosciences, Inc., Gyeonggi, Korea) provided by Green Cross

Genome Corporation (Gyeonggi, Korea). Collection kits containing fecal samples collected by the subjects were collected and sent to Green Cross Genome Corporation for analysis.

DNA extraction, amplification, and sequencing

DNA extraction from feces was performed using the Chemagic DNA Stool Kit (PerkinElmer, Inc., Waltham, MA, USA). A NEXTFLEX 16S V4 Amplicon-Seq kit (Bioo Scientific Corp., Austin, TX, USA) was used to create a library of the extracted DNA, which was evaluated for size and quality using the 2200 TapeStation (Agilent Technologies, Inc., Santa Clara, CA, USA). Sequencing was performed using a MiSeq Reagent Kit v2 (2×150 bp) and the MiSeq Sequencing System (Illumina, Inc., San Diego, CA, USA).

Bioinformatics analysis

Pre-processing and amplicon sequence variant clustering of the results file from the Illumina MiSeq Platform were performed using QIIME 2 (https://dev.qiime2.org). For microbial identification, BLCA software (https://github.om/qunfengdong/BLCA) and the NCBI RefSeq database (http://www.ncbi.nlm.nih.gov/refseq) were used. The final processed data were calculated as the number of reads per layer, and each calculated number of reads was converted into relative abundance for analysis.

Statistical analysis

All results were analyzed using SPSS, version 21.0 software (SPSS Inc., Chicago, IL, USA). Results were expressed as the mean±standard deviation (SD) or standard error of mean (SEM). Defecation habits and the relative abundances of gut microbiota before and after the intervention period were verified by paired Student's *t*-tests at $P<0.05$, $P<0.01$, and $P<0.001$. Statistical significance between experimental groups was analyzed using ANOVA ($P<0.05$).

RESULTS

General characteristics of participants

Table 2 provides information regarding the study participants. A total of 65 individuals were recruited, of which 63 were included in the analysis. The participants had an average age of 36.8±8.0 years, average weight of 65.0± 2.6 kg, average BMI of 23.7±0.8 mg/kg^2, and average body fat percentage of 30.2±1.4%. There were no significant differences between groups.

Table 2. General characteristics of study participants

	B-diet group	Probiotics group	Synbiotics group	*P*-value
Sex [n (%)]				
Male	8 (38.1)	7 (33.3)	7 (33.3)	
Female	13 (61.9)	14 (66.7)	14 (33.3)	
Age (yrs)	36.9±7.9	36.7±7.60	36.7±8.4	0.995
Body weight (kg)	64.8±2.60	65.1±2.56	65.3±2.78	0.992
BMI (kg/m^2)	23.9±0.84	23.4±0.64	23.6±0.78	0.898
Body fat (%)	30.6±1.22	30.5±1.41	29.7±1.56	0.881

Values are expressed as the mean±SD (n=21).
B-diet group, whole grains and vegetable-enriched diet; probiotics group, probiotic capsule containing *Lactobacillus plantarum* PMO 08; synbiotics group, whole grains and vegetable-enriched diet combined with *L. plantarum* PMO 08.
BMI, body mass index.

Table 3. Relative abundances of baseline gut microbiota communities at the phylum level in all participants

	All subjects (n=63)	Test group^{NS}		
		B-diet group	Probiotics group	Synbiotics group
Bacteroidetes	49.53±3.84	44.42±4.23	55.33±3.25	48.84±4.05
Firmicutes	41.87±3.37	45.17±3.51	37.45±2.85	42.98±3.75
Proteobacteria	3.58±0.65	4.01±0.68	4.14±0.97	2.60±0.32
Actinobacteria	1.03±0.38	0.87±0.20	0.89±0.28	1.32±0.65
Fusobacteria	1.21±0.97	2.67±2.30	0.32±0.25	0.63±0.35
Verrucomicrobia	1.35±0.98	1.47±0.98	0.44±0.32	2.13±1.64
Others	1.36±0.50	0.99±0.44	1.43±0.49	1.66±0.55
F/B ratio	1.15±0.23	1.34±0.23	0.80±0.12	1.31±0.29

Values are expressed as the mean±SEM (n=21).
F/B ratio, Firmicutes-to-Bacteroidetes ratio. B-diet group, whole grains and vegetable-enriched diet; probiotics group, probiotic capsule containing *Lactobacillus plantarum* PMO 08; synbiotics group, whole grains and vegetable-enriched diet combined with *L. plantarum* PMO 08. NS, no significant difference between groups.

Microbial composition at baseline

The main phyla analyzed in the feces of all subjects participating in the experiment are shown in Table 3. The gut microbiome of the participants was composed of the phyla Bacteroidetes (49.53±3.84%), Firmicutes (41.87±0.37%), Proteobacteria (3.58±0.65%), Actinobacteria (1.03±0.38%), Verrucomicrobia (1.35±0.98%), and Fusobacteria (1.21±0.97%), with the top six most abundant phyla accounting for 98.5% of the microbiota. The ratio of Firmicutes-to-Bacteroidetes (F/B) ratio was 1.15±0.23, and there were no significant differences between groups.

Changes in gut microbiota induced by dietary intake

Changes in the main phyla according to dietary intake are shown in Fig. 2. The B-diet group increased the abundance of Bacteroidetes by 113% (*P*=0.063), whereas the abundance of Firmicutes decreased to 84% (*P*<0.01). No significant changes were observed in the probiotics and synbiotics groups. The F/B ratio was reduced in the B-diet, probiotics, and synbiotics groups to 71.3%, 95.3%, and 88.6%, respectively. In particular, a significant decrease in the F/B ratio was observed in the B-diet group compared with baseline (*P*<0.05; Fig. 3).

Changes in the gut microbiota according to dietary intake at the family level are shown in Fig. 4. The B-diet group showed a significant increase in the abundance of Bacteroidaceae (*P*<0.05) and a significant decrease in the abundance of Clostridiaceae (*P*<0.001) and Lachnospiraceae (*P*<0.01). The probiotics group showed a significant decrease in the relative abundance of Clostridiaceae (*P*<0.001), and the synbiotics group showed a significant in-crease in the relative abundance of Lactobacillaceae (*P*<0.05) and a significant decrease in the abundance of Clostridiaceae (*P*<0.001).

Changes in the gut microbiota according to dietary intake at the genus level are shown in Fig. 5 and Fig. 6. The B-diet group showed an increase in the abundance of *Bacteroides* of 23.4% (*P*<0.05) and a significant decrease in the abundance of *Blautia* of 45.6% (*P*<0.01) compared to baseline levels. In addition, the abundances of *Anaerostipes* (*P*<0.01), *Lachnoclostridium* (*P*<0.05), *Dorea* (*P*<0.05),
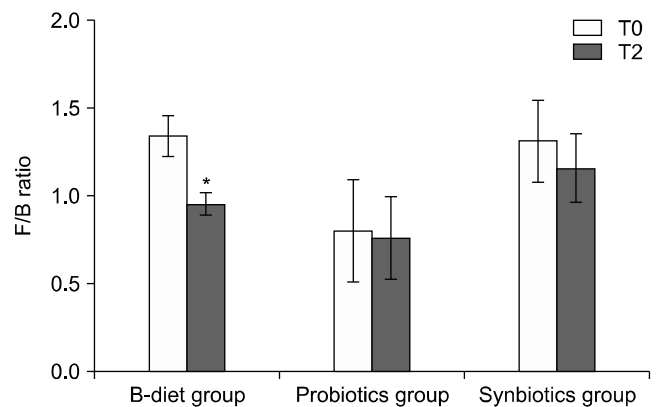

Fig. 3. Effect of each diet on changes in the Firmicutes-to-Bacteroidetes (F/B) ratio. T0, baseline value; T2, after 2 weeks of intervention. Values are expressed as the mean±SEM. B-diet group, whole grains and vegetable-enriched diet; probiotics group, probiotics capsule containing *Lactobacillus plantarum* PMO 08; synbiotics group, whole grains and vegetable-enriched diet combined with *L. plantarum* PMO 08. *P<0.05 compared with baseline (T0), calculated using paired *t*-tests.
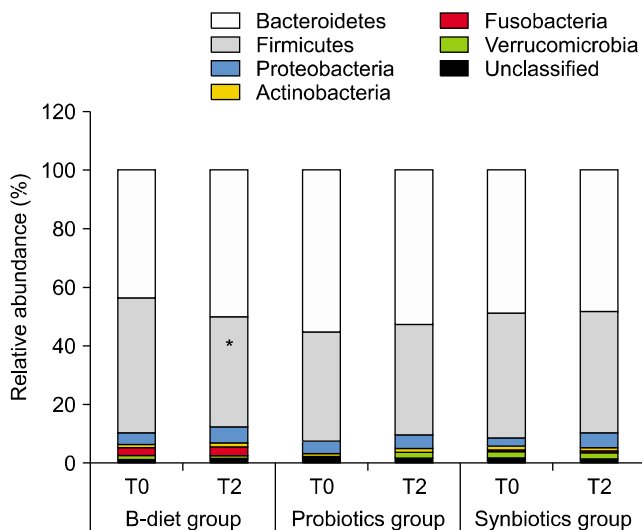

Fig. 2. Relative abundance of bacteria constituting the gut microbiome at the phylum level. T0, baseline value; T2, after 2 weeks of intervention. B-diet group, whole grains and vegetable-enriched diet; probiotics group, probiotics capsule containing *Lactobacillus plantarum* PMO 08; synbiotics group, whole grains and vegetable-enriched diet combined with *L. plantarum* PMO 08. *P<0.05 compared with baseline (T0), calculated using paired *t*-tests.
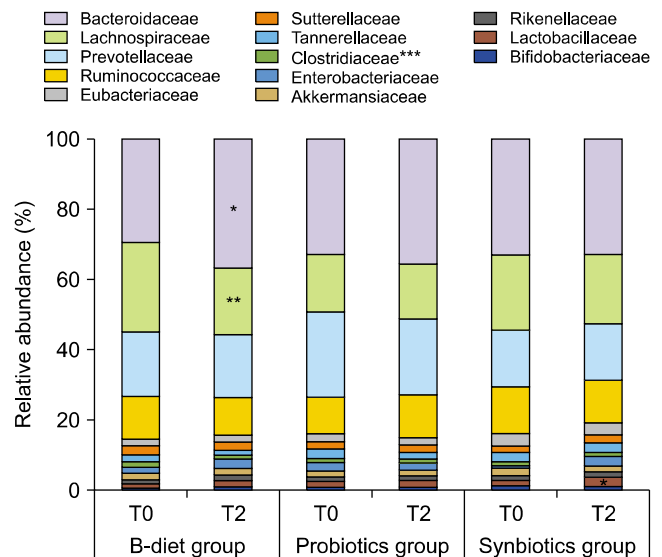

Fig. 4. Effect of each diet on relative bacterial abundances at the family level. T0, baseline value; T2, after 2 weeks of consumption. B-diet group, whole grains and vegetable-enriched diet; probiotics group, probiotics capsule containing *Lactobacillus plantarum* PMO 08; synbiotics group, whole grains and vegetable-enriched diet combined with *L. plantarum* PMO 08. *P<0.05, **P<0.01, and ***P<0.001 compared with baseline (T0), calculated using paired *t*-tests.

Cuneatibacter (*P*<0.05), and *Merdimonas* (*P*<0.05) were significantly decreased in the B-diet group (Fig. 6). Probiotics alone had little effect on *Bacteroides* and *Ruminococcus* levels, which are the major genera in the intestines, with the abundances of *Prevotella* only decreasing by

15.6% and 38.5%, respectively, in the probiotics group. However, in the probiotics group, the abundance of *Faecalibacterium*, a major butyrate producer, was significantly increased by 65.4% (*P*<0.05). In addition, the probiotics group showed significant increases in *Gemmiger* (difference=0.117, *P*<0.05). In the synbiotics group, there were few differences in the relative abundances of *Bacteroides* and *Prevotella*, and a 30.1% decrease in the relative abundance of *Ruminococcus*, although this was not considered statistically significant. However, participants in the synbiotics group showed a significant decrease in the abundance of *Blautia* (28.3%, *P*<0.05) and a significant increase in the abundance of *Lactobacillus* (*P*<0.05). In addition, the abundances of *Hespellia* (*P*<0.05) and *Phocea* (*P*<0.05) in the synbiotics group, and *Intestinomas* in B-diet group (*P*<0.05) were significantly decreased, but this finding was omitted from the data as the relative abundances were below 0.1%.

Relative abundances of *Lactobacillus* and *Bifidobacterium*
The relative abundances of the genus *Lactobacillus*, a beneficial intestinal bacteria, are shown in Fig. 7A. In the B-diet group, the initial (T0) relative abundance of *Lactobacillus* spp. was 1.00±0.31%, and increased to 1.48±0.39% after 2 weeks of dietary intake (difference=0.481, *P*= 0.09). In the probiotics group, the relative abundance of *Lactobacillus* spp. increased from 1.48±0.52% to 1.76± 0.46% after 2 weeks (difference=0.282, *P*=0.687), and in the synbiotics group, the relative abundance of *Lacto-*
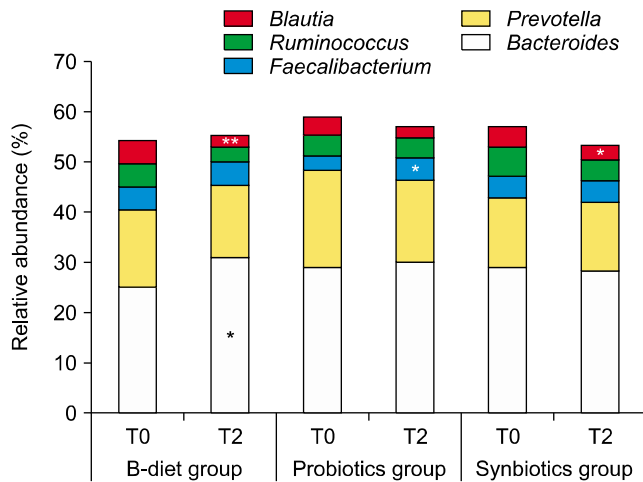

Fig. 5. Effect of each diet on relative abundances of bacteria in the major microbiota at the genus level. Relative abundances of the top five genera are shown. T0, baseline value; T2, after 2 weeks of consumption. Values are expressed as the mean± SEM. B-diet group, whole grains and vegetable-enriched diet; probiotics group, probiotics capsule containing *Lactobacillus plantarum* PMO 08; synbiotics group, whole grains and vegetable-enriched diet combined with *L. plantarum* PMO 08. *P*<0.05 and **P*<0.01 compared with values at baseline (T0), calculated by paired *t*-tests.
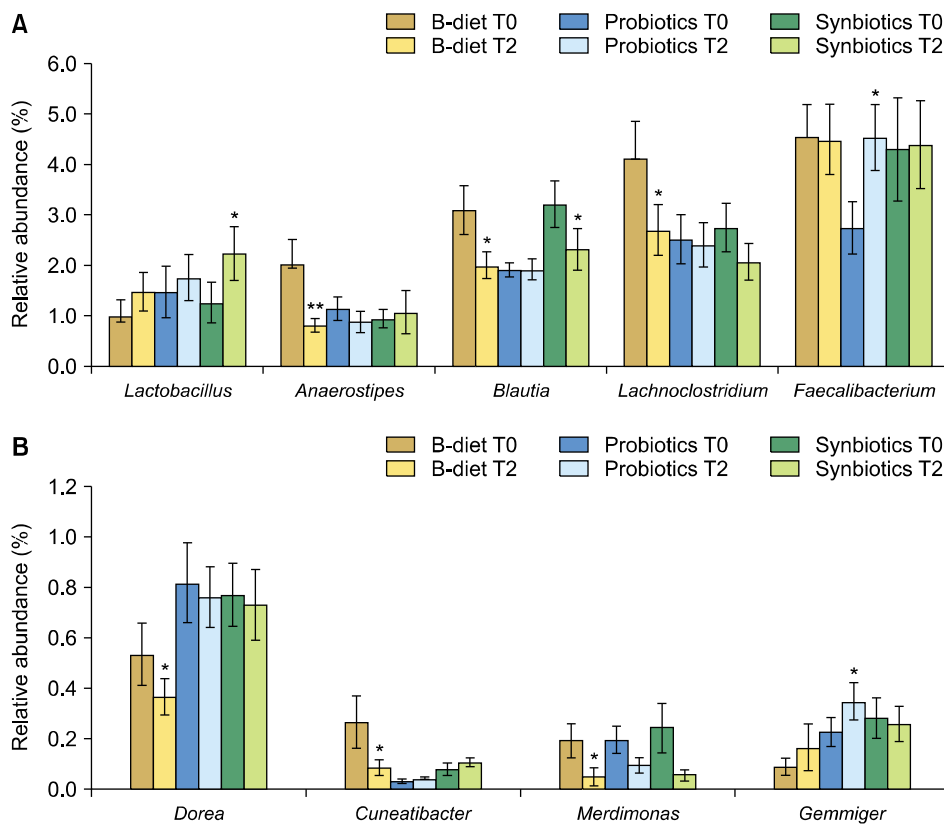

Fig. 6. Effect of each diet on relative abundances of bacteria in the microbiota at the genus level. Genera with significant differences in even one of the groups are shown. (A) Relative abundances of bacterial taxa accounting for more than 1%. (B) Relative abundances of bacterial taxa accounting for less than 1%. T0, baseline value; T2, after 2 weeks of consumption. Values are expressed as the mean±SEM. B-diet group, whole grains and vegetable-enriched diet; probiotics group, probiotics capsule containing *Lactobacillus plantarum* PMO 08; synbiotics group, whole grains and vegetable-enriched diet combined with *L. plantarum* PMO 08. *P*<0.05 and **P*<0.01 compared with values at baseline (T0), calculated by paired *t*-tests.

bacillus spp. increased by approximately 1.8-fold from 1.26±0.40% to 2.24±0.53% (difference=0.981, $P<0.05$). The relative abundances of the genus *Bifidobacterium* are shown in Fig. 7B. In the B-diet group, the relative abundance of *Bifidobacterium* spp. increased by 49.5%, although this increase was not significant. No changes in the abundances of *Bifidobacterium* spp. were observed in either the probiotics or synbiotics groups.

Changes in defecation habits induced by dietary intake

Defecation habits of the subjects are shown in Table 4. Before the dietary intervention, the average Bristol score of the subjects was 3.9±1.20 points, and 55.6% of the subjects scored 4 points with a normal fecal shape. An evaluation of each item (including frequency, regularity, transit time, the difficulty of defecation, incomplete evacuation, and bloating) showed that there were no significant differences between the groups at baseline (T0). The significance of dietary intake was evaluated using paired *t*-tests before and after the intervention period and showed that the "bloating" symptom was significantly alleviated in all groups. The score for bloating improved from 2.71±0.18 to 2.10±0.18 points ($P<0.01$) in the B-diet group, from 2.43±0.19 to 2.05±0.21 points ($P<0.05$) in
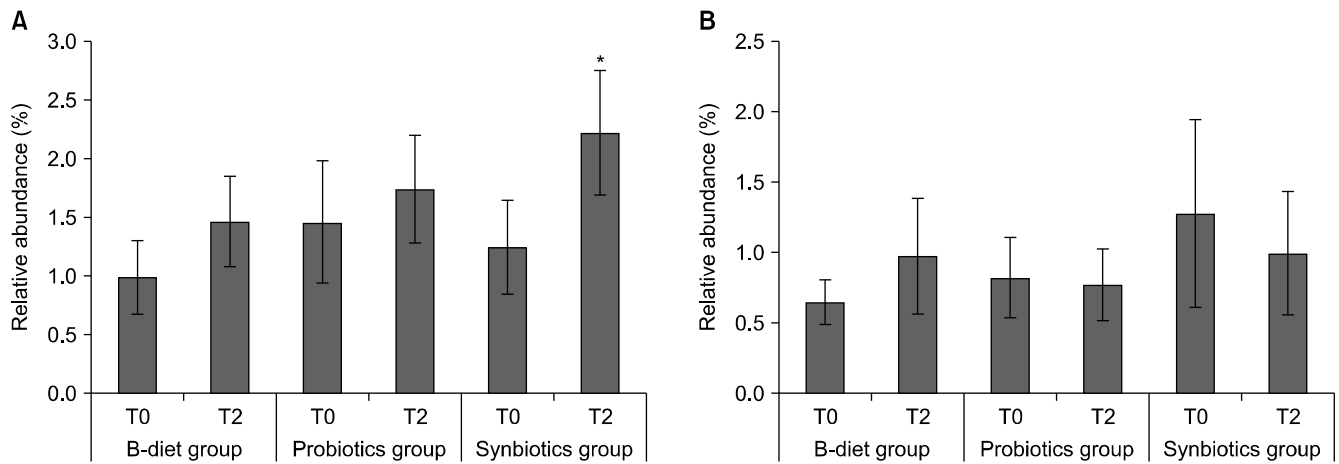

Fig. 7. Effect of each diet on (A) *Lactobacillus* and (B) *Bifidobacterium* composition of the microbiota. T0, baseline value; T2, after 2 weeks of consumption. Values are expressed as the mean±SEM. B-diet group, whole grains and vegetable-enriched diet; probiotics group, probiotics capsule containing *Lactobacillus plantarum* PMO 08; synbiotics group, whole grains and vegetable-enriched diet combined with *L. plantarum* PMO 08. *$P<0.05$ compared with values at baseline (T0), calculated by paired *t*-tests.

Table 4. Defecation habits and fecal symptoms of study participants

Items	Periods	B-diet group	*P*-value	Probiotics group	*P*-value	Synbiotics group	*P*-value
Bristol score	T0	4.24±0.23	0.590	4.00±0.26	0.760	3.48±0.28	0.162
	T2	4.10±0.23		4.10±0.27		3.86±0.21	
Stool frequency	T0	3.14±0.20	0.649	3.10±0.19	1.000	3.19±0.19	0.329
	T2	3.05±0.20		3.10±0.22		3.33±0.19	
Stool regularity	T0	2.05±0.13	0.666	2.19±0.15	0.493	2.14±0.13	0.055
	T2	2.00±0.14		2.10±0.15		1.86±0.14	
Gut transit time	T0	1.57±0.18	0.493	1.48±0.11	1.000	1.81±0.19	0.379
	T2	1.48±0.15		1.48±0.15		1.67±0.21	
Difficult of defecation	T0	1.81±0.18	0.540	1.86±0.16	0.104	2.05±0.16	0.030
	T2	1.90±0.14		1.67±0.16		1.76±0.15	
Incomplete evacuation	T0	2.29±0.18	0.025	1.86±0.16	0.056	2.29±0.12	0.004
	T2	1.86±0.14		1.62±0.16		1.81±0.16	
Bloating	T0	2.71±0.18	0.009	2.43±0.19	0.042	2.71±0.18	0.038
	T2	2.10±0.18		2.05±0.21		2.24±0.15	

Values are expressed as the mean±SD (n=21).
Significant differences were measured using paired *t*-tests.
The seven types of Bristol stool scale are as follows: type 1, separate hard lumps; type 2, lumpy and sausage-like; type 3, sausage-shape with cracks on the surface; type 4, like a smooth, soft sausage or snake; type 5, soft blobs with clear-cut edges; type 6, mushy consistency with ragged edges; type 7, liquid consistency with no solid pieces.
Constipated, including type 1 and type 2 on the Bristol stool scale; normal, including type 3, type 4, and type 5 on the Bristol stool scale; inflammation, including type 6 and type 7 on the Bristol stool scale.
B-diet group, whole grains, and vegetable-enriched diet group; probiotics group, *Lactobacillus plantarum* PMO 08-administered group; synbiotics group, whole grains, and vegetable-enriched diet group combined with *L. plantarum* PMO 08.

the probiotics group, and from 2.71±0.18 to 2.24±0.15 points ($P<0.05$) in the synbiotics group. Additionally, in the B-diet group, incomplete evacuation ($P<0.05$) was improved, and in the synbiotics group, incomplete evacuation ($P<0.01$) and difficulty in defecation ($P<0.05$) were improved, indicating a positive synergistic effect. Moreover, the regularity of defecation was relatively improved ($P=0.055$) in the synbiotics group. However, no significant changes in fecal shape (Bristol score), frequency, or transit time were observed for any of the three groups compared with baseline.

Dietary compliance
Through surveying intake of the diet provided during the experiment and any side effects, all participants were found to have complied with the provided diets and probiotic supplements during the study period, and no participants experienced any side effects (data not shown).

DISCUSSION

This study was conducted to evaluate the effect of a nutritionally balanced diet enriched in whole grains and vegetables alone or in combination with probiotics on the composition of the gut microbiota. Nutritionally balanced diets were provided to participants in lunch boxes. Meals provided on weekdays contained 561 kcal, with a ratio (%) of carbohydrate to protein to fat of 63:16:21 and 14 g of dietary fiber. Meals provided on weekends contained 592 kcal, with a ratio (%) of carbohydrates to protein to fat of 57:19:24 with 4 g of dietary fiber. We evaluated the synergistic effect of a nutritionally balanced diet in combination with a probiotic supplement containing *L. plantarum* PMO 08. The study showed that consumption of a nutritionally balanced diet decreased the F/B ratio and induced changes in major genera, including an increase in the abundance of *Bacteroides* and decreases in the abundances of *Blautia*, *Lachnoclostridium*, and *Dorea*, which belong to the family Lachnospiraeceae (phylum Firmicutes, class Clostridia).

Firmicutes and Bacteroidetes account for more than 90% of the gut microbiota, and a high F/B ratio is associated with IBD, type 2 diabetes, and obesity (Magne et al., 2020). The F/B ratio is an important indicator of body weight control as it is higher in patients with obesity and decreases with reduced caloric intake (Turnbaugh et al., 2006). Ley et al. (2006) first identified a causal relationship between the microbiome and obesity, which was confirmed by inducing obesity in lean mice via transplanting the feces of obese mice, showing that the ability to extract energy from food could be affected by intestinal microorganisms. Therefore, the nutritionally balanced diet used in the current study is expected to help prevent

chronic diseases by improving the composition of the gut microbiota.

In this study, the decrease in the F/B ratio induced by intake of a nutritionally balanced diet was due, in some cases, to a significant increase in the abundance of the genus *Bacteroides*. *Bacteroides* comprises Gram-negative bacteria that account for up to approximately 30% of the human fecal microbiota, and are present in feces at a concentration of $10^9 \sim 10^{11}$ CFU/g (Tan et al., 2019). *Bacteroides* appears to have anti-obesity effects, such as inhibition of fat absorption and blood sugar, regulation of insulin and leptin levels, and improvement in immune parameters related to being overweight (Chang et al., 2019). It can possibly be used to treat and prevent osteoporosis by improving bone density through the production of vitamin K (Fujita et al., 2012). In addition, low abundances of *Bacteroides coprophilus* in patients with multiple sclerosis and cardiac fibrillation (Mirza et al., 2020; Xu et al., 2020) were reported in case-control studies. Indeed, the antibiotic diarrhea-prevention effects of *Bacteroides fragilis* ZY312 28 (Zhang et al., 2018), anticancer and immune boosting effects of *Bacteroides xylanisolvens* DSM 23964 (Ulsemer et al., 2016), and metabolic syndrome-related function of *Bacteroides uniformis* CECT7771 studied for use as probiotics (Gauffin Cano et al., 2012). In this study, the abundance of *Bacteroides* increased from 25.1% to 30.9% after consumption of a nutritionally balanced diet ($P<0.05$). *Bacteroides* has a powerful polysaccharide breakdown system and provides nutrients and energy for the human body through breakdown of short chain fatty acids (Tan et al., 2019). The nutritionally balanced diet consisting of whole grains and vegetables is believed to have promoted the growth of *Bacteroides* by providing polysaccharides, such as glycan. Follow-up studies are needed to determine which dietary ingredients or nutrients in the diet contributed to the increase in the proportion of *Bacteroides*.

In the B-diet group, the abundance of the phylum Firmicutes, associated with obesity, type 2 diabetes, and IBD decreased, which we attributed to the significant reduction in the abundances of Clostridiaceae ($P<0.001$) and Lachnospiraceae ($P<0.01$), which belong to the class Clostridia and the order Clostridiales. It was reported that the abundance of Clostridiales is significantly increased in an animal model of high-fat diet-induced obesity (de La Serre et al., 2010) and can be reduced by high levels of vegetable intake (Kimura et al., 2013). Lachnospiraceae includes microorganisms associated with obesity, diabetes, and cardiovascular disease (Larsen et al., 2010; Cho and Blaser, 2012; Kameyama and Itoh, 2014; Liu et al., 2018). Kameyama and Itoh (2014) reported that the family Lachnospiraceae causes obesity and diabetes by isolating Lachnospiraceae microorganisms from hyperglycemic obese mice and injecting them into germ-

free mice to observe increases in fasting blood sugar and adipose tissue weight, and low insulin levels. The abundance of the family Lachnospiraceae is high in patients with obesity (Cho and Blaser, 2012). High levels of Lachnospiraceae in the microbiota have been proposed to be a predictor of cardiovascular disease as the bacteria accelerate the progression of diabetes and arteriosclerosis (Larsen et al., 2010; Liu et al., 2018). *Blautia*, the most abundant genus in the Lachnospiraceae family, is an acetic acid-producing microorganism that is highly associated with glucose tolerance and hypertension (Kakiyama et al., 2013). When analyzing the composition of the gut microbiota according to the presence or absence of metabolic diseases in patients with obesity, *Blautia* spp. have been positively correlated with waist circumference, BMI, low-density lipoprotein cholesterol, and triglycerides, and have been used as diagnostic markers to predict metabolic diseases (Ohigashi et al., 2013). *Dorea* spp. are positively correlated with secondary bile acids, such as deoxycholic and lithocholic acids production, body weight, and total cholesterol levels in the blood (Holscher et al., 2018; Zeng et al., 2019). A high-fat/low-fiber Western-type diet increases the levels of secondary bile acids produced by the gut microbiota, which induces intestinal cytotoxicity and ultimately leads to colon cancer or bowel diseases (O'Keefe, 2016). Thus, the reduction of *Dorea* spp. by intake of a nutritionally balanced diet is considered to be helpful in improving the intestinal environment.

In this study, intake of *L. plantarum* PMO 08 improved the gut microbiota by significantly increasing the abundance of *Faecalibacterium*, a beneficial bacterium found in the intestines, and decreasing the abundance of the family Clostridiaceae. *Faecalibacterium* is an anaerobic microorganism that produces butyrate and is present at abundances of approximately 5% in the gut microbiota of healthy adults (Martín et al., 2017). The abundance of *Faecalibacterium* is relatively low in weak elderly individuals and patients with obesity, irritable bowel syndrome, irritable colitis, colon cancer, or celiac disease (Martín et al., 2017). *F. prausnitzii* produces anti-inflammatory substances, such as shikimic and salicylic acids (Miquel et al., 2015), and induces Toll-like receptor 2-dependent immunomodulatory actions through interleukin (IL)-12 and IL-10 cytokines (Rossi et al., 2015). In an animal model of *Escherichia coli*-induced infection, administration of *L. plantarum* ZLP001 significantly increased the abundance of *F. prausnitzii*, increased the expression of barrier-enhancing proteins [claudin-1, occludin, and zonula occludens-1 (ZO-1)], and exerted an immunomodulatory effect on the expression of pro-inflammatory cytokines, such as IL-6 and tumor necrosis factor-α (Wang et al., 2018). In a mouse model of loperamide-induced constipation, administration of complex probiotics, including

L. plantarum, suppressed the decrease in stool water content due to constipation and resulted in a significant increase in the abundance of *F. prausnitzii* in the intestines (Eor et al., 2019). *L. plantarum* PMO 08 used in the current study may enhance barrier function, as it has been shown to increase ZO-1 expression in an obese mouse model (Oh et al., 2019) and exhibit antipruritic effects in a mouse model of drug-induced hypersensitivity (Jang et al., 2011b). Therefore, the gut barrier-enhancing function and immunomodulatory effects of *L. plantarum* PMO 08 may be explained in part by recovery of *F. prausnitzii* in the gut microbiota; however, further studies are needed to confirm this finding.

In the current study, a nutritionally balanced diet and probiotic supplements had a synergetic effect on significantly increasing the abundance of *Lactobacillus* spp. Moreover, in the synbiotics group, improvements were observed in defecation in addition to bloating and incomplete evacuation, which were also present in the B-diet group. Abdominal bloating might have been reduced by decreased levels of the gases CO_2, CH_4, and H_2 produced by intestinal microbes, and reduced stagnation of these gases due to intestinal motility promoted by propionic acid and acetic acid produced by intestinal microbes. Improved intestinal motility could have also improved the difficulty of defecation (Eor et al., 2019).

In this study, we conducted an analysis of gut microbiota according to dietary intake in healthy adults. Although the gut microbiota was not in a state of dysbiosis, replacing one meal per day for 2 weeks induced positive changes in the composition of the gut microbiota. However, since we did not measure hematological indicators, only limited physiological changes induced by alterations to the composition of the gut microbiota were observed. Therefore, to confirm the effect of a nutritionally balanced diet and probiotic supplementation on the human body, follow-up studies are needed to analyze the metabolites and physiological indicators together.

The objective of this study was to evaluate the effects of a nutritionally balanced diet consisting of whole grains and vegetables alone or in combination with probiotic supplements on the gut microbiota of healthy adults. Replacing one meal per day with a nutritionally balanced diet improved the intestinal environment by significantly lowering the F/B ratio, decreasing major gut microbiota, such as those of the families Clostridiaceae and Lachnospiraceae and the genera *Blautia*, *Dorea*, and *Lachnoclostridium*, and alleviating symptoms, such as bloating and incomplete evacuation. *L. plantarum* PMO 08 intake improved the gut microbiota by increasing the abundance of *Faecalibacterium*, *Ruminococcus*, and *Gemmiger*, and decreasing the abundance of the family Clostridiaceae. The synergetic effects of a nutritionally balanced diet in combination with probiotic supplementation were confirmed

by an increase in the abundance of *Lactobacillus* and improvements in bloating, incomplete evacuation, and difficulty of defecation. To our knowledge, this study is the first to show that a nutritionally balanced diet consisting of whole grains and vegetables may positively change the composition of the gut microbiota. Importantly, our results may support the design of healthy balanced diets to control the gut microbiota. In addition, this study shows that *L. plantarum* PMO 08 can be safely used as a probiotic supplement as it promotes colonization of beneficial bacteria and inhibits harmful bacteria without any side effects in humans.

AUTHOR DISCLOSURE STATEMENT

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