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ABOUT COVER

Editorial Board Member of *World Journal of Gastroenterology*, King-Wah Chiu is a Distinguished Professor at the Cheng Shui University in Kaohsiung, Taiwan, Republic of China. Having received his Bachelor's degree from China Medical University College of Medicine in 1985, he rose to Chief in the Gastroenterology Division of the Kaohsiung Chang Gung Memorial Hospital Affiliated to Chang Gung University of College of Medicine in 2002. Dr. Chiu is a recognized expert in hepato-gastroenterology, having practiced for 30 years, and the pioneer of transplant hepatology in the field of liver transplantation, practicing in Kaohsiung Chang Gung Memorial Hospital since 1998. His ongoing research interests involve the application of molecular biology in transplant hepatology, particularly to study the effects of integrative basic medicine on and management of living-donor liver transplantation establishment. (L-Editor: Filipodia)

AIMS AND SCOPE

The primary aim of *World Journal of Gastroenterology* (*WJG*, *World J Gastroenterol*) is to provide scholars and readers from various fields of gastroenterology and hepatology with a platform to publish high-quality basic and clinical research articles and communicate their research findings online. *WJG* mainly publishes articles reporting research results and findings obtained in the field of gastroenterology and hepatology and covering a wide range of topics including gastroenterology, hepatology, gastrointestinal endoscopy, gastrointestinal surgery, gastrointestinal oncology, and pediatric gastroenterology.

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ORIGINAL ARTICLE

Randomized Controlled Trial

Effect of probiotic *Lactobacillus plantarum* Dad-13 powder consumption on the gut microbiota and intestinal health of overweight adults

Endang Sutriswati Rahayu, Mariyatun Mariyatun, Nancy Eka Putri Manurung, Pratama Nur Hasan, Phatthanaphong Therdtatha, Riko Mishima, Husnita Komalasari, Nurul Ain Mahfuzah, Fathyah Hanum Pamungkaningtyas, Wahyu Krisna Yoga, Dina Aulia Nurfiana, Stefanie Yolanda Liwan, Mohammad Juffrie, Agung Endro Nugroho, Tyas Utami

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Author contributions: Rahayu ES leaded the research project and together with Utami T designed the study; Juffrie M supervised the ethical approval and acted as the consultant for the clinical trial; Nugroho AE supervised the production of probiotic powder; Mariyatun M and Hasan PN supervised the on-site study; Putri Manurung NE, Komalasari H, Yoga WK, Therdtatha P, Mishima

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Abstract

BACKGROUND

Shifting on lifestyle, diet, and physical activity contributed on increasing number of obese people around the world. Multiple factors influence the development of obesity. Some research suggested that gut microbiota (GM) plays an important role in nutrient absorption and energy regulation of individuals, thus affecting their nutritional status. Report of Indonesia Basic Health Research showed that the prevalence of obesity in every province tended to increase. Although the root

R performed the work on-site and maintained the coordination with subject as well as conducted sampling; Nurfiana DA, Mahfuzah NA, Liwan SY performed analysis at the laboratory; Mariyatun M and Pamungkaningtyas FH analysed the laboratory data and took part in preparing the manuscript.

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cause of obesity is excessive calorie intake compared with expenditure, the differences in gut microbial ecology between healthy and obese humans may affect energy homeostasis. GM affect body weight, especially obesity. Probiotics that are consumed while alive and able to colonize in the intestine are expected to increase the population of good bacteria, especially *Bifidobacteria* and Lactobacilli, and suppress pathogens such as *Enterobacteriaceae* and *Staphylococcus*. The strain of *L. plantarum* Dad-13 has been demonstrated to survive and colonize in the gastrointestinal tract of healthy Indonesian adults who consume fermented milk containing *L. plantarum* Dad-13. The consumption of probiotic *L. plantarum* Dad-13 powder decreased *E. coli* and non-*E. coli* coliform bacteria in school-aged children in Indonesia. *L. plantarum* is a dominant bacterium in the average Indonesian's GM. For this reason, this bacterium is probably a more suitable probiotic for Indonesians.

AIM

To determine the effect of the consumption of indigenous probiotic *Lactobacillus plantarum* Dad-13 powder in overweight adults in Yogyakarta (Indonesia).

METHODS

Sixty overweight volunteers with a body mass index (BMI) equal to or greater than 25 consume indigenous probiotic powder *L. plantarum* Dad-13 (2×10^9 CFU/gram/sachet) for 90 d. The study was a randomized, double-blind, placebo-controlled study. The volunteers filled in a diary on a daily basis, which consisted of questions on study product intake (only during ingestion period), other food intake, number of bowel movements, fecal quality (consistency and color), any medications received, and any symptom of discomfort, such as diarrhea, constipation, vomiting, gassing, sensation of illness, *etc.* Fecal samples and the subjects' diaries were collected on the morning of day 10 + 1, which was marked as the end of the baseline period and the start of the ingestion period. During the ingestion period (from day 11 to day 101), several parameters to measure and analyze the results included body weight and height (once a month), the lipid profile, GM analysis using MiSeq, short-chain fatty acid (SCFA) analysis using gas chromatography, and the measurement of fecal pH using a pH meter.

RESULTS

The consumption of indigenous probiotic powder *L. plantarum* Dad-13 caused the average body weight and BMI of the probiotic group to decrease from 84.54 ± 17.64 kg to 83.14 ± 14.71 kg and 33.10 ± 6.15 kg/m^2 to 32.57 ± 5.01 kg/m^2, respectively. No significant reduction of body weight and BMI in the placebo group was observed. An analysis of the microbiota showed that the number of *Bacteroidetes*, specifically *Prevotella*, increased significantly, while that of *Firmicutes* significantly decreased. No significant change in lipid profile in both groups was found. Also, no significant change in SCFAs (*e.g.*, butyrate, propionate, acetic acid) and pH level was found after the consumption of the probiotic.

CONCLUSION

No significant differences in pH before and after ingestion were observed in both the probiotic and placebo groups as well as in the lipid profile of both cholesterol and triglyceride, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and the LDL/HDL ratio. In addition, no significant changes in the concentration of SCFAs (*e.g.*, acetic acid, propionate, and butyrate) were found after consumption. Interestingly, a significant decrease in body weight and BMI ($P < 0.05$) was determined in the treatment group. An analysis of GM shows that *L. plantarum* Dad-13 caused the *Firmicutes* population to decrease and the *Bacteroidetes* population (especially *Prevotella*) to increase.

Key Words: Obesity; Body mass index; Lipid profile; Gut microbiota; Short chain fatty acid; Probiotic *Lactobacillus plantarum* Dad-13

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Core Tip: Obesity and overweight are corelated with unhealthy lifestyle that affect the health of intestine and affect the ecosystem of gut microbiota (GM). Consumption of probiotics help to maintain the ecosystem of GM to stay balance and healthy. *L. plantarum* Dad-13 is potential probiotics for Indonesians to maintain health of the gastrointestinal ecosystem. This research was conducted to investigate and determine the effect of consumption of indigenous probiotic *L. plantarum* Dad-13 powder in overweight adults in Yogyakarta (Indonesia). The results show decreasing body mass index and weight on overweight subject and increasing of *Bacteroidetes* specifically *Prevotella*.

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INTRODUCTION

Changes in lifestyle, diet, and physical activity have resulted in an exponential increase in the number of obese people around the world. Multiple factors influence the development of this disease, and gut microbiota (GM) have been suggested to play an important role in the nutrient absorption and energy regulation of individuals, thus affecting their nutritional status. Different levels of GM have been observed between individuals with normal nutritional status and those who are obese.

The World Health Organization defines obesity as an accumulation of abnormal or excessive fat that can interfere with health[1]. Body mass index (BMI) is the easiest way to identify whether someone is obese or not, namely, by calculating body weight (kg) divided by height squared (m^2). A person is categorized as overweight if his/her BMI is greater than or equal to 25.0, while an obese person is someone with a BMI greater than or equal to 30.0[2]. A report from Indonesia Basic Health Research showed that the prevalence of obesity in every province tended to increase from 2007 to 2013 to 2018. In addition, it reported that adult women had higher obesity prevalence compared with adult men[3].

Although the root cause of obesity is excessive calorie intake compared with expenditure, the differences in gut microbial ecology between healthy and obese humans may affect energy homeostasis. In other words, individuals predisposed to obesity may have gut microbial communities that promote the more efficient extraction and/or storage of energy from a given diet compared with the communities in lean individuals[4].

GM affect body weight, especially obesity. A study showed that after the GM of fat mice were moved to thin mice, the latter gradually increased in weight and became fat[5]. A link is presumed to exist between GM and body weight. The biomarker of the GM of obesity is high-phylum *Firmicutes* bacteria[6]. Delzenne *et al*[7] found that the number of *Bifidobacteria* in obese individuals is lower than that in normal individuals. Other bacteria that were reported to consistently increase in obese individuals include *Enterobacteriaceae*, *Escherichia coli*, and *Staphylococcus* aureus[7].

Bifidobacteria are known as good bacteria as they produce short-chain fatty acids (SCFAs), such as acetate, propionate, butyrate, and lactate. This metabolite result has important effects on host metabolism. SCFAs can regulate (suppress or activate) the expression of specific genes associated with adiposity and inflammation that somewhat benefits the host. Given this description, we know that the population of *Bifidobacteria* is decreasing and that some pathogen bacteria, such as *Enterobacteriaceae* and *Staphylococcus*, are inclined to increase in obese individuals. Probiotics that are consumed while alive and able to colonize in the intestine are expected to increase the population of good bacteria, especially *Bifidobacteria* and Lactobacilli, and suppress pathogens such as *Enterobacteriaceae* and *Staphylococcus*. Kobyliak *et al*[8] proved that the consumption of probiotics, especially Lactobacilli, for nine weeks could suppress body weight gain and reduce adipose tissue in obese mice.

The probiotic research team of Universitas Gadjah Mada (UGM) came up with an indigenous probiotic from Indonesia that was obtained from various sources, but it has not been thoroughly studied. A study by Rahayu *et al*[9] revealed that the Indonesian indigenous probiotic strains have been molecularly confirmed and could inhibit the growth of pathogenic bacteria. In addition, the indigenous probiotic strains were shown to be resistant to pH 2 and bile salt of 3% concentration. Some probiotic cultures owned by the UGM research team are *Lactobacillus plantarum* Dad-13 (obtained from dadih, fermented buffalo milk), *L. plantarum* Mut-7 and Mut-13 (taken from gatot, fermented cassava), *L. plantarum* T3 (obtained from growol, fermented raw cassava), and *Lactobacillus paracasei* SNP-2 (taken from healthy baby feces).

The strain of *L. plantarum* Dad-13 has been demonstrated to survive and colonize in the gastrointestinal tract of healthy Indonesian adults who consume fermented milk containing *L. plantarum* Dad-13[10]. A safety assessment of *L. plantarum* Dad-13 on Sprague Dawley rats reported no adverse effects in general health, organ weight, leukocyte profiles, GOT activity, MDA concentration, and intestinal morphology after the consumption of the probiotic[11]. The indigenous probiotic *L. plantarum* Dad-13 also did not cause translocation in the organs and blood of the rats[11].

Apart from significantly increasing the population of *L. plantarum* and *Lactobacillus*, the consumption of probiotic *L. plantarum* Dad-13 powder decreased *E. coli* and non–*E. coli* coliform bacteria in school-aged children in Indonesia[12]. *L. plantarum* is a dominant bacterium in the average Indonesian's GM[13]. For this reason, this bacterium is probably a more suitable probiotic for Indonesians. Thus, this study aimed to determine the effect of the consumption of indigenous probiotic *L. plantarum* Dad-13 powder in overweight adults in Yogyakarta (Indonesia).

MATERIALS AND METHODS

Study subjects

This study involved 60 overweight volunteers, consisting of 24 males and 36 females. The age of the subjects ranged between 35 and 56 years old. The inclusion criteria of the subjects covered having a BMI equal to or greater than 25, no history of gastrointestinal disorder (such as constipation, diarrhea, abdominal pain, and irritable bowel syndrome), and no allergies to certain foods. The subjects had not taken antibiotics/antimycotics or any specific drugs and did not consume antidiarrheal or laxative medicine for 100 d during the study.

Ethical approval

This study was conducted in accordance with Good Clinical Practice (GCP) as defined by the International Conference of Harmonization (ICH) and in accordance with the Indonesian National Agency for Drug and Food Control Guidance. Approval by the Ethics Committee of the Faculty of Medicine, Public Health, and Nursing of UGM, Yogyakarta, was received on January 2, 2018, as stated in the committee's letter, with reference number KE/FK/0002/2018.

Study products

The product of this study was 1 g of skimmed milk powder containing the probiotic *L. plantarum* Dad-13 of 2×10^9 CFU in sachet packing. The product was prepared using a halal medium by the Center for Food and Nutrition Studies, UGM. One gram of skimmed milk obtained from a local supermarket was used in the placebo group. The study products were stored in a refrigerator (< 4 °C) before being consumed. *L. plantarum* Dad-13, the indigenous probiotic strain, was deposited in ampoules at the Food and Nutrition Culture Collection (FNCC), Center for Food and Nutrition Studies, UGM. Labelling and product blinding were prepared by the Unit Production of Probiotics and Starter Cultures, Center for Food and Nutrition Studies, UGM.

Materials

DNA fecal extraction was performed using phenol–chloroform extraction. The SsoFast™ Evagreen®Supermix Kit from PT Sciencewerke (Indonesia) was used as a mixture of DNA extracts in super-mix real-time PCR. The primers for qPCR analysis consisted of *Bifidobacteria*[14], the *L. plantarum* subgroup[15], *Clostridium coccoides*[15], and *Enterobacteriaceae*[14]. The main instrument used for GM analysis was real-time PCR. Stool sampling equipment included stool tubes, sterile tissue paper, gloves, masks, ice gel, and cool boxes. DNA extraction equipment included a centrifuge, a vortex,

analytical scales, and other kinds of glassware. The equipment for the probiotic powder included freeze dryers and vacuum sealing.

Study design

The study was a randomized, placebo-controlled study; 60 volunteers were divided into an intervention group (probiotic) and a control group (placebo). All the subjects and the researcher were blinded to the treatment administrated (double-blind study). This study used simple randomization, performed in such a way that leaves no significant difference between the study groups (BMI, age, or sex). The placebo product used was skimmed milk without probiotics. The study consisted of 10 d without the consumption of the study product (baseline period) followed by 90 d of ingestion, as shown in Figure 1. During the baseline period, the volunteers consumed their normal dietaries with the exception of probiotic products. The baseline period was a "washout" period to eliminate the effect of previously used probiotics. The volunteers filled in a diary on a daily basis, which consisted of questions on study product intake (only during the ingestion period), other food intake, number of bowel movements, fecal quality (consistency and color), any medications received, and any symptom of discomfort, such as diarrhea, constipation, vomiting, gassing, sensation of illness, *etc*. Fecal samples and the subjects' diaries were collected on the morning of day 10 ± 1, which was marked as the end of the baseline period and the start of the ingestion period. During the ingestion period (from day 11 to day 101), the volunteers consumed one sachet of the study product per day after having lunch for 90 consecutive days. The volunteers were not allowed to consume any other probiotic products. They were requested to fill in a new diary on a daily basis. Upon the completion of the ingestion period, on the morning of day 100 ± 1, fecal samples and the subjects' diaries were collected.

Fecal collection

A fecal sample was collected into a sterile tube with a kind of scoop built into the inside of the lid by the subjects at home, and the sample was immediately transported to the laboratory in a cold storage container (< 10 °C). Two tubes were used to collect the samples, containing buffer/stabilizer RNA later and glass beads, one tube for GM analysis and the other for SCFA analysis. The materials and instructions for fecal sample collection were provided to the subjects prior to the fecal collection schedule. The subjects were instructed to defecate on the trail paper (smooth side up) and were prevented from wetting the fecal paper with urine or water. Then they were required to immediately take a sample by scraping the feces with the scooper and capping the tube tightly.

Analysis

Several parameters to measure and analyze the results included: (1) The measurement of body weight and height once a month; (2) The lipid profile; (3) GM analysis; (4) SCFA analysis using gas chromatography; and (5) The measurement of fecal pH using a pH meter.

GM analysis using next-genome sequencing - MiSeq

A high-throughput analysis of 16 rRNA gene sequences was carried out according to the previous method. Areas V3-V4 of the sequences from the bacteria were amplified with the fecal DNA genome (approximately 1 ng) using TaKaRa Ex TaqTM HS (Takara Bio, Japan) and universal primer Bakt_341F (5'-CGCTCTTCCGATCTCTG CCTACGGGGGGGCWGCAG-355)GGCTATICCCACCATTCCCCATTCCACCA CCACCACCACCACCACCACCACCA UTAA. The amplification results were used as a template for the second PCR using barcode-tag primers. The second PCR results were purified using the FastGene Gel/PCR Extraction Kit (NIPPON Genetics, Japan) according to company protocol. The purified products were quantified using the PicoGreen® dsDNA Assay Kit (Life Technologies, United States) based on company protocol. All the PCR samples were of the same total amount (approximately 200 ng total), and they were purified using electrophoresis in 2% (w/v) agarose gel (classic-type Agarose-LE: Nacalai Tesque, Japan), followed by extraction from the gel by the FastGene Gel/PCR Extraction Kit. The purified mixture was applied to the final-pair sequence of Illumina MiSeq v3 (Illumina, United States).

Statistical analysis

The data was displayed as mean ± standard deviation unless stated otherwise. IBM Statistic SPSS 20.0 with a 95% confidence interval (a = 5%) was used to perform

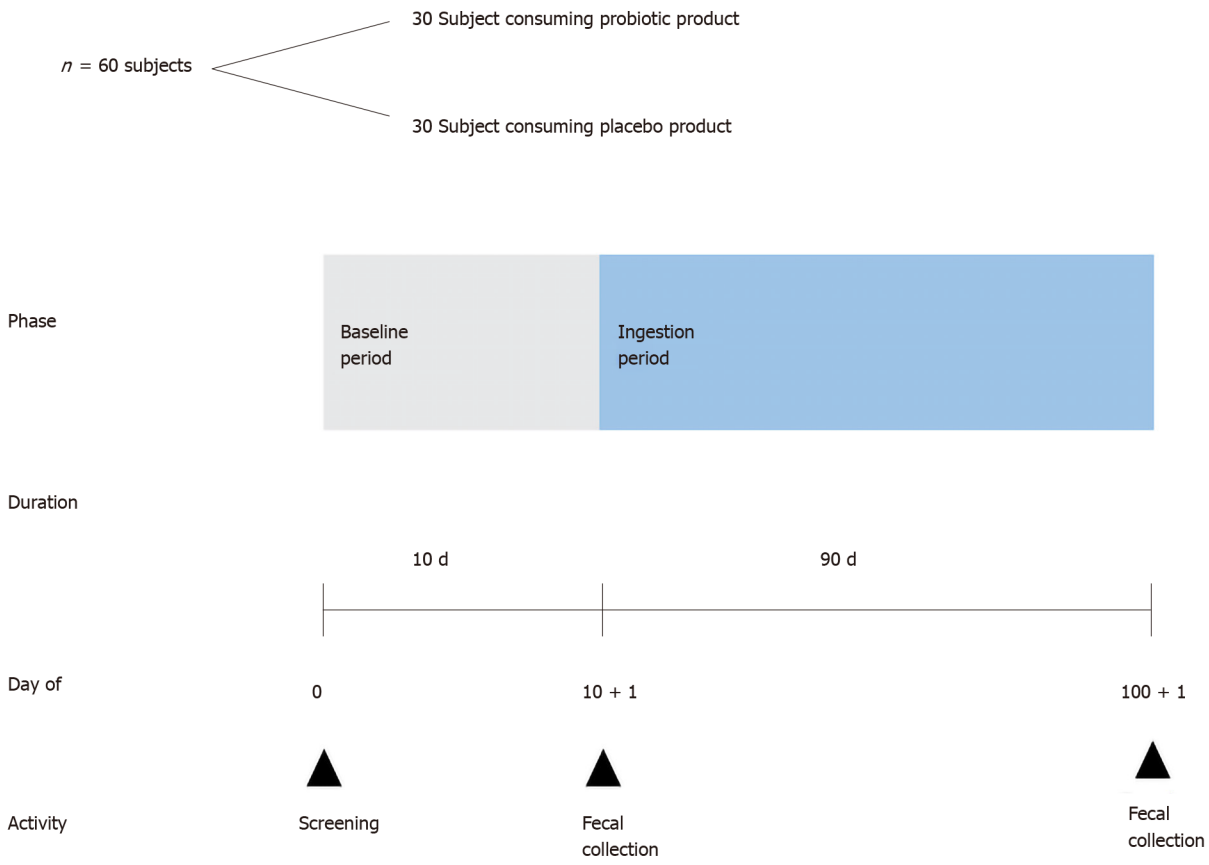


Figure 1 Study design.

statistical analysis. A chi-square test or independent *t*-test or Wilcoxon test was carried out to evaluate the significant differences of the observed parameters between the probiotic-treated group and the placebo group depending on the normality and equality of variance of the data. In addition, a paired *t*-test was used to analyze the observed parameters before and after the consumption of the indigenous probiotic powder or placebo powder.

RESULTS

Demographic data of study subjects

Sixty overweight subjects who participated in the research signed informed consent forms. The subjects were divided into two groups, namely, the probiotic-treated group and the placebo group. Neither the researcher nor the participants knew which subject entered the probiotic group or the placebo group. The research began on January 5, 2019. Fifteen days were allotted for the prescreening period, and the baseline period started on January 21-30, 2019, the intervention period started on January 31 and ended on April 30, 2019. The research ended when all the subjects finished giving their fecal samples to the researcher. The demographic data of the subjects showed no significant differences in age, height, weight, and BMI, and the number of female participants was higher than that of male participants (Table 1).

Body weight, height, and BMI

The body weight, height, and BMI of the subjects were measured every 10 d. Table 2 presents a significant decrease ($P < 0.05$) in the body weight and BMI of the subjects after 90 d of probiotic ingestion. Table 3 further shows the different effects of consuming probiotics between the female and male subjects.

Some studies also reported that probiotics could reduce body weight. Kadooka *et al*[16] found that the probiotic *Lactobacillus gasseri* SBT2055 (LG2055) caused abdominal adiposity, body weight, and other measures to decrease, suggesting its

Table 1 Demographic data of study subjects

	Probiotic-treated group (*n* = 30)	Placebo group (*n* = 30)	*P* value
Age (yr)	44.07 ± 6.23	44.67 ± 5.66	0.42[1]
Height (cm)	159.66 ± 8.27	157.92 ± 9.58	0.40[1]
Weight (kg)	83.45 ± 14.61	79.58 ± 11.79	0.21[1]
BMI (kg/m^2)	32.69 ± 5.07	31.88 ± 3.77	0.18[1]
Women, *n* (%)	18 (60)	18 (60)	0.00[2]
Men, *n* (%)	12 (40)	12 (40)	0.00[2]

[1]Independent sample *t*-test.
[2]Chi-square. BMI: Body mass index.

Table 2 Changes of body weight, height, and body mass index

	Group	Baseline period	Ingestion period	*P* value
Weight	Probiotic-treated	84.54 ± 17.62	83.14 ± 14.71	0.04[2a]
	Placebo	79.37 ± 11.76	78.80 ± 11.77	0.12[1]
Height	Probiotic-treated	159.66 ± 8.27	159.66 ± 8.27	1.00[2]
	Placebo	157.92 ± 9.58	157.92 ± 9.58	1.00[2]
BMI	Probiotic-treated	33.10 ± 6.15	32.57 ± 5.01	0.04[2a]
	Placebo	31.80 ± 3.71	31.56 ± 3.67	0.18[1]

[1]Independent sample *t*-test.
[2]Wilcoxon signed-rank test. A significantly different ([a]$P < 0.05$). BMI: Body mass index.

beneficial influence on metabolic disorders. According to Higashikawa *et al*[17], the heat-killed *Pediococcus pentosaceus* LP28 displayed an anti-obesity effect that reduced BMI, body fat, and waist circumference. Another study revealed that the mean of weight loss in female subjects consuming *Lactobacillus rhamnosus* CGMCC1.3724 (LPR) supplementation was significantly higher than that in women who belonged to the placebo group after the first 12 wk. The body weight and fat mass of the male subjects were not affected by the treatment[18].

Lipid profile

The lipid profile showed that in both groups, there was no significant difference in each parameter measured after consuming the study product. The results of the lipid profile are shown in Table 4.

Fecal characteristics and defecation frequency

Fecal characteristics indicate intestinal conditions in humans. These characteristics include volume, type, color, odor, and pH. The fecal volume of 1 is equal to the volume of a chicken egg. The color is indicated in four scales (1: yellow; 2: brownish yellow; 3: brown; 4: green). The Bristol stool chart was used to identify the type of feces. The aroma of the feces was expressed using a three-point scale (1: normal; 2: strong; 3: very strong). Table 5 shows the fecal characteristics and defecation frequency.

Table 5 indicates that in both the probiotic-treated group and the placebo group, the volume, type, color, odor, and pH of the feces during the baseline and ingestion periods were not significantly changed. The defecation frequency was expressed as the total number or frequency of defecation in 10 d. Overall, the fecal samples from both groups had the following characteristics: Banana-like shape, brownish yellow color, normal odor, and pH of 5.58-5.76.

SCFA

An analysis of SCFAs was performed using gas chromatography. The SCFAs analyzed

Table 3 Different changes of body weight, height, and body mass index in female and male subjects

Gender		Group	Baseline period	Ingestion period	*P* value
Women	Weight	Probiotic-treated	77.91 ± 14.16	77.08 ± 13.68	0.01[1a]
		Placebo	73.20 ± 9.93	72.69 ± 9.93	0.33[1]
	Height	Probiotic-treated	153.82 ± 4.05	153.82 ± 4.05	1.00[2]
		Placebo	151.42 ± 5.92	151.42 ± 5.92	1.00[2]
	BMI	Probiotic-treated	32.90 ± 5.73	32.58 ± 5.58	0.02[1a]
		Placebo	31.96 ± 4.25	31.72 ± 4.14	0.31[1]
Men	Weight	Probiotic-treated	94.48 ± 18.11	92.22 ± 11.45	0.38[1]
		Placebo	88.63 ± 7.51	87.97 ± 7.77	0.16[1]
	Height	Probiotic-treated	168.42 ± 3.92	168.42 ± 3.92	1.00[2]
		Placebo	167.67 ± 3.87	167.67 ± 3.87	1.00[2]
	BMI	Probiotic-treated	33.39 ± 6.98	32.54 ± 4.25	0.37[1]
		Placebo	31.56 ± 2.87	31.32 ± 2.96	0.15[1]

[1]Independent sample *t*-test.
[2]Wilcoxon signed-rank test. A significantly different ([a]$P < 0.05$). BMI: Body mass index.

Table 4 Lipid profile

Lipid profile	Group	Baseline period	Ingestion period	*P* value
Cholesterol (mg/dL)	Probiotic-treated	194.93 ± 37.64	192.20 ± 36.55	0.46[2]
	Placebo	193.70 ± 29.47	192.37 ± 29.75	0.41[2]
Triglyceride (mg/dL)	Probiotic-treated	151.50 ± 63.92	166.83 ± 75.02	0.16[2]
	Placebo	191.40 ± 133.60	187.73 ± 111.58	0.54[2]
HDL (mg/dL)	Probiotic-treated	40.33 ± 9.77	40.00 ± 9.28	0.69[1]
	Placebo	39.93 ± 7.29	40.60 ± 8.18	0.39[1]
LDL (mg/dL)	Probiotic-treated	141.43 ± 32.17	136.97 ± 33.12	0.18[2]
	Placebo	134.50 ± 24.84	133.50 ± 27.06	0.71[1]
Ratio of LDL/HDL	Probiotic-treated	3.63 ± 0.95	3.55 ± 0.87	0.38[1]
	Placebo	3.44 ± 0.70	3.39 ± 0.84	0.61[1]

[1]Paired *t*-test.
[2]Wilcoxon signed-rank test. A significantly different ($P < 0.05$). LDL: Low-density lipoprotein; HDL: High-density lipoprotein.

in this study included acetic acid, propionate, and butyrate. Table 6 shows the SCFA concentration of the probiotic-treated and placebo groups. It also shows that the SCFAs did not significantly change ($P > 0.05$) in both groups after the ingestion period. The SCFAs function *via* diverse host molecular mechanisms to regulate host energy intake, energy expenditure, and storage[19]. The production of SCFAs by bacteria that ferment carbohydrates contribute 10% of the total energy to be absorbed in the colon, and the rest would be lost through the feces[20]. One study proved that the administration of *L. salivarius* Ls-33 to obese adolescent subjects did not have a significant effect[21]. Likewise, the administration of *L. plantarum* Dad-13 in this study did not affect the SCFA concentration of the overweight subjects. The pH value in the treatment group was 5.72 ± 0.31 before ingestion and 5.76 ± 0.28 after ingestion. The pH value in the placebo group was 5.58 ± 0.40 before ingestion and 5.75 ± 0.34 after ingestion. This insignificant change of fecal pH was attributed to insignificant SCFA concentration, so the intestines' condition did not change.

Table 5 Fecal characteristic (volume, type, color, odor, pH) and defecation frequency

	Group	Baseline period	Ingestion period	*P* value
Volume	Probiotic-treated	2.20 ± 0.79	2.23 ± 0.96	0.86[1]
	Placebo	2.37 ± 0.95	2.29 ± 0.90	0.23[2]
Type	Probiotic-treated	3.59 ± 0.93	3.22 ± 1.17	0.08[1]
	Placebo	4.02 ± 1.14	3.89 ± 0.93	0.34[2]
Color	Probiotic-treated	1.86 ± 0.57	1.73 ± 0.55	0.20[1]
	Placebo	2.00 ± 0.59	1.95 ± 0.54	0.70[1]
Odor	Probiotic-treated	1.11 ± 0.37	1.10 ± 0.28	0.93[2]
	Placebo	1.26 ± 0.35	1.32 ± 0.41	0.57[2]
pH	Probiotic-treated	5.72 ± 0.31	5.76 ± 0.28	0.51[3]
	Placebo	5.58 ± 0.40	5.75 ± 0.34	0.07[3]
Defecation frequency[4]	Probiotic-treated	12.93 ± 3.61	13.40 ± 4.52	0.58[2]
	Placebo	14.67 ± 4.93	15.70 ± 7.57	0.51[2]

[1]Independent sample *t*-test.
[2]Wilcoxon signed-rank test.
[3]Paired *t*-test.
[4]Every 10 d.

Table 6 Short-chain fatty acid (acetic acid, propionic acid, and butyrate acid) of feces

	Group	Baseline period	Ingestion period	*P* value[a]
Acetic acid (mmol/kg)	Probiotic-treated	63.19 ± 34.97	64.76 ± 17.61	0.89
	Placebo	67.09 ± 19.56	63.76 ± 13.05	0.65
Propionic acid (mmol/kg)	Probiotic-treated	22.02 ± 14.17	20.65 ± 9.76	0.67
	Placebo	21.98 ± 10.44	17.16 ± 2.04	0.17
Butyrate acid (mmol/kg)	Probiotic-treated	14.78 ± 6.68	14.97 ± 7.24	0.95
	Placebo	19.45 ± 8.60	15.59 ± 9.40[a]	0.33

[a]Paired *t*-test with significance level of 5%.

Diet profile

Diet or food intake is associated with obesity. This study also recorded the daily diet of the subjects. The dietary records were analyzed using the NutriSurvey 2007 software. The household size based on the standard issued by the Republic of Indonesia Ministry of Health in 2014 was used to measure the amount of food intake. Table 7 below summarizes the diet profile of the subjects.

Based on the analysis of the dietary patterns of the subjects, the standard deviation was high, which indicates that the nutrient intake of the subjects was very diverse. Compared to the intake during the baseline period, both the probiotic-treated group and the placebo group consumed less energy, protein, lipid, carbohydrate, and PUFA sources in the last month of the ingestion period. In addition, the average daily energy intake of the subjects was around 1518.17-1642.88 kcal/d, less than that of a normal adult (around 2000 kcal/d)[22]. The consumption of dietary fiber sources experienced a gradual drop from day 41 to the end of the study period. Meanwhile, no significant differences were observed in the intake of cholesterol between the baseline period and the end of the ingestion period in both the probiotic-treated group and the placebo group.

Table 7 Diet profile of subjects

	Group	Baseline period	Ingestion day 11-20	Ingestion day 21-30	Ingestion day 31-40	Ingestion day 41-60	Ingestion day 61-80	Ingestion day 81-100
Energy (kcal)	Probiotic-treated	1518.17 ± 484.46	1322.04 ± 431.90	1338.23 ± 376.17	1274.13 ± 390.37	1124.89 ± 378.82[a]	1060.65 ± 286.86[a]	1103.54 ± 311.74[a]
	Placebo	1642.88 ± 599.33	1660.02 ± 611.62	1562.79 ± 1144.53	1396.65 ± 476.01[a]	1085.16 ± 346.08[a]	1047.90 ± 313.37[a]	1105.73 ± 313.58[a]
Water (g)	Probiotic-treated	744.06 ± 526.92	633.17 ± 397.27	593.60 ± 377.72	691.70 ± 470.44	720.66 ± 551.62	656.24 ± 419.94	712.81 ± 438.11
	Placebo	1122.19 ± 615.82	1016.66 ± 665.44	1041.80 ± 568.23	1100.16 ± 601.35	688.97 ± 533.39[a]	813.86 ± 565.46	841.27 ± 595.64
Protein (g)	Probiotic-treated	52.72 ± 19.49	44.69 ± 16.73	46.23 ± 14.61	46.81 ± 12.61	38.49 ± 14.10[a]	38.30 ± 10.23[a]	39.62 ± 11.30[a]
	Placebo	53.92 ± 24.02	55.03 ± 25.52	47.12 ± 33.52	47.02 ± 18.70	38.38 ± 12.95[a]	35.67 ± 10.98[a]	39.09 ± 9.72[a]
Lipid (g)	Probiotic-treated	61.41 ± 30.24	47.15 ± 26.32	47.60 ± 21.41	49.86 ± 19.25	40.78±15.24[a]	39.63 ± 12.02[a]	40.78 ± 12.99[a]
	Placebo	64.15 ± 34.36	63.17 ± 38.78	54.22 ± 36.98	52.15 ± 25.09[a]	36.99 ± 13.85[a]	36.88 ± 12.74[a]	37.68 ± 9.70[a]
Carbo-hydrate (g)	Probiotic-treated	190.85 ± 68.00	179.93 ± 57.44	180.95 ± 65.23	160.50 ± 59.23	150.80 ± 56.88	137.97 ± 43.53[a]	144.63 ± 46.33[a]
	Placebo	216.39 ± 88.21	221.71 ± 83.07	224.10 ± 190.74	186.27 ± 71.98	149.82 ± 48.91[a]	144.70 ± 49.45[a]	153.74 ± 60.37[a]
Fiber (g)	Probiotic-treated	10.96 ± 5.88	8.66 ± 3.04	8.71 ± 2.91	8.49 ± 2.63	7.08 ± 2.16[a]	6.24 ± 2.01[a]	6.61 ± 2.48[a]
	Placebo	12.17 ± 7.15	13.34 ± 9.00	11.90 ± 8.61	10.20 ± 4.67	6.96 ± 2.99[a]	7.17 ± 2.30[a]	7.45 ± 2.30[a]
PUFA (g)	Probiotic-treated	16.87 ± 13.02	12.80 ± 7.73	12.17 ± 5.87	13.03 ± 7.64	12.17 ± 7.36	10.91 ± 4.50	10.86 ± 4.31
	Placebo	18.20 ± 13.73	18.49 ± 15.71	15.70 ± 13.47	16.29 ± 13.52	10.50 ± 5.94[a]	10.03 ± 4.70[a]	9.73 ± 3.1[a]
Choles-terol (mg)	Probiotic-treated	191.39 ± 163.58	163.28 ± 83.55	168.44 ± 92.31	182.29 ± 68.95	160.32 ± 84.56	172.70 ± 77.78	179.23 ± 57.13
	Placebo	170.42 ± 154.32	168.91 ± 169.50	142.45 ± 159.96	164.19 ± 124.86	173.22 ± 86.80	165.31 ± 85.03	182.64 ± 81.91

[a]$P < 0.05$ based on Independent sample *t*-test compared to the nutrient intake in baseline. PUFA: Polyunsaturated fatty acid.

Population of GM in overweight subjects

Based on the results of the 16 RNA sequences using MiSeq performed in both the probiotic-treated and placebo groups, the bacterial group was dominated by the phyla *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, *Fusobacteria*, and *Verrucomicrobia* (Figure 2). A small portion of the phyla *Cyanobacteria*, *Lentisphaerae*, *Elusimicrobia*, and *Synergistetes* appeared in both the treatment and control groups (Table 8).

The phylum distribution composition of each subject from both the treatment and control groups can be seen in Figure 3. Three genera - *Firmicutes*, *Bacteroidetes*, and *Actinobacteria* - are the most dominant genera appearing on almost all the subjects, while some phyla, such as *Proteobacteria* and *Fusobacteria*, appear dominantly in only a few subjects.

Concerning the two dominant phyla, the number of *Bacteroidetes* significantly increased ($P < 0.05$) in both the treatment and placebo groups after the ingestion period (Table 9), and the number of *Firmicutes* significantly decreased ($P < 0.05$) in the treatment group (Figure 4). Meanwhile, the *Fusobacteria* population was only found in a few subjects. The *Verrucomicrobia* population significantly decreased in both the treatment and placebo groups after the ingestion period. *Verrucomicrobia* was often associated with gastrointestinal health and glucose homeostasis. No significant changes ($P > 0.05$) were found in the phyla of *Cyanobacteria*, *Elusimicrobia*, *Lentisphaerae*, and *Synergistetes* in the treatment and placebo groups before and after the ingestion period. The changes in phylum of bacterial composition in both the probiotic-treated and placebo groups before and after the ingestion period are presented in Figure 4.

At the genus level (as shown in Table 8), some microbiota showed some changes in

Table 8 Gut microbiota composition based on genus

Phylum	Genus	Probiotic-treated			Placebo		
		Baseline, mean (%) ± SD	Ingestion, mean (%) ± SD	*P* value	Baseline, mean (%) ± SD	Ingestion, mean (%) ± SD	*P* value
Firmicutes	*Faecalibacterium*	11.43 ± 5.03	10.94 ± 4.21	0.614	15.30 ± 7.07	11.82 ± 5.50	0.01[a]
	Coprococcus	7.53 ± 3.55	6.23 ± 1.85	0.037[a]	7.34 ± 3.71	5.82 ± 2.67	0.116
	Other	7.63 ± 5.05	8.22 ± 4.15	0.491	6.03 ± 4.13	8.40 ± 4.56	0.012[a]
	Ruminococcus	4.49 ± 4.06	3.69 ± 2.90	0.271	3.80 ± 3.84	3.70 ± 4.79	0.572
	Roseburia	1.36 ± 1.31	1.54 ± 1.10	0.072[b]	1.49 ± 1.55	2.04 ± 1.28	0.037[a]
	Clostridium	0.16 ± 0.22	0.16 ± 0.27	0.829	0.25 ± 0.53	0.35 ± 0.49	0.202
	Paenibacillus	0.01 ± 0.01	0.00 ± 0.01	0.1	0.03 ± 0.06	0.00 ± 0.01	0.002[a]
Bacteroidetes	*Prevotella*	14.56 ± 11.57	19.25 ± 13.03	0.066[b]	14.15 ± 13.69	14.28 ± 14.13	0.75
	Bacteroides	3.78 ± 5.43	5.57 ± 8.21	0.019[a]	5.59 ± 9.97	10.30 ± 13.83	0.04[a]
Actino-bacteria	*Bifidobacterium*	3.38 ± 4.96	2.74 ± 4.01	0.6	3.07 ± 3.95	2.71 ± 2.91	0.957
	Collinsella	2.02 ± 1.40	1.52 ± 0.94	0.069[b]	2.00 ± 1.80	1.51 ± 0.98	0.271
	Brevibacterium	0.01 ± 0.01	0.00 ± 0.01	0.307	0.03 ± 0.07	0.01 ± 0.02	0.082[b]
Proteo-bacteria	*Succinivibrio*	2.19 ± 4.51	1.44 ± 3.37	0.548	2.06 ± 5.29	2.51 ± 6.89	0.534
	Phyllobacterium	0.02 ± 0.04	0.02 ± 0.02	0.037[a]	0.01 ± 0.03	0.01 ± 0.03	0.167
	Sphingomonas	0.02 ± 0.03	0.02 ± 0.02	0.75	0.01 ± 0.03	0.01 ± 0.03	0.833
Verrucomicrobia	*Akkermansia*	0.13 ± 0.56	0.04 ± 0.18	0.028[a]	0.35 ± 1.64	0.02 ± 0.07	0.024[a]

[a]Paired *t*-test with significance level of 5% ($P < 0.05$).
[b]Significance level 10% ($P < 0.1$).

composition. However, not all genera of the whole phylum experienced some changes. In the phylum *Firmicutes*, *Faecalibacterium* was quite significant in the placebo group compared with the treatment group ($P < 0.05$). One species of *Faecalibacterium* that is quite abundant in the human digestive tract is *Faecalibacterium prausnitzii*. *Faecalibacterium* is a fairly dominant digestive microbiota, as indicated by the fact that 5%-15% of total bacteria are *F. prausnitzii* species[23]. *F. prausnitzii* is also considered one of the health indicators of gastrointestinal health. Healthy subjects normally showed an abundance of *F. prausnitzii* compared with subjects with Crohn's disease[24]. The genus *Coprococcus* showed a significant decrease in the treatment group ($P < 0.05$). *Coprococcus* is characterized as comprising anaerobic microbes able to produce butyrate acid. Countless studies have associated *Coprococcus* with the health conditions of the human digestive tract. One study showed that its healthy subjects had a high abundance of *Coprococcus* compared with subjects with colorectal cancer[25]. Other studies showed that the number of *Bacteroides* and *Coprococcus* in subjects with colorectal pre-cancerous conditions was much lower than that in healthy subjects[26]. Body conditions in humans, such as obesity or being overweight, can also affect the conditions of microbiota. The populations of *Blautia*, *Coprococcus*, and *Enterobacteriaceae* were quite high in overweight children in Mexico compared with those with normal conditions[27].

Table 8 shows that *Roseburia* significantly increased in both the treatment ($P < -0.01$) and placebo groups ($P < 0.05$). *Roseburia* is a microbiota of the genus *Firmicutes* that has the characteristics of gram-positive, obligate anaerobes and can produce the SCFA butyrate. In the human digestive tract, one of the species of *Roseburia* - namely, *Roseburia hominis* - can regulate immunity[28]. An increase in the population of *Roseburia* can also be attributed to the type of food consumed. The consumption of resistant starch is said to increase *Eubacterium rectale* and *Roseburia*[29]. The *Roseburia* population in humans is quite varied. A study of groups of obese and overweight children showed a fairly high *Roseburia* population compared with the normal group[27].

The *Paenibacillus* genus, as shown in Table 8, decreased in both groups, but only the placebo control group experienced a significant decrease. The genus *Clostridium* did not significantly change in both groups, but the placebo group experienced a slight

Table 9 Composition of gut microbiota (based on the most abundant in phylum population) in the probiotic-treated and placebo groups before and after ingestion period

No.	Phylum	Group	Baseline period	Ingestion period	*P* value[a]
1	*Firmicutes*	Probiotic-treated	69.90 ± 15.95	64.13 ± 15.22	0.037[a]
		Placebo	70.66 ± 14.41	65.30 ± 14.52	0.153
2	*Bacteroidetes*	Probiotic-treated	20.63 ± 11.49	28.27 ± 14.26	0.008[a]
		Placebo	21.30 ± 12.72	26.71 ± 13.93	0.045[a]
3	*Actinobacteria*	Probiotic-treated	6.11 ± 5.37	5.07 ± 5.48	0.237
		Placebo	5.55 ± 4.90	4.63 ± 3.61	0.453
4	*Proteobacteria*	Probiotic-treated	2.87 ± 6.39	2.04 ± 6.05	0.491
		Placebo	1.84 ± 4.32	1.69 ± 3.77	0.572
5	*Fusobacteria*	Probiotic-treated	0.23 ± 0.57	0.34 ± 0.86	0.701
		Placebo	0.20 ± 0.94	1.58 ± 6.57	0.044[a]
6	*Verrucomicrobia*	Probiotic-treated	0.13 ± 0.57	0.04 ± 1.66	0.028[a]
		Placebo	0.35 ± 0.19	0.02 ± 0.07	0.024[a]
7	*Cyanobacteria*	Probiotic-treated	0.09 ± 0.29	0.07 ± 0.21	0.537
		Placebo	0.06 ± 0.24	0.04 ± 0.10	0.427
8	*Lentisphaerae*	Probiotic-treated	0.02 ± 0.05	0.01 ± 0.08	0.681
		Placebo	0.03 ± 0.02	0.03 ± 0.11	0.334
9	*Elusimicrobia*	Probiotic-treated	0.00 ± 0.01	0.00 ± 0.03	0.655
		Placebo	0.01 ± 0.02	0.00 ± 0.00	0.317
10	*Synergistetes*	Probiotic-treated	0.00 ± 0.00	0.00 ± 0.00	0.655
		Placebo	0.00 ± 0.00	0.00 ± 0.01	0.273

[a]Paired *t*-test with significance level of 5%.

increase in abundance. The genus *Ruminococcus* decreased significantly in the probiotic-treated group.

The *Coprococcus* genus experienced a significant decrease in the treatment group after consumption, inversely proportional to the genus *Roseburia*, which experienced a significant increase in the treatment group after consumption. *Roseburia* also experienced a significant increase in the control group after the ingestion period. The genera *Faecalibacterium* and *Paenibacillus* experienced a decrease in the placebo group after the ingestion period. No significant differences were observed in the genera *Clostridium* and *Ruminococcus* in both the treatment and placebo groups after the ingestion period.

The second dominant phylum (Table 9) is *Bacteroidetes*, which increased in the treatment group. The genus *Bacteroides* significantly increased in both the probiotic-treated and placebo groups ($P < 0.05$). However, the genus *Prevotella* significantly increased ($P < 0.1$) in the probiotic-treated group. Previous papers mentioned that *Prevotella* is the dominant genus in the phylum *Bacteroidetes* for healthy school-aged children (Murugesan *et al*[27] 2015) and adult Indonesians (Rahayu *et al*[13] 2019). In this study, the population of *Prevotella* is much higher than that of *Bacteroides*. This finding supports previous reports stating that Indonesians have the *Prevotella* enterotype.

The relative abundance of the phylum *Actinobacteria* - namely, the genera Brevibacterium, *Bifidobacteria*, and *Collinsella* - is shown in Table 8. *Brevibacterium* in the treatment group significantly decreased after the ingestion period and showed a significant decrease in the genus *Collinsella* in the treatment group ($P < 0.1$) compared with the placebo group. *Collinsella* is a microbiota of the phylum *Actinobacteria*. These microbiota were often said to be pathobionts, which have the potential to influence the nature of the pathogen to its host[30]. Subjects with obesity and having type 2 diabetes are said to have a high abundance of *Collinsella* compared with healthy people[31].

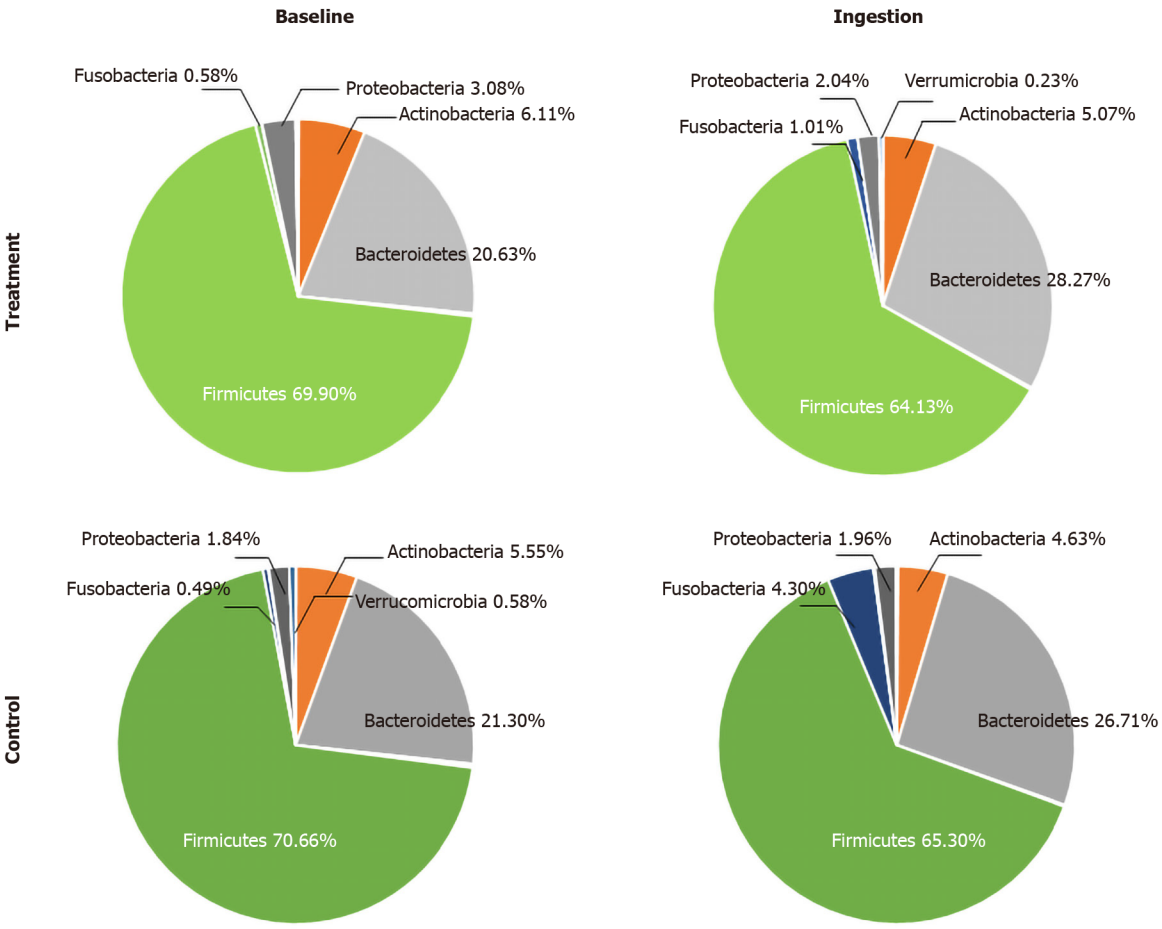


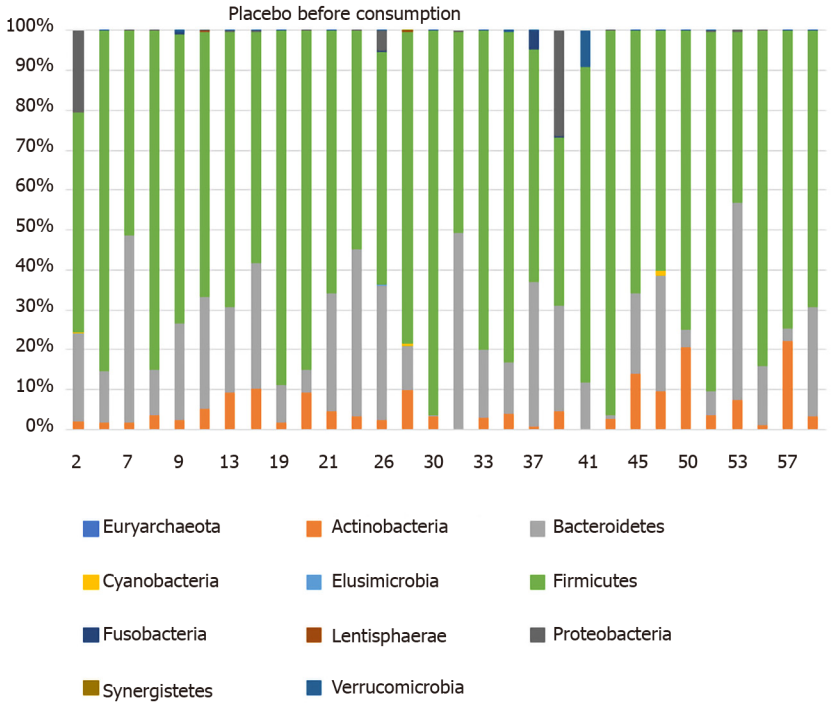
Figure 2 The composition of gut microbiota [relative abundance (%) in the probiotic-treated and placebo groups before and after ingestion period].

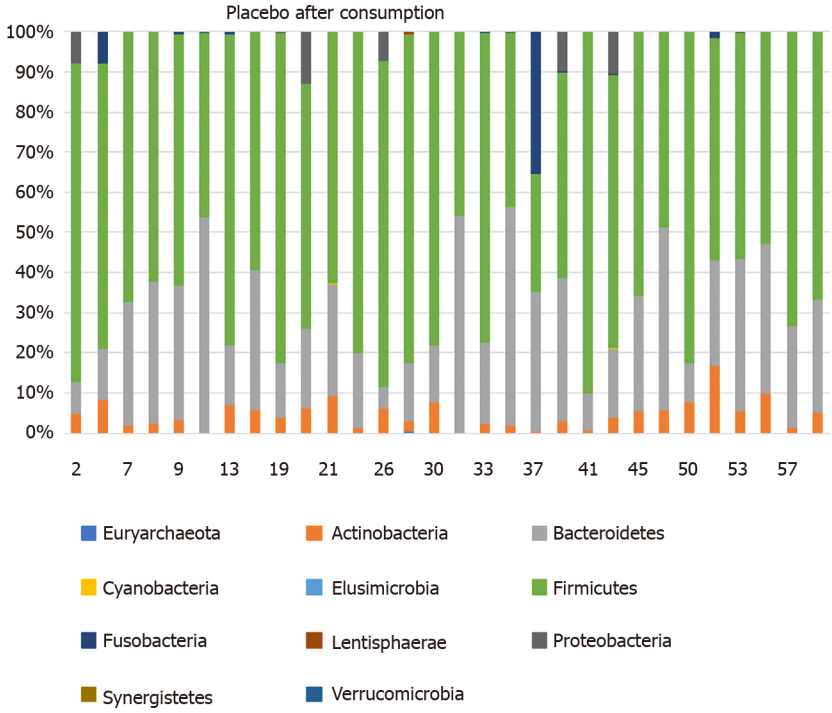
Table 8 shows that the genus Phyllobacterium of the phylum *Proteobacteria* increased significantly in the treatment group, while no significant change in the genera *Succinivibrio* and *Sphingomonas* was found in both the treatment and placebo groups. Meanwhile, the abundance of the genus *Akkermansia* in the phylum *Verrucomicrobia* decreased significantly.

From the analyses using LeSfe with a *P* value of < 0.05 (LDA > 2.0), one against all showed a significant difference in bacterial abundance in the probiotic-treated and placebo groups before and after the ingestion period. Alpha diversity analysis (Figure 5) showed that the abundance of bacteria in the probiotic-treated group significantly increased after they consumed the probiotic powder. This indicates that the consumption of probiotics could increase the abundance of bacteria in obese people, who have a diversity and wealth of microbiota gut components compared with eutrophic subjects[32]. At the genus level, a significant increase was observed in the abundance of the genus Phyllobacterium in the probiotic-treated group after consumption, whereas in the control group, *Roseburia* abundance increased significantly after consumption. The Brevibacterium, *Paenibacillus*, *Bacillales*, and *Faecalibacterium* groups were abundant in the placebo group before the consumption of the placebo product.

DISCUSSION

Ley *et al*[33] authored one of the first studies linking GM to obesity in humans[4]. The results from the 16 rRNA gene sequences in mouse models indicated that the two most abundant bacterial phyla were *Firmicutes* (60%-80%) and *Bacteroidetes* (20%-40%). In particular, the *ob*/*ob* mice had a 50% decrease in the population of *Bacteroidetes* and a proportional increase in *Firmicutes*. These changes indicate that obesity affects the diversity of GM and suggest that the intentional manipulation of the community


Placebo before consumption
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
2 7 9 13 19 21 26 30 33 37 41 45 50 53 57
Euryarchaeota
Actinobacteria
Bacteroidetes
Cyanobacteria
Elusimicrobia
Firmicutes
Fusobacteria
Lentisphaerae
Proteobacteria
Synergistetes
Verrucomicrobia


Placebo after consumption
100%
90%
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30%
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0%
2 7 9 13 19 21 26 30 33 37 41 45 50 53 57
Euryarchaeota
Actinobacteria
Bacteroidetes
Cyanobacteria
Elusimicrobia
Firmicutes
Fusobacteria
Lentisphaerae
Proteobacteria
Synergistetes
Verrucomicrobia

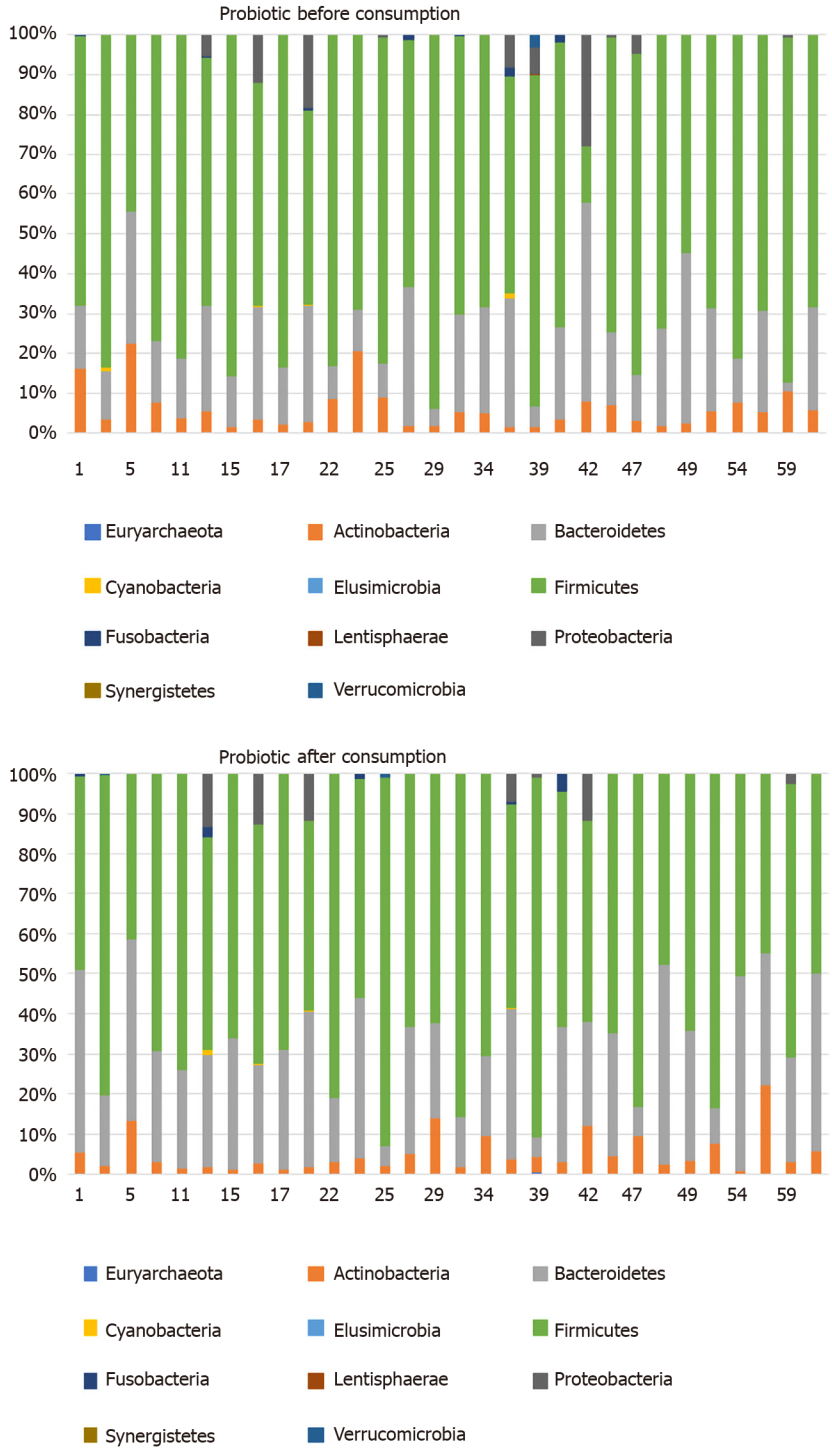

Figure 3 The phylum distribution composition of each subject from both probiotic-treated and placebo groups. The number on the X-axis represents the code of subject.

structure may be useful to regulate the energy balance in the obese individual[4,33]. Meanwhile, Turnbaugh *et al*[34] and Furet *et al*[35] found a lower representation of *Bacteroidetes* (*Bacteroides*/*Prevotella*) in obese individuals, with no differences in the *Firmicutes phylum*.

In addition, an ongoing review of GM and obesity found evidence of the association between gut bacteria and obesity[36,37]. Normally, the subclass distributions of GM are composed of the following: *Bacteroidetes* (23%), comprising the genus *Bacteroides*; *Firmicutes* (64%), including *Bacilli*, *Clostridia*, and *Mollicutes*; *Proteobacteria* (8%), gram-negative bacteria, such as *E. coli* and *Helicobacter pylori*; *Fusobacteria*, *Verrucomicrobia*, and *Actinobacteria* (3%), which include species such as *Bifidobacteria*; and only about 2% of other phyla. Our findings also indicate that an obese person has a different microbial proportion of the dominant phyla, which consists of the higher *Firmicutes* of

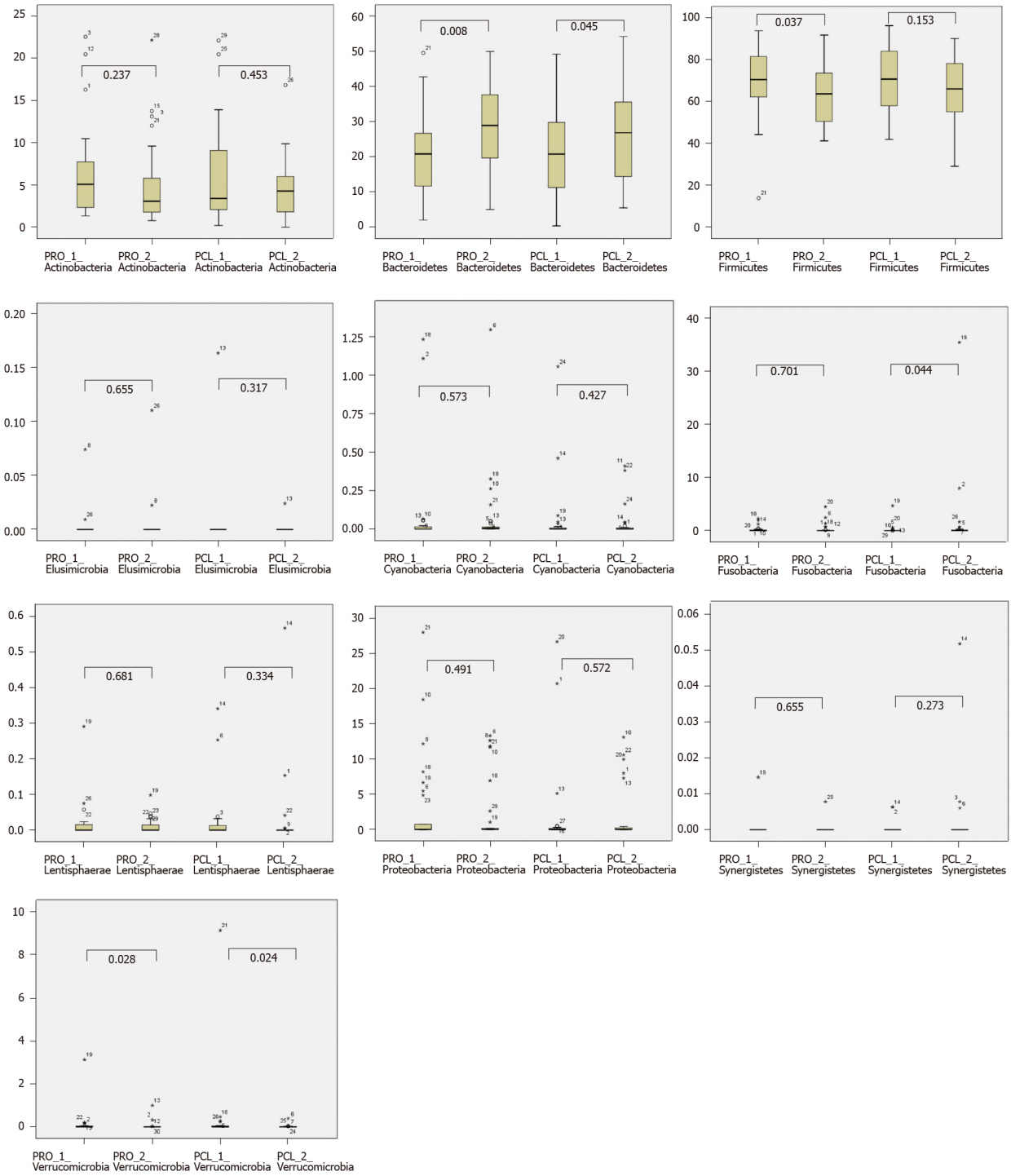

Figure 4 The changes in phylum of bacterial composition in both probiotic-treated and placebo groups before and after ingestion period.
PRO1: Probiotic before ingestion; PRO2: Probiotic after ingestion; PLC1: Placebo before ingestion; PLC2: Placebo after ingestion.

about 70% and the lower *Bacteroidetes* of about 21%, compared with a normal person, as mentioned in another study by Abenavoli *et al*[37]. The other phyla comprise *Actinobacteria* at about 6%, *Proteobacteria* at 3%, and less than 1% of other bacteria, such as *Fusobacteria*, *Verrucomicrobia*, *Cyanobacteria*, and *Lentisphaerae*.

Jumpertz *et al*[38] investigated the dynamic changes of GM during diets that varied in caloric content in the feces of lean and obese individuals by measuring ingested and stool calories using bomb calorimetry. The alteration of the nutrient load induced rapid changes in the GM. These changes were directly correlated with stool energy loss in lean individuals, such as a 20% increase in *Firmicutes* and a corresponding decrease in *Bacteroidetes*, which were associated with an increased energy harvest. A high degree of overfeeding in lean individuals was accompanied by a greater fractional decrease in stool energy loss. These results show that the nutrient load is a

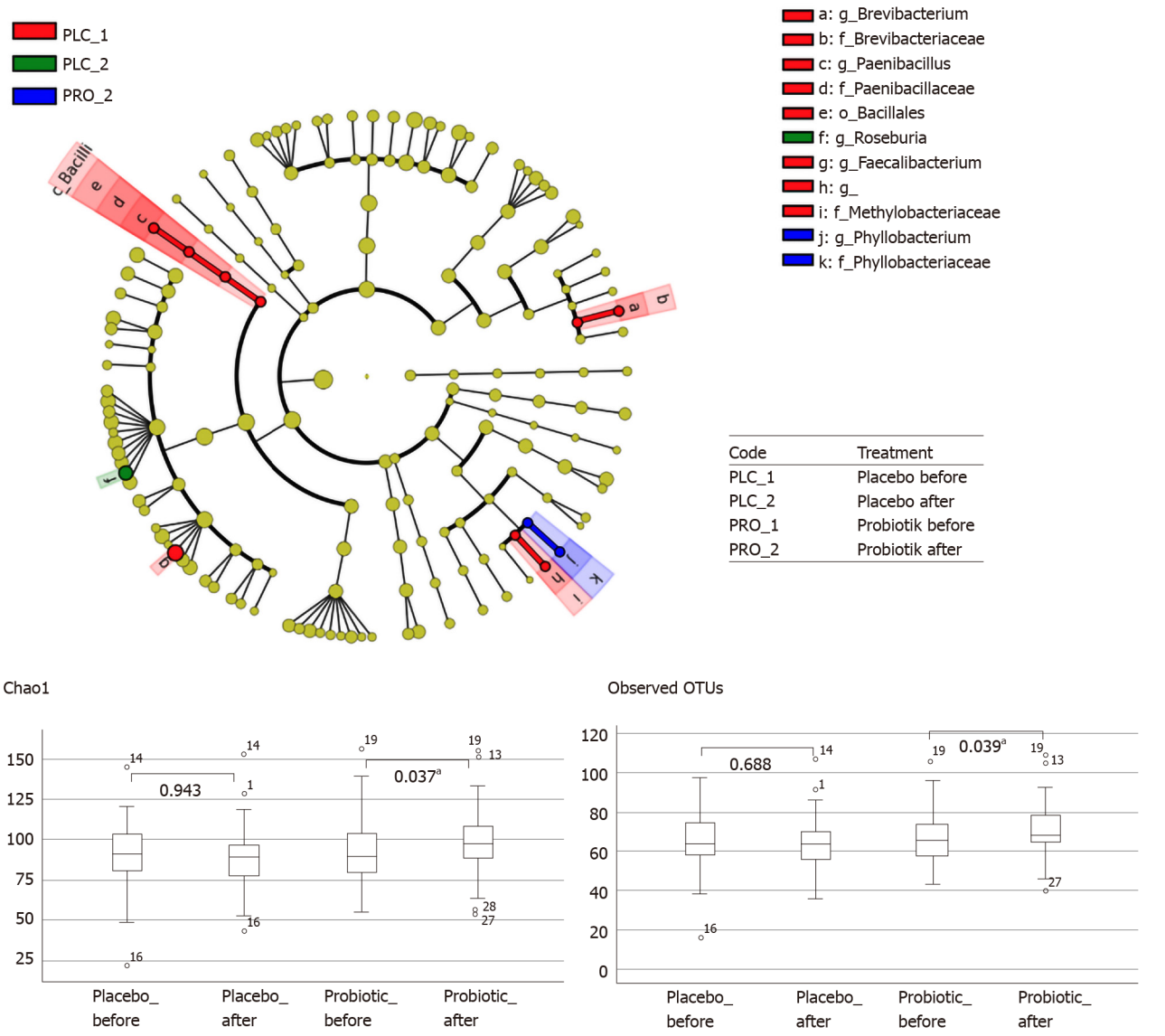

Figure 5 Cladogram and Alpha diversity on the treatment group and placebo group before and after the ingestion period. PRO1: Probiotic before ingestion; PRO2: Probiotic after ingestion; PLC1: Placebo before ingestion; PLC2: Placebo after ingestion.

key variable that can influence the gut (fecal) bacterial community structure over short periods. Furthermore, the observed associations between gut microbes and nutrient absorption indicate a possible role of the human GM in the regulation of the nutrient harvest. Recent studies have shown that the increase of bile acids in the intestine when comparing sterile rats with normal rats would show that the GM are related to not only obesity but also a diverse range of metabolic diseases[39].

Several mechanisms have been proposed for GM causative action in obesity physiopathology. In fact, gut commensal bacteria interact with our metabolism at several points. They help convert ingested complex nutrients to SCFAs, transform mucins and dietary fibers into simple sugars ready for absorption, stimulate intestinal epithelial proliferation, and favor nutrient absorption and metabolism. They are the main actor in shaping the gut crucial defense barrier constituted by the systemic and mucosal immune system and activate bio-inactive compounds[40]. Nevertheless, GM play an important role in human adipose tissue formation and deposition. Indeed, our intestinal bacteria can maintain the human body's energy balance mainly because of their ability to share the otherwise indigestible components of a mammalian's diet[41]. In this study, the average daily energy intake of the subjects was around 1518.17-1642.88 kcal/d, less than that of a normal adult (around 2000 kcal/d). No significant differences were observed in the diet profile of the subjects in both the probiotic-treated and placebo groups.

Abenavoli *et al*[37] mentioned in their review that evidence of the association between gut bacteria and obesity exists in both infants and adults. Several genetic, metabolic,

and inflammatory pathophysiological mechanisms are involved in the interplay between gut microbes and obesity. Microbial changes in the human gut can be considered a factor in obesity development in humans. The modulation of the bacterial strains in the digestive tract can help reshape the metabolic profile in the human obese host, as suggested by data from several animal and human studies. Several reports have also been conducted on the probiotic treatment of obese individuals. In adults, different strains of *Lactobacillus* and *Bifidobacterium*, alone or in combination, as well as *P. pentosaceus* led to a significant reduction of body weight, BMI, waist circumference, and fat mass[17,42-46].

As the administered dosage of probiotics affects the efficacy of the treatment, reduced visceral adiposity and waist circumference were observed after exposure to a high dose of *L. gasseri* BNR17[44]. These results were not so unambiguous given the different doses of Ecologic® (a mixture of multi-strains of *Lactobacillus* and *Bifidobacterium*), although this study was only conducted on obese women[47]. Interestingly, a report by Sanchez *et al*[48] showed the gender-specific effects of probiotics in human obese subjects. Indeed, the administration of *L. rhamnosus* CGMCC1.3724 and a restricted caloric diet resulted in significantly higher weight loss in obese women than in men. This finding can be explained by a greater impact on satiety, eating habits, and mood in women *vs* men[48]. Finally, scant evidence exists on the potential preventive effect on obesity of some probiotics in non-obese subjects. Specifically, VSL#3 can reduce body weight and fat accumulation *via L. gasseri* SBT2055 administration[16,49].

CONCLUSION

No significant differences in pH were found before and after ingestion in both the probiotic and placebo groups as well as in the lipid profile of both cholesterol and triglyceride, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and the LDL/HDL ratio. In addition, no significant changes in the concentration of SCFAs (acetic acid, propionate, and butyrate) were observed after the consumption of the probiotic powder *L. plantarum* Dad-13.

An interesting finding is a significant decrease in body weight and BMI ($P < 0.05$) in the treatment group. This weight loss was particularly observed in the female subjects. GM analysis shows that *L. plantarum* Dad-13 was able to decrease *Firmicutes* and increase *Bacteroidetes* (especially *Prevotella*).

ARTICLE HIGHLIGHTS

Research background

Gut microbiota (GM) play an important role in the nutrient absorption and energy regulation of individuals, thus affecting their nutritional status. GM also affect body weight, especially obesity, a condition wherein the accumulation of abnormal or excessive fat can interfere with health. Obesity in Indonesia showed an increasing prevalence in every province from 2007 to 2018. One study found a link between GM and body weight. Probiotics, as healthy bacteria, can improve an individual's health status by affecting GM composition. The consumption of probiotics may maintain this status and reduce the weight gain of adults with obesity in Indonesia.

Research motivation

This research aimed to investigate the effect of the consumption of an indigenous probiotic on overweight people. The results obtained may be used to determine the condition of GM in overweight people and the effect of indigenous probiotics on the GM of overweight adults. These results may also be used to determine the treatment of probiotic consumption that is most suitable and effective for overweight individuals in Indonesia to improve their health status.

Research objectives

The objective of this study was to determine the effect of the consumption of the indigenous probiotic powder *L. plantarum* Dad-13 on overweight adults in Indonesia.

Research methods

Sixty overweight volunteers with body mass index (BMI) equal to or greater than 25 consumed indigenous probiotic powder *L. plantarum* Dad-13 (2×10^9 CFU/gram/sachet) for 90 d. The study was a randomized, double-blind, placebo-controlled study. The volunteers filled in a diary on a daily basis, which consisted of questions on study product intake (only during the ingestion period), other food intake, number of bowel movements, fecal quality (consistency and color), any medications received, and any symptom of discomfort, such as diarrhea, constipation, vomiting, gassing, sensation of illness, *etc*. Fecal samples and the subjects' diaries were collected on the morning of day 10 + 1, marked as the end of the baseline period and the start of the ingestion period. During the ingestion period (from day 11 to day 101), several parameters to measure and analyze the results included body weight and height (once a month), the lipid profile, GM analysis using MiSeq, short-chain fatty acid (SCFA) analysis using gas chromatography, and the measurement of fecal pH using a pH meter.

Research results

The consumption of indigenous probiotic powder *L. plantarum* Dad-13 by overweight people caused the average body weight and BMI of the probiotic group to decrease from 84.54 ± 17.64 kg to 83.14 ± 14.71 kg and from 33.10 ± 6.15 kg/m^2 to 32.57 ± 5.01 kg/m^2, respectively. No significant reduction in the body weight and BMI of the placebo group was found. An analysis of the microbiota showed that the number of *Bacteroidetes*, specifically *Prevotella*, increased significantly, while *Firmicutes* significantly decreased. No significant change in lipid profile was observed in both groups. Also, no significant change in SCFAs (butyrate, propionate, acetic acid) and pH level were found after the consumption of the probiotic.

Research conclusions

No significant differences in pH were found before and after ingestion in both the probiotic and placebo groups as well as in the lipid profile of both cholesterol and triglyceride, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and the LDL/HDL ratio. In addition, no significant changes were observed in the concentration of SCFAs (acetic acid, propionate, and butyrate) after consumption. Interestingly, a significant decrease in body weight and BMI ($P < 0.05$) was found in the treatment group. An analysis of the GM shows that *L. plantarum* Dad-13 was able to decrease *Firmicutes* and increase *Bacteroidetes* (especially *Prevotella*).

Research perspectives

These results proved that the consumption of probiotics among overweight adults helps significantly reduce body weight, especially in women, and affects the composition of GM.

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