

Research Article

Effects of Consumption of Probiotic Powder Containing *Lactobacillus Plantarum* Dad-13 on Fecal Bacterial Population in School-Age Children in Indonesia

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Consumption of probiotics is known to influence the gut microbiota. The aim of this study was to assess the effect of probiotic powder containing *Lactobacillus plantarum* Dad-13 on bacterial composition in the gut by examining fecal samples of school-age children in Yogyakarta, Indonesia. This is a randomized, double-blind, placebo-controlled study. A total of 40 healthy subjects were recruited for this study and were divided into two groups: placebo group and probiotic group. The placebo group consumed skim milk and the probiotic group consumed probiotic powder containing *L. plantarum* Dad-13 (2×10^9 CFU/g) for 65 days. The results showed that placebo intake had no significant effect on gut microbiota; however, probiotic caused a significant increase in *L. plantarum* and *Lactobacillus* population, while decreasing the population of *E. coli* and non-*E. coli* coliform bacteria by 55% and 75%, respectively and *Bifidobacteria* count did not change significantly. The study concluded that consumption of probiotic powder *L. plantarum* Dad-13 could increase propionic acid thereby decreasing the gut pH which has an effect on the microbial population.

Keywords: Bifidobacterium, Coliform, *Lactobacillus*, *Lactobacillus plantarum* Dad-13, Probiotic powder, SCFA

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INTRODUCTION

The human body is inhabited by a vast number of bacteria which are collectively known as human microbiota (Young and Schmidt, 2008). The beneficial bacteria present in the colon produce antimicrobial compounds and protect the body against infections. For example, *Lactobacilli* and *Bifidobacteria* can inhibit harmful microbes. In addition to producing antimicrobials, beneficial bacteria competitively excludes pathogenic bacteria by occupying receptor sites and competes for space and nutrients (Fooks and Gibson, 2002).

The composition of intestinal microbiota varies greatly among individuals. This variation is caused by several factors such as age, diet, and environment. Nakayama et al. (2015) studied the diversity of gut microbiota of urban school-age children in five countries in Asia. They found that the majority of children from China, Japan, and Taiwan had higher population of *Bifidobacterium* and *Bacteroides* whereas children from Indonesia and Thailand had

higher *Prevotella* population. The higher *Prevotella* count reflects the intake of foods rich in resistant starches (Nakayama et al., 2015). Thus, dietary habits play a major role in shaping the composition and activity of gut microbiota. However, to balance gut microbiota, consuming probiotics has been proved to be effective.

Probiotics are live microorganisms which when administered in adequate amounts confer health benefits on the host (FAO/WHO, 2011). *Lactobacillus plantarum* Dad-13 is a lactic acid bacteria isolated from curd (traditional Indonesian fermented buffalo milk). It shows stronger resistance at low gastric pH, can inhibit pathogenic bacteria, and is a potential probiotic strain (Rahayu et al., 2015). Rahayu et al. (2016) found that consuming fermented milk containing *L. plantarum* Dad-13 can increase *Lactobacillus* count and decrease the population of enterobacteriaceae, *E. coli* and non-*E. coli* coliform bacteria in the feces of healthy Indonesian volunteers. Similarly, Goossens et al. (2016) reported that consumption of *L. plantarum* 299v can significantly increase the number of lactic acid bacteria and *Clostridia* in the feces.

The beneficial bacteria act as immunomodulators and protect against harmful bacteria. *Lactobacillus* and *Bifidobacterium* are beneficial bacteria that can suppress the growth of enteropathogenic bacteria (Sekirov et al., 2010). These bacteria produce short-chain fatty acids (SCFA) that lower the intestinal pH thereby inhibiting the growth of harmful bacteria. By improving blood flow and peristaltic activity of the colon, the transit time of harmful bacteria can be reduced so that they will exit with feces (Picard et al., 2005).

The aim of this study was to investigate the effect of probiotic powder containing *L. plantarum* Dad-13 on gut microbiota by examining the feces of healthy school-age children in SMPN 1 Ngemplak Yogyakarta.

MATERIALS AND METHODS

Subjects

The study consisted of 40 healthy school-age children (12 male and 28 female) aged between 13 to 16 years. The subjects were randomly divided into two groups: placebo group and probiotic group, with 20 participants in each group. Inclusion criteria were normal body mass index (BMI 15.4–22.9), no history of gastro-intestinal disorder, and no allergies to special food. Subjects were not allowed to use antibiotics, antimicrobics, antidiarrheals or any specific drugs for 30 days during the study period. As the subjects were minors, the informed consent was obtained from their parents or legal guardians. The study protocol was approved by the Ethical Committee, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia.

Research Products

The study product was a probiotic powder containing 2×10^9 CFU *Lactobacillus plantarum* Dad-13 per gram. The indigenous probiotic strain was deposited in ampoules at the Food and Nutrition Culture Collection (FNCC), Center for Food and Nutrition Studies, Universitas Gadjah Mada. The placebo group consumed skim milk as much as 1 gram.

Research Design

This study was randomized, placebo-controlled and double-blind. The total duration of the study period was 65 days. During the study period, the subjects in the probiotic group consumed 1 gram of probiotic powder each day, while subjects in the placebo group consumed 1 gram of skim milk each day (Fig. 1). Subjects were asked to fill daily food record. The fecal samples were collected in the morning from day 0 to day 66. All fecal samples were analyzed for *Lactobacillus plantarum*, *Lactobacillus*, *E. coli*, non-*E. coli* coliform bacteria, *Bifidobacteria*, SCFA, pH and quality of feces.

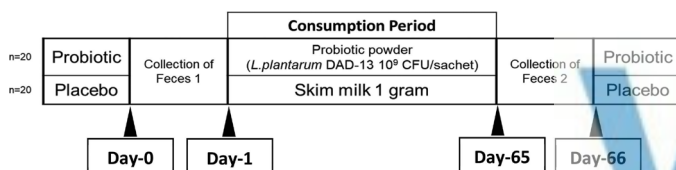


FIGURE 1 | Research design.

Fecal Sample Collection

Subjects were asked to collect their fecal samples using a stool kit provided by the laboratory. Collection process involved defecation on a biodegradable paper, so that it will not be contaminated. Then the feces was placed in sterile bottles using a spoon and firmly closed. The samples was transferred to the laboratory in cold storage containers ($< 10^{\circ}\text{C}$) within one hour.

Enumeration of Fecal Microbiota with Culture Method

Enumeration of *Lactobacillus*, *L. plantarum*, *E. coli* and non-*E. coli* coliform bacteria was performed using culture methods. *Lactobacillus* was enumerated by dilution and pour plate method using Rogosa media, while *L. plantarum* was enumerated by dilution and spread plate method using *Lactobacillus plantarum* selective media (LPSM). *E. coli* and non-*E. coli* coliform bacteria were counted using Oxoid Brilliance *E. coli*/coliform selective agar (Rahayu et al., 2016).

Analysis of Fecal Microbiota with Real-Time PCR

DNA extraction

A 100 mg of fecal sample was mixed with 800 μL lysis buffer and vortexed for 30 min followed by addition of 20 μL proteinase K. Next, 40 μL lysozyme was added, vortexed and placed in a water bath at 55°C for 30 min. The heated mixture was centrifuged at 13,000 rpm for 5 min to separate supernatant and residue. To 800 μL of the supernatant, 900 μL phenol was added, vortexed briefly followed by centrifugation at 13,000 rpm for 10 min. The supernatant was vortexed with 900 μL chloroform followed by centrifugation at 13,000 rpm for 5 min. The process of chloroform extraction was repeated once. Finally, the supernatant was mixed with 700 μL of absolute ethanol and incubated for 24 h at -20°C . After 24 h, the sample was centrifuged at 13,000 rpm for 10 min. The resulting supernatant was removed and pellet washed with 70% ethanol, then dried for 1–2 h. After drying, the 100 μL of TE 50 was added to dissolve the pellet. The quality of DNA preparation was checked by electrophoresis.

DNA amplification with real-time PCR

DNA amplification was performed using a thermal cycler. Amplification cycle consisted of initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 20 sec, annealing at 58°C for 20 sec, extension at 72°C for 50 sec and 1 cycle of final extension at 95°C for 15 sec (Matsuki et al., 2004). The Real Time PCR process used SYBR Green as a fluorescence marker. Specific primers were used for *Bifidobacteria* as described elsewhere (Matsuda et al., 2009).

Short Chain Fatty Acids Analysis

Concentrations of SCFA (acetic, propionic and butyric acids) were determined using gas chromatography (GC), according to the method by Salazar et al. (2015) with slight modifications. A total of 0.5–1 gram of feces was diluted with aquadest (1:3), vortex-mixed and centrifuged at 3000 rpm for 40 min. Then, 1 mL of supernatant was collected and re-centrifuged at 14,000 rpm for 20 min at 4°C . 1 μL of supernatant was subjected to SCFA analysis.

Determination of pH

About 0.2–1.0 gram of feces was mixed with aquadest in the ratio of 1:5 and vortexed until homogeneous. The pH of the homogenized sample was measured by direct contact of a glass electrode with the sample (Shiori et al., 2006).

Quality of Feces

Fecal quality including consistency and color, and daily frequency of defecation was noted. Stool consistency was measured using the Bristol Stool Scale (scale 1–7) and stool color was evaluated on a scale of 1–4 as described elsewhere (Utami et al., 2015).

Carbohydrate Intake Analysis

Carbohydrate intake was analyzed using NutriSurvey 2007 software (EBISpro, Willstaett, Germany) based on the participant's food record.

Data Analysis

All statistical analysis was performed using SPSS v. 24.0 (IBM Corp., Armonk, NY, USA). A P value of <0.05 was considered statistically significant. Data ratio with normal distribution was tested by Paired t-test, while for abnormal distribution was tested using Wilcoxon signed-rank and Independent t-test.

RESULTS AND DISCUSSION

Carbohydrate Intake Values in Research Subjects

The data presented in Table 1 show changes in carbohydrate intake values in the control (placebo) and probiotic group. In the placebo group, carbohydrate intake decreased in 11/20 subjects (55%)

TABLE 1 | Carbohydrate intake. *mean value; **n (mean value); †Significant at $P < 0.05$.

	Group	Carbohydrate (g)
Placebo	Before consumption*	259.7
	During consumption*	255.6
	Increment**	9 (41.2)
	Decrease**	11 (–41.2)
Probiotic	Before consumption*	229.1
	During consumption*	263.1†
	Increment**	14 (62.1)
	Decrease**	6 (–31.9)

whereas the amount of carbohydrate intake increased in 14/20 (70%) subjects receiving probiotics. In summary probiotics treatment promotes carbohydrate intake. During the study, the choice of food by subjects was not controlled except intake of foods or beverages containing probiotics other than *L. plantarum* Dad-13. The subjects dominantly consumed rice as carbohydrate source.

Nakayama et al. (2015) reported that intestinal microbiota in school-age children in the Yogyakarta Indonesia and Khon Kaen Thailand was dominated by *Prevotella* bacteria (P-type). The higher *Prevotella* count reflects the intake of food rich in resistant starches.

Effects of Probiotic Powder Containing *L. plantarum* Dad-13

The placebo group did not show a significant change in *L. plantarum* count. The average *L. plantarum* population was $5.33 \pm 0.86 \log_{10}$ CFU/g in feces before consuming probiotic (in 5 subjects), and after consuming probiotic was $5.00 \pm 1.44 \log_{10}$ CFU/g (in 7 subjects) (Fig. 2A). The probiotic group exhibited a significant increase *L. plantarum* count ($P < 0.05$) after treatment (Fig. 2A). Only 4/20 subjects had *L. plantarum* Dad-13 in their stool before treatment;

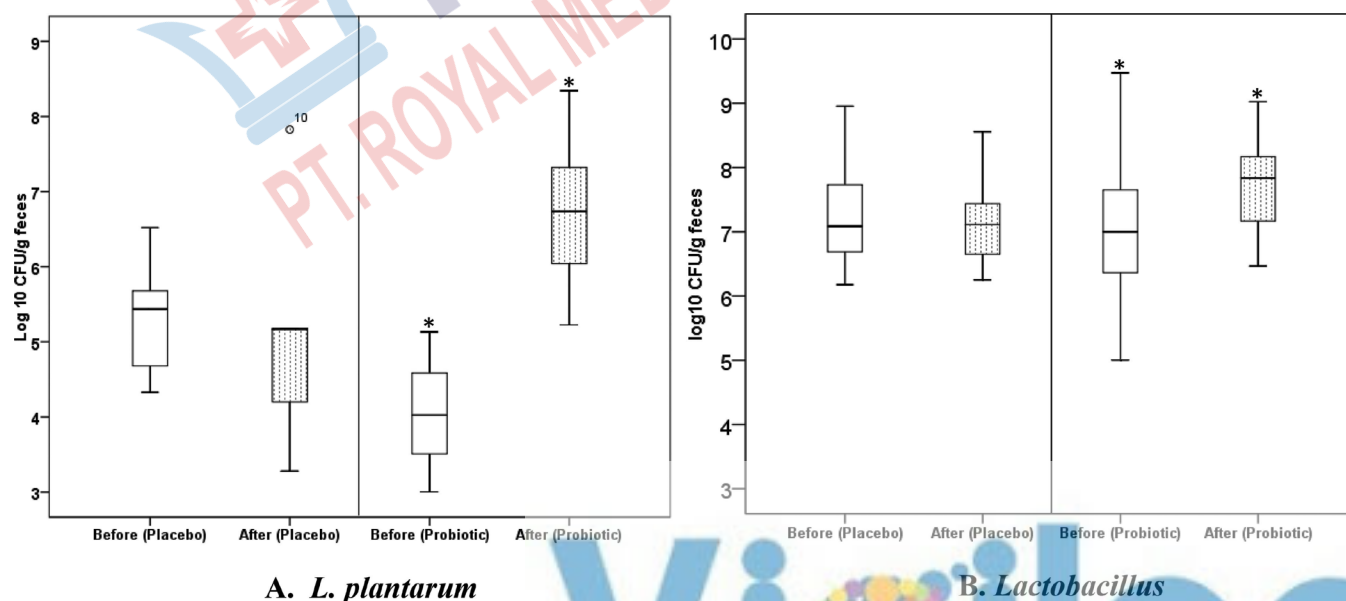


FIGURE 2 | Population of *L. plantarum* and *Lactobacillus* in subjects' feces in placebo and probiotic groups before and after consumption period. Data are presented as mean $\pm$ SD. * $P < 0.05$, mean value significantly different from before and after in *L. plantarum* and *Lactobacillus*.

Note: the plot number in the picture indicates the subject number.

however, this number increased in 20/20 after probiotic treatment. An average of $-4.05 \pm 0.86 \log_{10}$ CFU/g ($n = 4$) of feces was found before treatment and $6.75 \pm 0.93 \log_{10}$ CFU/g ($n = 20$) after treatment. This shows that *L. plantarum* Dad-13 is able to survive in the human digestive tract.

The number of *Lactobacillus* in the placebo group did not change significantly between before ($7.29 \pm 0.86 \log_{10}$ CFU/g of feces) and after ($7.12 \pm 0.61 \log_{10}$ CFU/g of feces) treatment. In the probiotic group, however, there was a significant increase ($P < 0.05$) in population before ($6.96 \pm 1.13 \log_{10}$ CFU/g) and after ($7.74 \pm 0.68 \log_{10}$ CFU/g) treatment (Fig. 2B). These results showed that significant increase of *L. plantarum* in subjects' feces can change the *Lactobacillus* population.

The results of this study are similar to an earlier report by Rahayu et al. (2016) showing consumption of fermented milk containing *L. plantarum* Dad-13 led to increase in the number of *L. plantarum* and *Lactobacillus* in subjects' feces on day 10 and 20. These observations are further supported by results of a study on 26 healthy Vietnamese adults consuming 65 mL of fermented beverages containing *Lactobacillus casei* Shirota (LcS) that show that LcS was able to survive in the gastrointestinal tract and increase its population (Mai et al., 2017).

Consumption Effects of Probiotic Powder Containing *L. plantarum* Dad-13 on the Number of *E. coli* and non-*E. coli* Coliform Bacteria in Subjects' Feces

The population of *E. coli* in the placebo group did not change significantly after treatment (Before: $7.71 \pm 0.76 \log_{10}$ CFU/g of feces; After: $8.18 \pm 0.74 \log_{10}$ CFU/g of feces). Probiotic group also did not decrease significantly between before and after the $8.20 \pm 0.65 \log_{10}$ CFU/g of feces and $7.91 \pm 0.83 \log_{10}$ CFU/g of feces (Fig. 3A).

In placebo group, the population of *E. coli* tend to increase in 12/20 subjects. While in the probiotic group, the population of *E. coli* tend to decrease in 11/20 subjects (55%) (Table 2).

The population of non-*E. coli* coliform bacteria in the placebo group did not significantly change between after treatment (Before: $7.79 \pm 0.79 \log_{10}$ CFU/g of feces; After: $7.99 \pm 0.78 \log_{10}$ CFU/g of feces) (Fig. 3B). In the placebo group, the population of non-*E. coli* coliform bacteria tend to increase in 12/20 subjects. In the probiotic group, non-*E. coli* coliform bacteria populations did not significantly decrease between before and after probiotic consumption $8.19 \pm 0.77 \log_{10}$ CFU/g of feces to $7.83 \pm 0.80 \log_{10}$ CFU/g of feces (Fig. 3B). However, there was a decrease in the number of non-*E. coli* coliform bacteria in 15/20 subjects (75%) with average $-0.79 \log_{10}$ CFU/g (Table 2). This show that consumption of probiotic powder *L. plantarum* Dad-13 can decrease the population of *E. coli* and non-*E. coli* coliform bacteria in the feces of some individuals.

The number of *E. coli* (Before: $7.93 \log_{10}$ CFU/g; After: $7.30 \log_{10}$ CFU/g) and non-*E. coli* coliform bacteria (Before: $7.87 \log_{10}$ CFU/g; After: $7.62 \log_{10}$ CFU/g) in feces decreased after 10 days of consumption of fermented milk containing *L. casei* strain Shirota (Utami et al., 2015). Out of 26 volunteers, 18 and 14 showed a decrease in *E. coli* and non-*E. coli* coliform bacteria, respectively (Utami et al., 2015). Rahayu et al. (2016) also reported that consumption of fermented milk containing *L. plantarum* Dad-13 for 10 days could decrease the population of *E. coli* (average amount of $0.69 \log_{10}$ CFU/g of feces) in 23 of 30 subjects. The consumption of fermented milk containing *L. plantarum* Dad-13 was also able to decrease the number of non-*E. coli* coliform bacteria in some individuals, but not in others. This may be influenced by other microbiota compositions in the intestinal tract.

The decrease of *E. coli* and non-*E. coli* coliform bacteria could result from increase in *Lactobacillus* population. *Lactobacillus* population from consuming probiotic powder containing *L. plantarum*

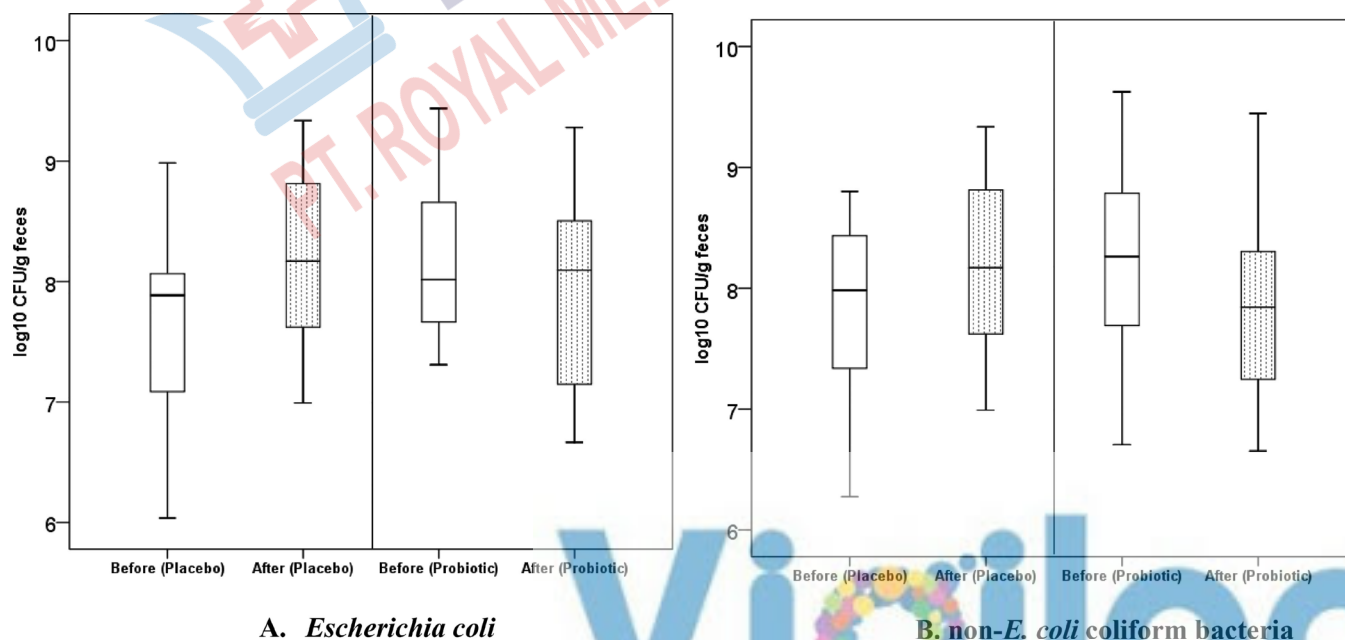
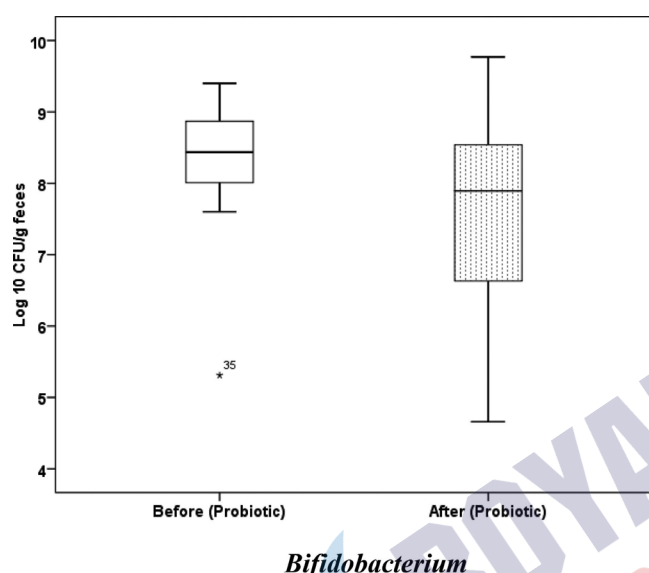


FIGURE 3 | Population of *E. coli* and non-*E. coli* coliform bacteria in subjects' feces in placebo and probiotic groups before and after consumption period. Data are presented as mean $\pm$ SD. Differences were statistically not significant at P -value ≤ 0.05 .

TABLE 2 | Changes in microbiota in subjects' feces using culture method. *Data are presented as n (%); **Unit value log₁₀ CFU/g of feces.

Variable	Placebo group				Probiotic group			
	Increment* (subject)	Average** (min-max)	Descent* (subject)	Average** (min-max)	Increment* (subject)	Average** (min-max)	Descent* (subject)	Average** (min-max)
<i>L. plantarum</i>	6 (30%)	5.29 (3.83 to 7.83)	5 (25%)	-4.67 (-3.24 to -5.68)	20 (100%)	6.14 (0.87 to 8.34)	–	–
<i>Lactobacillus</i>	9 (45%)	0.65 (0.20 to 1.08)	11 (55%)	-0.84 (-0.14 to -1.79)	14 (70%)	1.55 (0.05 to 3.84)	6 (30%)	-1.00 (-0.23 to -2.27)
<i>Escherichia coli</i>	12 (60%)	1.13 (0.13 to 2.80)	8 (40%)	-0.5 (-0.08 to -1.21)	9 (45%)	0.6 (0.20 to 1.25)	11 (55%)	-1.03 (-0.11 to -2.73)
Non- <i>E. coli</i> coliform bacteria	12 (60%)	0.68 (0.01 to 1.94)	8 (40%)	-0.51 (-0.05 to -1.10)	5 (25%)	0.96 (0.10 to 2.38)	15 (75%)	-0.79 (-0.05 to -2.46)

**FIGURE 4** | Population of *Bifidobacterium* in probiotic groups before and after consumption period using real time PCR. Data (n=35) are presented as mean ± SD. Differences were statistically not significant at P-value ≤ 0.05.

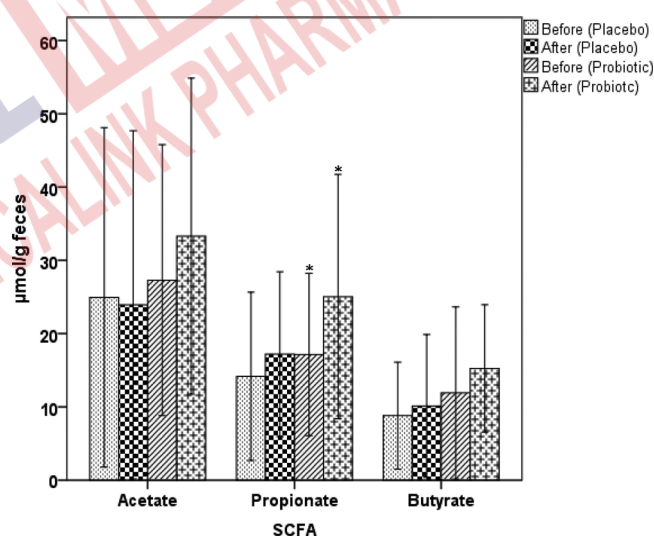
Dad-13. *L. plantarum* Dad-13 could also stimulate the growth of indigenous *L. plantarum* in the gastrointestinal tract of the subjects, by decreasing the population of pathogenic bacteria (Rahayu et al., 2016). Thirabunyanon (2011) explains in his study that probiotics can reduce the population of pathogenic bacteria by one or more mechanisms including i) production of antimicrobial agents, ii) competition between probiotic bacteria and pathogenic bacteria for adhesion receptors in the intestinal mucosa, iii) competition for nutrients and iv) immune stimulation.

Effects of Probiotic Powder Containing *L. plantarum* Dad-13 on *Bifidobacteria* in Subjects' Feces

The population of *Bifidobacterium* in the probiotic group did not change significantly between after treatment (Before: 8.27 ± 0.91 log₁₀ CFU/g; After: 7.53 ± 1.49 log₁₀ CFU/g) (Fig. 4). The increase and decrease in the population of *Bifidobacterium* in subjects' feces are summarized in Table 3. Wang et al. (2015) reported that consumption of *L. casei* strain Shirota in children (4–12 years) did not show any significant change in *Bifidobacteria* population

TABLE 3 | Changes in the number of *Bifidobacterium* in probiotic group using RT-PCR. *Data are presented as n (%); **Unit value log₁₀ CFU/g of feces.

Item	<i>Bifidobacterium</i>
Increment* (subject)	4 (20%)
Average** (min-max)	0.92 (0.28 to 1.68)
Descent* (subject)	14 (70%)
Average** (min-max)	-1.22 (-0.08 to -3.92)

**FIGURE 5** | Concentration of short-chain fatty acid in the placebo and probiotic groups before and after consumption period. The data are presented as mean ± SD. *P < 0.05.

initially but increased after 3 and 6 months. Consumption of *L. plantarum* Dad-13 did not have significant effects on some bacterial populations.

Consumption Effects of Probiotic Powder Containing *L. plantarum* Dad-13 on Short Chain Fatty Acid Concentration

Figure 5 shows that there were no significant change in the concentration of acetic acid (Before: 24.95 ± 23.15 μmol/g of feces; After: 23.93 ± 23.75 μmol/g of feces), propionic acid (Before:

14.16 ± 11.49 µmol/g of feces; After: 17.20 ± 11.24 µmol/g of feces) or butyric acid (Before: 8.25 ± 6.52 µmol/g of feces; After: 9.35 ± 9.19 µmol/g of feces) in the placebo group after 65 days of treatment. The result showed that the consumption of skim milk did not alter SCFA concentration. In the probiotic group, however, there was a significant increase ($P < 0.05$) in propionic acid concentration (Before: 17.15 µmol/g of feces; After: 25.05 µmol/g of feces) but not acetic acid (Before: 27.29 µmol/g of feces; After: 33.31 µmol/g of feces) or butyric acid (Before: 11.91 µmol/g of feces; After: 15.25 µmol/g of feces). The concentrations of acetic acid and butyric acid increased in 13/20 subjects and 12/20 subjects, respectively (Table 4).

Short chain fatty acids (SCFAs) are volatile fatty acids produced by intestinal microbiota in the colon as a fermentation product of undigestible food component in the small intestine, characterized by less than six carbons mostly present in straight chain conformations. These include acetic acid (C2), propionic acid (C3), and butyric acid (C4) that are the most abundant, representing 90–95% of SCFA in the colon (Rios-Covian et al., 2016).

The concentration of propionic acid significantly increased after consumption of probiotic powder containing *L. plantarum* Dad-13. Propionic acid is produced through three different pathways to be used by colonic bacteria. These include succinate pathway, acrylic stripe, and propanediol pathway (Reichardt et al., 2014). The succinate pathway uses succinate as a substrate for propionate formation and involves decarboxylation of methylmalonyl-CoA to propionyl-CoA. This pathway is present in some Firmicutes and Bacteroidetes. In the propanediol pathway, characterized by the conversion of deoxy-sugars into propionate, CoA-dependent dehydrogenase propionaldehyde, which converts propionaldehyde to propionyl-CoA (Louis et al., 2014; Reichardt et al., 2014).

In the acrylic pathway, lactate is converted to propionate through lactoyl-CoA dehydratase activation and downstream enzymatic reactions (Flint et al., 2015). In this pathway, propionic acid could increase due to higher population of *Lactobacillus* in feces from the

consumption of lactic acid bacteria (Louis et al., 2014). Main groups of fermentative gut microorganisms in the formation of propionic acid include *Bacteroides*, *Propionibacteria*, *Veillonella*, *Clostridia*, *Prevotella*, and *Megasphaera* (Macfarlane and Macfarlane, 2012). The concentration of acetic acid and butyric acid in feces showed no significant change. However, the change in acetic and butyric acid concentrations were influenced by the absorption and metabolism of total SCFA in the host (Hamer et al., 2010). In an earlier study (Goossen et al., 2003), consumption of oatmeal-fermented beverages containing *L. plantarum* 299v changed *Lactobacilli* population, but not SCFA concentrations.

Consumption Effects of Probiotic Powder Containing *L. plantarum* Dad-13 on pH Value and Quality of Feces

The data on the pH value, quality of feces (consistency, color), daily frequency of defecation and number of days' defecation is summarized in Table 5. The placebo group had no significant change in the quality of feces, whereas the probiotic group showed significant change only in the pH value ($P < 0.05$).

Shioiri et al. (2006) reported that the pH of feces decreased in volunteers consuming synbiotic fermented milk containing *L. casei* strain Shirota and galactooligosaccharides. The decrease in pH value was attributed to organic acids released by bacteria that stimulate motility in the colon. The change in pH of the feces was influenced by the amount and activity of microorganisms in the colon that produced acids such as lactic acid and others which in turn can decrease the population of unfavorable bacteria such as *Enterobacteriaceae*, *Clostridium*, and *Staphylococcus*. The fecal characteristics of both groups did not change significantly as judged by the quality of stool, fecal color, frequency of defecation/10 days and the number of days defecated/10 days.

Utami et al. (2015) reported that there was no significant difference in the quality of feces before and after the intake of fermented

TABLE 4 | Changes in SCFA concentrations in the placebo and probiotic groups. *Data are presented as n (%); **Unit value (µmol/g of feces).

Item	Placebo group			Probiotic group		
	Acetate	Propionate	Butyrate	Acetate	Propionate	Butyrate
Increment* (subject)	13(65%)	12 (60%)	13(65%)	13(65%)	15 (85%)	12 (60%)
Average**	9.86	11.51	4.50	13.33	13.11	10.88
(min-max)	(0.29 to 28.74)	(0.12 to 52.14)	(0.07 to 17.53)	(1.19 to 42.13)	(0.12 to 54.65)	(0.31 to 27.68)
Descent* (subject)	7(35%)	8 (40%)	7(35%)	7(35%)	5 (25%)	8 (40%)
Average**	-17.35	-9.67	-5.17	-7.55	-7.72	-7.96
(min-max)	(-2.40 to -44.63)	(-0.12 to -38.34)	(-1.47 to 11.60)	(-3.61 to -13.46)	(-1.17 to -20.70)	(-1.25 to -24.18)

TABLE 5 | The pH value and quality of subjects' feces in placebo and probiotic groups. *Data are presented as Mean ± SD; **Significant $P < 0.05$.

Item	Placebo group		Probiotic group	
	Before	After	Before	After
pH*	6.47 ± 0.59	6.46 ± 0.64	6.33 ± 0.51	6.03 ± 0.48**
Consistency (1–7)*	3.96 ± 0.37	3.95 ± 0.37	3.88 ± 0.42	4.10 ± 0.37
Color (1–4)*	2.25 ± 0.53	2.34 ± 0.49	2.00 ± 0.51	2.16 ± 0.35
Frequency of defecated* (number/10 days)	8.60 ± 3.51	7.79 ± 1.55	8.35 ± 2.25	8.22 ± 1.69
Day of defecated* (day/10 days)	7.65 ± 1.98	7.41 ± 1.57	7.60 ± 1.98	7.70 ± 1.56

milk containing LcS. The stool color and consistency scores showed no significant difference in the pH range of 4.2–4.1 and 2.3–2.2, respectively. Based on the Bristol Stool Form Scale, consistency of feces of all subjects was considered as type 4—sausage or snake, smooth and soft. In addition, Rahayu et al. (2016) reported that the quality of feces before and after the consumption of fermented milk containing *L. plantarum* Dad-13 had no significant difference when the stool consistency score was 4 and the color score was 2. The subjects in the study were healthy school-age children with normal fecal quality and frequency of defecation; hence, the consumption of probiotic powder for 65 days did not significantly affect the stool consistency, fecal color, frequency and number of days of defecation.

CONCLUSION

This study shows that consuming probiotic powder containing *L. plantarum* Dad-13 has been able to increase the population of *L. plantarum* and *Lactobacillus* but had no significant effect on *Bifidobacteria*. Also, consumption of probiotic powder decreases the number of *E. coli* and non-*E. coli* coliform bacteria by 55% and 75%, respectively. Furthermore, *L. plantarum* Dad-13 probiotic significantly increases the production of propionic acid in all subjects, while acetic and butyric acids are increased in 65% and 60% of subjects, respectively.

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CONFLICT OF INTEREST

All authors report that there is no conflict of interest.

REFERENCES

FAO/WHO. (2011). Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. World Health Organization. http://www.who.int/foodsafety/publications/fs_management/en/probiotics.pdf. <https://doi.org/10.1109/ISI.2013.6578843>

Flint, H. J., Duncan, S. H., Scott, K. P. and Louis, P. (2015). Links between diet, gut microbiota composition and gut metabolism. *Proceedings of the Nutrition Society* **74**:13–22. <https://doi.org/10.1017/S0029665114001463>

Fooks, L. J. and Gibson, G. R. (2002). Probiotics as modulators of the gut flora. *British Journal of Nutrition* **88**(S1), s39. <https://doi.org/10.1079/BJN2002628>

Goossens, D. S., Jonker, D. S., Russel, M., Stobberingh, E. and Van Den Bogaard, A. (2003). The effect of *Lactobacillus plantarum* 299v on the bacterial composition and metabolic activity in faeces of healthy volunteers. *Alimentary pharmacology and therapeutics* **18**:495–505. <https://doi.org/10.1046/j.0269-2813.2003.01708.x>

Goossens, D. A. M., Jonkers, D. M. A. E., Russel, M. G. V. M., Stobberingh, E. E. and Stockbrügger, R. W. (2006). The effect of a probiotic drink with *Lactobacillus*

plantarum 299v on the bacterial composition in faeces and mucosal biopsies of rectum and ascending colon. *Alimentary Pharmacology and Therapeutics* **23**:255–263. <https://doi.org/10.1111/j.1365-2036.2006.02749.x>

Hamer, H. M., Jonkers, D., Brummer, R. J., Troost, F. J., Vanhoutvin, S. and Venema, K. (2010). Review article: the role of butyrate on colonic function. *Alimentary Pharmacology and Therapeutics* **27**:104–119. <https://doi.org/10.1111/j.1365-2036.2007.03562.x>

Louis, P., Hold, G. L. and Flint, H. J. (2014). The gut microbiota, bacterial metabolites and colorectal cancer. *Nature Reviews Microbiology* **12**:661–672. <https://doi.org/10.1038/nrmicro3344>

Macfarlane, G. T. and Macfarlane, S. (2012). Bacteria, Colonic Fermentation, and Gastrointestinal Health. *AOAC International* **95**:50–60. <https://doi.org/10.5740/jaoacint.SGE>

Mai, T. T., Van Hop, D., Van Anh, T. T. and Lam, N. T. (2017). Recovery of *Lactobacillus casei* strain Shirota (LcS) from the intestine of healthy Vietnamese adults after intake of fermented milk. *Asia Pacific Journal of Clinical Nutrition* **26**:72–77. <https://doi.org/10.6133/apjcn.112015.09>

Matsuda, K., Tsuji, H., Asahara, T., Matsumoto, K., Takada, T. and Nomoto, K. (2009). Establishment of an analytical system for the human fecal microbiota, based on reverse transcription-quantitative PCR targeting of multicopy rRNA molecules. *Applied and Environmental Microbiology* **75**:1961–1969. <https://doi.org/10.1128/AEM.01843-08>

Matsuki, T., Watanabe, K., Fujimoto, J., Takada, T. and Tanaka, R.T. (2004). Use of 16S rRNA gene-targeted group-specific primers for real-time PCR analysis of predominant bacteria in human feces. *Applied and Environmental Microbiology* **70**:7220–7228. <https://doi.org/10.1128/AEM.70.12.7220>

Nakayama, J., Watanabe, K., Jiang, J., Matsuda, K., Chao, S. H., Haryono, P., La-ongkham, O., Sarwoko, M.-A., Sujaya, I.N., Zhao, L., Chen, K.-T., Chen, Y.-P., Chiu, H.-H., Hidaka, T., Huang, N.-X., Kiyohara, C., Kurakawa, T., Sakamoto, N., Sonomoto, K., Tashiro, K., Tsuji, H., Chen, M.-J., Leelavatcharamas, V., Liao, C.-C., Nitisinprasert, S., Rahayu, E. S., Ren, F.-Z., Tsai, Y.-C. and Lee, Y. K. (2015). Diversity in gut bacterial community of school-age children in Asia. *Scientific Reports* **5**:1–11. <https://doi.org/10.1038/srep08397>

Picard, C., Fioramonti, J., Francois, A., Robinson, T., Neant, F. and Matuchansky, C. (2005). Review article: Bifidobacteria as probiotic agents – Physiological effects and clinical benefits. *Alimentary Pharmacology and Therapeutics* **22**:495–512. <https://doi.org/10.1111/j.1365-2036.2005.02615.x>

Rahayu, E. S., Cahyanto, M., Windiarti, L., Sutriyanto, J., Kandarina, T. and Utami, T. (2016). Effects of consumption of fermented milk containing indigenous probiotic *Lactobacillus plantarum* Dad-13 on the fecal microbiota of healthy Indonesian volunteers. *International Journal of Probiotics and Prebiotics* **11**:91–98.

Rahayu, E. S., Yogeswara, A., Mariyatun, Windiarti, L., Utami, T. and Watanabe, K. (2015). Molecular characteristic of indigenous probiotic strain from Indonesia. *International Journal of Probiotics and Prebiotics* **10**:1–7.

Reichardt, N., Duncan, S. H., Young, P., Belenguer, A., McWilliam Leitch, C., Scott, K. P., Flint, H. J. and Louis, P. (2014). Phylogenetic distribution of three pathways for propionate production within the human gut microbiota. *ISME Journal* **8**:1323–1335. <https://doi.org/10.1038/ismej.2014.14>

Rios-Covian, D., Ruas-Madiedo, P., Margolles, A., Gueimonde, M., de los Reyes-Gavilán, C. G. and Salazar, N. (2016). Intestinal short chain fatty acids and their link with diet and human health. *Frontiers in Microbiology* **7**:1–9. <https://doi.org/10.3389/fmicb.2016.00185>

Salazar, N., Dewulf, E. M., Neyrinck, A. M., Bindels, L. B., Cani, P. D., Mahillon, J., de Vos, W. M., Thissen, J. P., Gueimonde, M., de Los R.-G., C. G. and Delzenne, N. M. (2015). Inulin-type fructans modulate intestinal Bifidobacterium species populations and decrease fecal short-chain fatty acids in obese women. *Clinical Nutrition* **34**:501–507. <https://doi.org/10.1016/j.clnu.2014.06.001>

Sekirov, I., Russell, S., Antunes, L. C. M. and Finlay, B. (2010). Gut microbiota in health and disease. *Physiological Reviews* **90**:859–904. <https://doi.org/10.1159/000481627>

Yshagi, K., Nakayama, S., Asahara, T., Yuki, N., Kawakami, K., Yamaoka Y., Sakai Y., Nomoto K. and Totani, M. (2006). The effects of a synbiotic fermented milk beverage containing *Lactobacillus casei* strain Shirota and transgalactosylated oligosaccharides on defecation frequency, intestinal microflora, organic acid concentrations, and putrefactive metabolites of sub-opt. *Bioscience and Microflora* **25**:137–146. <https://doi.org/10.12938/bifidus.25.137>

- Thirabunyanon, M. (2011). Biotherapy for and protection against gastrointestinal pathogenic infections via action of probiotic bacteria. *Maejo International Journal of Science and Technology* **5**:108–128.
- Utami, T., Cahyanto, M. N., Juffrie, M. and Rahayu, E. S. (2015). Recovery of *Lactobacillus Casei* Strain Shirota (Lcs) From the intestine of healthy Indonesian volunteers after intake of fermented milk and its impact on the enterobacteriaceae faecal microbiota. *International Journal of Probiotics and Prebiotics* **10**:77–84.
- Wang, R., Chen, S., Jin, J., Ren, F., Li, Y., Qiao, Z., Wang, Y. and Zhao, L. (2015). Survival of *Lactobacillus casei* strain Shirota in the intestines of healthy Chinese adults. *Microbiology and Immunology* **59**:268–276. <https://doi.org/10.1111/1348-0421.12249>
- Young, V. B. and Schmidt, T. M. (2008). Overview of the gastrointestinal microbiota. *GI Microbiota and Regulation of the Immune System* **635**:29–40. <https://doi.org/10.1007/978-0-387-09550-9>



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