Research Article

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The Consumption Effect of Indigenous Probiotic Powder *Lactobacillus plantarum* Dad-13 on Gut Microbiota Population and Short Chain Fatty Acids in Students of SMPN 1 Pangururan, Samosir

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ABSTRACT: The human intestine is a diverse ecosystem populated by microbiota affected by several factors, including age. The aim of this research was to evaluate the effects of the probiotic powder *Lactobacillus plantarum* Dad-13 on the numbers of gut microbiota, short-chain fatty acids (SCFA), and fecal characteristics in healthy adolescents. This research was conducted at SMPN 1 Pangururan, Samosir, with a randomized, double-blind, parallel placebo-controlled trial. 54 healthy adolescents aged 13 to 14 were divided into two groups, one consumed a gram of skimmed milk powder (placebo group) and the other ingested powder containing *L. plantarum* Dad-13 with 1.18×10^9 CFU/gram (probiotic group). After 33 days of intervention, the height of placebo group (149.42 ± 5.03 cm) and probiotic group (154.37 ± 4.67 cm) increased significantly. Significant increases in body weight (44.35 kg ± 4.61 to 45.20 kg ± 4.78) and BMI (and 18.77 ± 2.12 to 18.99 ± 2.11) were observed in the probiotic group. In the probiotic group, the numbers of gut microbiota were not significantly affected ($p > 0.05$). The amount of SCFA and fecal characteristics of both groups showed no significant differences. Thus, the consumption of *L. plantarum* Dad-13 increased weight, height, and BMI but could not influence the numbers of gut microbiota, SCFA, and the fecal characteristics of healthy adolescents.

Keywords: *Lactobacillus plantarum* Dad-13, gut microbiota, short chain fatty acid, fecal characteristics

INTRODUCTION

The colon is a part of the gastrointestinal tract inhabited by various microorganism populations known as gut microbiota. These can ferment carbohydrates and proteins, which are then absorbed by the intestine during digestion. Analysis using 16S rRNA shows that gut microbiota in human feces is dominated by four phylum bacteria, which are *Firmicutes*, *Bacteroidetes*, *Proteobacteria,* and *Actinobacteria* (Rios-Covian *et al*., 2009; Panda *et al*., 2014). Many factors can affect gut microbiota composition, such as the region where hosts reside and dietary habits. Wu *et al*. (2011) stated that *Bacteroides* are related to a high intake of protein and animal fats, while *Prevotella* is related to a high carbohydrate intake. Another study reported by (Nakayama *et al*., 2015) displays how dietary habits and geographical location influence microbiota. The study reported that gut microbiota in children from five different countries, Japan, China, Taiwan, Thailand, and Indonesia, contained various kinds of bacteria and were classified as enterotype-like clusters (*Prevotella* and *Bifidobacterium/Bacteroides* type). Other results from this study show that Indonesia and Thailand share the same characteristics of enterotype P-type, which is dominated by *Prevotella*.

Having a broad geographic component, Indonesia encompasses great diversity in its culture, climate, and dietary habits, which can encourage a wide variety of microbiota. One of many diverse regions in Indonesia is Samosir. According to data from the Health Research and Development Agency and its study of the total diets of Indonesian people in the year 2014 (Anonymous[a], 2014), Food in the Province of Samosir, North Sumatra, contains animal protein such as pork, buffalo, and fish taken from the lake. On average, individuals between the ages of 13-18 consumed 63.2 grams of protein per day, which is higher than the average national consumption of only 56.3 grams/day. On the other hand, the average consumption of carbohydrates in North Sumatra was lower than the national average, with consumption at 239.3 grams per day. In comparison, the average national consumption of carbohydrates equaled 241.7 grams per day. Consumption of a specific diet may lead to a specific composition of microbiota in the gut. Another factor that

has an impact on gut microbiota composition is the consumption of probiotics.

Probiotics are a group of living organisms that give health benefits to the human body if consumed adequately (FAO and WHO., 2011). Fermented milk containing the *Lactobacillus casei* strain Shirota probiotic showed effects on the number of *Lactobacillus* and *Bifidobacteria*. *Bifidobacteria* and *Lactobacillus*, as probiotics, will ferment carbohydrates to produce acids. *Bifidobacteria* will produce lactic acid and acetic acid, while *Lactobacillus* will produce several acids, including short-chain fatty acids (SCFA), such as acetate, propionate, and butyrate, that will provide an advantage in the fight against *Enterobacteriaceae* (*E. coli*, *K. pneumoniae*, and *P. mirabilis*). Probiotic consumption of *Lactobacillus kefir* LKF01 on healthy subjects also displayed efficacy on the microbiota composition *Prevotella, Bacteroides,* and *Clostridium*. (Toscano *et al*., 2017; Kleerebezem and Vaughan, 2009; Levison, 1973). Probiotics are also known to influence body weight and BMI in subjects. Consumption of probiotics *L. gasseri* SBT2055 in the form of yogurt (viability of 5×10^{10} CFU / ml) in adults who tend to be overweight can cause reductions in both weight and BMI (Kadooka *et al.*, 2010). Consumption of probiotics also has a healthy effect on bones. Administration of *L. reuteri* 6475 probiotics to healthy male mice 3× / week with 109 viability for 4 weeks can increase mineral density, bone volume, and trabecular bone thickness (McCabe *et al*., 2013).

Research on probiotic effects in subjects of the teenage age category is very scarce. A study by (Agans *et al*., 2011) reported that the composition of microbiota in adolescents (11-18 years old) differs from that of adults (22-61 years old), in whom the levels of *Bifidobacterium* and *Clostridium* are higher in the adolescents. Therefore, it is highly crucial that studies on gut microbiota composition between the two different age categories are not considered the same. Most of the studies discussing the probiotic's role in adolescence focused on their ability to decrease the prevalence of intestinal tract diseases (cholic and diarrhea) and respiratory illness (Gilbowski & Turcyzn, 2013; Silva *et al*., 2020). Based on the Central Agency of Statistics in North Sumatra province, Indonesia, the disease with the highest prevalence recorded was diarrhea (205,155 cases). Therefore, a therapeutic alternative to decrease the rate of diarrhea cases in North Sumatra was vital (Central Agency of Statistics, 2022).

The probiotic used in this research is an indigenous bacterium, *L. plantarum* Dad-13. Tari *et al.* (2016) conducted research that revealed that the addition of *L. plantarum* culture to yogurt with purple sweet potato extract can provide health advantages to recipients, including decreased diarrhea and free radicals. Another research by (Rahayu *et al*., 2015) showed that when compared with other indigenous probiotics, *L. plantarum* Dad-13 has the highest antimicrobial content to fight against pathogens (*A. hydrophilla* dky-5, *S. dysentriae* dky-4, *S. typhi* dky-3, *E. coli* OK 111, *E. coli* ST). There are a lot of potentials and advantages that can be developed from the local isolate *L. plantarum* Dad-13 as a probiotic and for its effects on gastrointestinal microbiota. The objective of this study was to examine the impact of *L. plantarum* Dad-13 powder, an indigenous probiotic, on junior high school students in Pangururan, Samosir Regency, North Sumatra.

MATERIALS AND METHOD

Production of Probiotic Powder

The probiotic powder used in this research was packed in units of 1 gram and stored in a refrigerator to maintain the viability of 10^9 CFU/gram. A probiotic culture was obtained in the form ofR pure, freeze-cultured powder from The Food Nutrition and Culture Collection (FNCC) PSPG UGM. The culture used was *L. plantarum* Dad-13. 50 µL of pure probiotic culture was incubated in a halal medium for 24 hours at 30 °C. According to a preceding study, the halal media was created with natural ingredients, including sucrose, meat peptone, mung bean sprout extract, tomato extract, and young coconut water (Utami *et al*., 2020). From the incubated culture, about 1.5 mL was taken and incubated in 150 mL of halal medium for 24 hours at 30 °C. About 10 mL of the incubated culture was then incubated in 1 L of halal medium for another 24 hours at 30 °C. The final incubated culture was then centrifuged at 3000 rpm for 20-30 minutes to produce supernatant and pellets. The pellets were then added to a solution of 10% skimmed milk and 1% sucrose. The mixture was then frozen at -40 °C for 12 hours, followed by freeze drying at -40 °C for 72 hours to produce a probiotic powder with a viability of 10^9 CFU. The probiotic powder was stored in a freezer at 0-4 °C to keep the CFU viability unchanged until the packing process.

Research subjects

Subjects in this research were screened using inclusion criteria: (1) age range 13 – 18 years, (2) confirmed

physically and mentally healthy by the doctor, (3) able to eat regularly (breakfast, lunch, and dinner), (4) live in Pangururan, Samosir, North Sumatra, (5) not allergic to probiotics nor the placebo ingredients used in the research, (6) agreed to obey the protocol applied, (7) agreed to and be capable of consumption of probiotic powder, (8) be able to read, understand and approve the requirements.

Clinical trial

This research used the Randomized Double-Blind Parallel Placebo-Controlled method. Two groups were created from a total of 54 subjects. The first group was given a probiotic powder called *Lactobacillus plantarum* Dad-13 with 1.18×10^9 and it was referred to as the probiotic group. The second group was given a placebo which was skimmed milk powder and it was named the placebo group. For the consumption phase, the product was given to subjects to be consumed in the class at recess. Subjects were also given food and medical records and a subject's diary to keep track of their daily diets, medicine intake, and defecation frequency. This research was conducted for 43 days, consisting of 10 days for the preliminary phase and 33 days for the consumption phase.

Fecal Sampling

The fecal sampling of subjects was conducted before (10th day) and after consumption (43rd day). An icebox containing a fecal sampling kit was distributed to each subject. The fecal kit consisted of a fecal tube, ice gel, trail paper, rubber gloves, and a surgical mask for fecal sampling. Iceboxes were distributed to the subjects one day before the day of fecal sampling, and the subjects had the sampling techniques explained to them. Fecal samples were collected the following morning, and the received samples were stored in a refrigerator.

Gut Microbiota Population Analysis

DNA extraction from fecal samples

The extraction of DNA was conducted using ZymoBIOMICS™ DNA Miniprep Kit (D4300). 15 samples from subjects were chosen for isolation. The isolation was done following the instructions from the kit with a minor modification.

Microbiota population analysis using qPCR on fecal sample

The primers used in this research are g-Prevo-F (CACRGTAAACGATGGATGCC) and g-Prevo-R (GGTCGGGTTGCAGACC) for *Prevotella*, g-Bfra-F2 (AYAGCCTTTCGAAAGRAAGAT) and g-Bfra-R (CCAGTATCAACT GCAATTTTA) for *Bacteroides fragilis*, g-Ccoc-F (AAATGACGG TACCTGACTAA) and g-Ccoc-R (CTTTGAGTTTCATTCTTGCGAA) for *Clostridium coccoides*. g-Bifid-F (CTCCTGGAA ACGGGTGG) and g-Bifid-R (GGTGTTCTTCCC GATATCTACA) for *Bifidobacterium*, sg-Lpla-F (CTCTGGTATTGATTGGTGCTTGCAT) and sg-Lpla-R (GTTCGCCACTCACTCAAATGTAAA) for *L. plantarum* subgroup, and En-lsu-3F (TGCCGT AACTTCGGGAGAAGGCA) and En-lsu-3R (TCAAGG ACCAGTGTTCAGTGTC) for *Enterobacteriaceae* (Matsuda *et al.*, 2009). The settings of specification for qPCT used were: initial denaturation at 98 °C for 120 seconds; denaturation at 98 °C for 1-5 seconds; annealing at 55-60 °C for 1-5 seconds and melt curve at 65-98 °C for 2-5 seconds.

Short-chain fatty acids (SCFA) compounds analysis

The SCFA analysis used the methods explained by Salazar *et al.* (2014). Gas chromatography (GC) (Shidmadzu, GC 2010 plus series) was used to take measurements. The instrument was equipped with a 240 °C injector, RTX-Wax column, 30 m column length, 145 °C column temperature, 0.25 diameter, 0.8 minutes column flow, and helium as the carrier gas. The measurements were detected using a flame ionization detector (FID) at 240 °C. About 0.5 – 1 gram of fecal sample was added to aquadest at a ratio of 1:3. Afterward, the combination was agitated for five minutes using a vortex, and then subjected to centrifugation at a speed of 10000 rotations per minute for ten minutes. The supernatant produced was then analyzed using gas chromatography.

Fecal Sample Characteristics Analysis

The characteristics analyzed in this research were pH, consistency, color, and defecation frequency over a 10-day period and total days of defecation over 10 days. Fecal pH analysis was conducted according to Matsumoto *et al.* (2006). About 0.5 - 1 gram of fecal sample was added to aquadest at a ratio of 1:5 in a falcon tube. The mixture was then measured using a pH meter electrode.

Fecal consistency was recorded every time subjects defecated in the Subject's daily record that had been distributed to each subject, and self-measurement of consistency was undertaken using the Bristol Stool Form Scale. Fecal color was also recorded by subjects in their daily records. The scale used was in accordance with the four scales explained by Utami *et al.* (2015).

Data Analysis

The data obtained were processed using a completely randomized design. Statistical analysis was conducted

using the SPSS V.20 data processing program (paired t-test analysis and Wilcoxon signed-rank test with $\alpha = 0.05$.

RESULT AND DISCUSSION

Research Subjects

After subject screening, according to inclusion criteria throughout the research, there were 54 subjects. The data can be seen in Table 1. The 54 subjects at the beginning of the research were reduced to 47 due to 7 subjects being refused permission to continue by their parents during the research. Therefore, at the end of the research, the 47 remaining subjects were divided into two groups comprising 23 subjects from the placebo group and 24 subjects from the probiotic group. Of the total of 54 subjects, 35 subjects were male, and 12 subjects were female. Subject data are displayed in Table 1.

Table 1. The data subject followed study

Category	Amount
Subject	54
Gender	
• Female	12
• Male	35
Discontinued	7
Age (years)	13-14
Groups	
• Placebo	23
• Probiotic	24
Undergo medical treatment	0 (0%)
Previous medical history	0 (0%)

The Effects of Probiotic Powder Consumption on Subjects' Weight and Height

Subjects were measured for their weight and height data before and after the consumption phase to investigate the effects of probiotic powder consumption on subjects' weight and height. The data for height and weight difference after the intervention period is presented in Figure 1.

In the placebo group, the results experienced a significant increase in height from before (149.05 ± 5.19 cm) and after (149.42 ± 5.03 cm) the consumption period following statistical analysis with $p < 0.05$. For the weight data in the placebo group, the increase was insignificant, i.e., from 42.55 ± 4.52 kg to 43.18 ± 5.03 kg. In the probiotic group that had consumed the probiotic powder, significant increases in both weight and height were shown in the data. The height data increased from 153.85 ± 4.63 cm to 154.37 ± 4.67 cm. At the same time, the weight data showed a climb from 44.35 ± 4.61 kg to 45.20 ± 4.78 kg. Previous research that studied probiotics' effects on weight also showed an increase after the probiotics intervention. Angelakis *et al.* (2012) reported an increase in the weight of mice that had been given *L. ingluvei* probiotics with a viability of 4×10^{10}. The weight increase only manifested in the group that consumed the probiotic powder, while height data showed increases in both placebo and probiotic groups. However, there were no significant differences in the weight and height gained between groups. The findings indicate that consuming probiotic was insignificant towards body growth in students SMPN 1, Samosir regency. This could be due to the subjects still being in their growing period. A review by Onubi *et al.* (2012) discussed that five papers showed a positive effect of probiotics on child growth, while the other seven studies reported no significant difference in children's growth. The review also concluded that the scarce amount of evidence suggesting probiotics can boost child growth in poor countries and malnourished children indicates the importance of further research to obtain a deeper understanding of the role of probiotics in children.

The Effects of Probiotic Powder Consumption on Gut Microbiota

Probiotic consumption was also analyzed to discover its effects on the population of gut microbiota. The data on the effects of consumption of probiotics on gut microbiota *Prevotella, Bacteroides fragilis, Clostridium coccoides, L. plantarum, Bifidobacterium*, and *Enterobacteriaceae* are displayed in Figure 2.

The data we collected not only described the microbiota populations before and after taking probiotics but also enabled us to obtain subject data that shows experiences of decrease and increase in microbiota numbers. The data are shown in Table 2.

The consumption of indigenous probiotic powder containing *L. plantarum Dad-13* influences the number of *Prevotella*. Before consumption, the amount of *Prevotella* was 9.26 ± 0.82 Log_{10} cells/g, and after consumption, it decreased insignificantly to 9.15 ± 0.91 Log_{10} cells/g. The prevalence data showed an increase from 87% to 100% despite a decrease in the average number of *Prevotella*. From Table 2, it can be seen that 9 subjects experienced

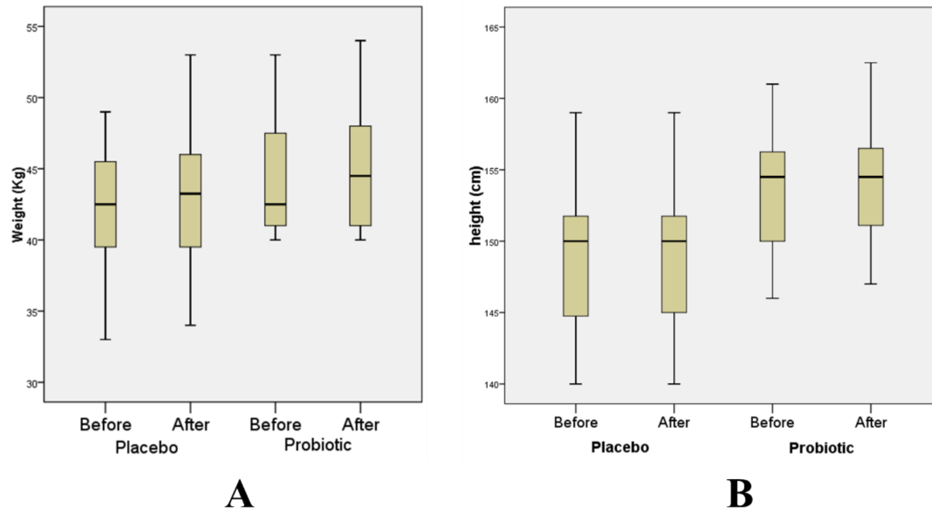

Figure 1. The change of height (A) and weight (B) of subjects after the intervention period

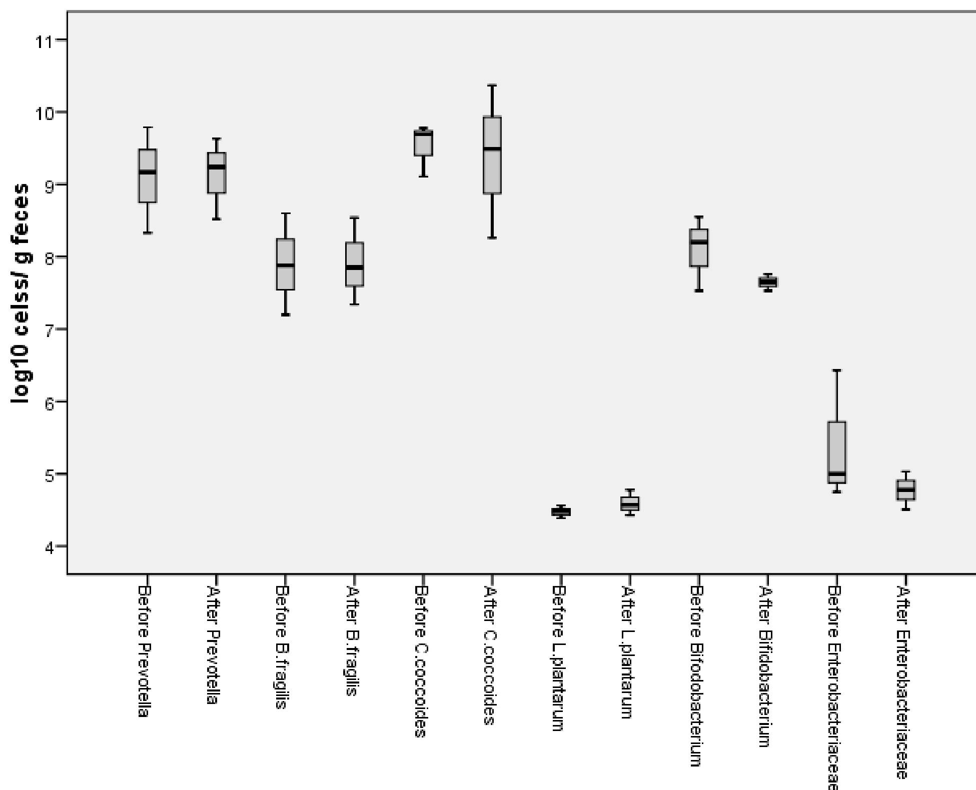

Figure 2. The effect of probiotic consumption on gut microbiota on probiotic group

an increase in *Prevotella* population with an average increase of 1.56 Log_{10} cells/g, while the number of *Prevotella* decreased in 6 subjects with an average decrease of 1.03 Log_{10} cells/g. On average, the population of *Prevotella* experienced a decline, but from Table 2, more subjects experienced an increase in *Prevotella* levels than a decrease. The downturn in population can be seen both at a minimum and a maximum before and after the consumption period. After the consumption of probiotic powder by subjects, the number of *Prevotella* underwent a fall. This decline in *Prevotella* due to probiotic consumption is also shown in research by Toscano *et al*. (2017). The population of *Prevotella* was declining in the subjects that had consumed *Lactobacillus kefiri* LKF01 probiotics. Within a month after probiotics consumption, *Prevotella* population re-analysis showed a slight increase, though not as much as before. Another research to examine the impact of indigenous powder *L. plantarum* Dad-13 on adolescent subjects in Yogyakarta was conducted for 65 days, and the results were as

Table 2. Increase, decrease, and prevalence of microbiota population

	Increment	Average (min-max)*	Descent	Average (min-max)*
Prevotella	9	1.56 (0.08 to 6.07)	6	1.03 (-1.72 to -0.11)
Bacteroides fragilis	7	1.04 (0.13 to 1.92)	8	1.71 (-4.83 to -0.14)
Clostridium coccoides	9	1.67 (0.11 to 5.61)	5	2.64 (-5.66 to -0.29)
L. plantarum	5	0.56 (0.01 to 0.53)	4	-0.12 (-0.33 to -0.04)
Bifidobacterium	6	2.36 (0.55 to 5.43)	5	-0.53 (-0.79 to -0.11)
Enterobacteriaceae	4	0.35 (0.06 to 0.52)	6	-0.93 (-1.92 to -0.30)

*Log_{10} cells/g fecal

follows: the number of *Prevotella* before consumption was 10.45 Log10 cells/g while after consumption of probiotics, it was 9.60 Log_{10} cells/g. The population of *Prevotella* was seen to be higher in Yogyakarta than in Samosir, both before and after the consumption of probiotic powder, although the research in Yogyakarta did not show a significant result ($p > 0.05$) (Panjaitan *et al*., 2018).

From the results shown, it can be re-iterated that the intake of indigenous probiotic powder containing *L. plantarum* Dad-13 cannot affect the population of *Prevotella*. This could be due to *Prevotella* being an enterotype for Indonesians. *Prevotella* is usually connected to dietary habits that incorporate a high carbohydrate intake. This is also supported by Nakayama *et al.* (2015), who stated that in children within the age range of 7-11 years, the most dominant enterotype is *Prevotella*. Data from the Health Research and Development Agency (Anonymous., 2014) revealed that in a study of the total diets of Indonesian people in the year 2014, protein intake was 63.2 grams/day, which was higher than the average national consumption of only 56.3 grams/day. Compare this with figures for carbohydrate consumption based on data from the same study. Carbohydrate consumption in North Sumatra was 239.3 grams/day, which was lower than the national consumption of carbohydrates at 241.7 grams/day. Even though carbohydrate consumption was lower than the national average, consumption of carbohydrates was still higher than protein. Gut microbiota composition can be affected by the dominance of *Prevotella*, which is marked as gut microbiota from a diet rich in carbohydrates.

Another microbiota studied in this research is *Bacteroides fragilis.* This microorganism is usually related to animal protein consumption. From the data from Figure 1, the population of *B. fragilis* before consumption was 7.52 ± 0.95 Log_{10} cells/g and 8.01 ± 0.68 Log10 cells/g after consumption. However, the incline was insignificant ($p > 0.05$) and prevalence data displayed a reduction from 100% to 80%. Table 2 showed that 7 subjects experienced an increase in levels of *B. fragilis* while the other 8 subjects registered a decrease. The average increase of *B. fragilis* was 1.04 Log_{10} cells/g, while the average decrease was 1.71 Log_{10} cells/g. According to Table 2, although there was an incline in the number of *B. fragilis*, more subjects showed a decrease than an increase. The population that was detected before and after the intervention had different minimum and maximum values. Panjatan *et al.* (2018) also investigated the effects of consuming indigenous probiotic powder containing *L. plantarum* Dad-13. From the research, the amount of *B. fragilis* also showed an insignificant result ($p > 0.05$), where the population before consumption was 9.67 Log_{10} cells/g and after consumption was 9.68 Log_{10} cells/g. Based on that result, probiotic powder *L. plantarum* Dad-13 cannot affect the population, but it can change the prevalence of *B. fragilis* by 20%. From Figure 6, it can be seen that the population of *B. fragilis* was lower than that

of *Prevotella*. The dominance of *Prevotella* can be linked to the consumption of high carbohydrate sources. Although protein consumption is high compared to the national figures, consumption of carbohydrate sources in Samosir is still relatively high. This causes the *Prevotella* population to remain high when compared to *B. fragilis*.

The other microbiota analyzed was *Clostridium coccoides*. Analysis of fecal samples to investigate the amount of *C. coccoides* before and after probiotic powder consumption can be seen in Figure 6. Figure 6 shows that consumption of *L. plantarum* Dad-13 probiotic powder did not have sufficient effect to show any significant changes in *C. coccoides* levels. Before the consumption period, the amount of *C. coccoides* was 9.06 ± 0.69 Log_{10} cells/g. After consuming probiotic powder for 33 days, it increased to 9.21 ± 0.74 Log_{10} cells/g. However, the incline of the *C. coccoides* population was insignificant, as shown in the statistical analysis results ($p > 0.05$). The data also showed that the prevalence of *C. coccoides* did not show any alteration; therefore, the number of *C. coccoides* did not change with the addition of *L. plantarum* Dad-13 intake. From the data obtained and shown in Table 2, it can be seen that 9 subjects experienced an increase of *C. coccoides* with an average climb of 1.67 Log_{10} cells/g, while 5 subjects had a decrease in *C. coccoides* population with an average drop of 2.64 Log_{10} cells/g. Although more subjects were shown to have had an increase in the population of *C. coccoides* after probiotic consumption, there was no significant difference in the average population of *C. coccoides*, even after consuming probiotic powder.

Probiotic powder with *L. plantarum* Dad-13 did not have any significant effect on the *C. coccoides* population. According to research by Matsuki *et al.* (2004), the population of *C. coccoides* in a healthy human adult gastrointestinal tract is Log 10.3 ± 0.3 Cells/g feces. Other research by (Kurakawa *et al.*, 2015) showed the number of *C. coccoides* in healthy children to be Log 9.8 ± 0.3 Cells/g feces. These data support the results obtained from this research by showing that the microbiota composition of *C. coccoides* is the same as in an average healthy human.

The numbers of *Lactobacillus plantarum* before and after the consumption period were 4.67 Log_{10} cells/g ± 0.35 and 4.71 Log_{10} cells/g ± 0.33, respectively (Figure 6). There was no significant ($p > 0.05$) change in the *Lactobacillus plantarum* population after consuming *L. plantarum* Dad-13. Rahayu *et al.* (2016) reported no significant change in the *L. plantarum* population of healthy adult subjects who had consumed *L. plantarum* Dad-13 for 10 and 20 days. 5 subjects showed an average increase of 0.56 Log_{10} cells/g fecal matter, while 4 others displayed an average decrease of 0.12 Log_{10} cells/g fecal matter. More subjects showed an increase than a decrease after consuming probiotic powder containing *L. plantarum* Dad-13. However, 6 subjects showed no change in the number of *L. plantarum* Dad-13 after the consumption period. This demonstrated that *L. plantarum* Dad-13 only increased *L. plantarum* in certain subjects. *L. plantarum* could not survive in the colons of North Sumatran subjects. It was shown that prevalence subjects were 7 out of 15 before consuming probiotic powder and 9 out of 15 after. This was contrary to subjects from Yogyakarta, where Marta *et al.* (2018) stated that 20 out of 20 subjects, consisting of healthy adolescents, displayed an incline of *L. plantarum* (average increase 6.14 Log_{10} cells/g fecal matter) after consuming *L. plantarum* Dad-13 for 2 months. These results showed that *L. plantarum* Dad-13 can live inside the human gastrointestinal tract and provide health advantages.

The incompatibility with survival by *L. plantarum* in the colons of subjects in North Sumatra could be caused by the high consumption of animal proteins in the area, as indicated by the percentage of protein intake in Pangururan being similar to the percentage of protein intake of children in the Ormoc region, who consumed a western diet of 13%, higher protein intake than children in the Baybay area, who consume 11% more carbohydrate source foods. (Nakayama *et al*., 2017). It can also be seen from the figures for consumption of animal protein according to Anonymous[b] (2017) and Anonymous[c] (2018) for North Sumatra, which increased from 18.38 to 19.87 grams per capita per day from 2017 to 2018. This resulted in a higher number of pathogenic bacteria than good bacteria, such as *Bifidobacterium* and *L. plantarum*, which prevented an increase in these two bacteria. De Filippo *et al.* (2010) stated that increasing high-fat and animal protein diet consumption could potentially increase *Enterobacteriaceae*. Finegold *et al.* (1974) reported that in two gut microbiota groups consuming a Japanese diet and a Western diet, the populations of *L. plantarum* and *E. coli* were 8.65 log_{10} CFU/g fecal matter and 6.3 log_{10} CFU/g fecal matter for those consuming the Japanese diet and the populations of *L. plantarum* and *E. coli* were 0 log_{10} CFU/g fecal matter and 7.05 log_{10} CFU/g fecal matter for those consuming the western diet. Hentges *et al.* (1977) added that the number of

Bifidobacterium adolescents significantly decreased while consuming a high-beef diet compared to a low-meat-beef diet.

The numbers of *Bifidobacterium* before and after the consumption period were 7.42 ± 1.12 and 7.62 ± 0.90 Log_{10} cells/g fecal matter, respectively (Figure 6). There was no significant ($p > 0.05$) change in the *Bifidobacterium* population after the consumption of *L. plantarum* Dad-13. 6 subjects showed an increase of 2.36 Log_{10} cells/g fecal matter, while 5 other subjects displayed an average decrease of 0.53 Log_{10} cells/g fecal matter. There were more subjects that showed an increase than a decrease. Moreover, 4 subjects did not show any change after the consumption period. This could be due to the constant number of bacteria even after consuming probiotic powder. Because the number of subjects that displayed an increase in *Bifidobacterium* levels was less than half of the total number of subjects, it can be concluded that probiotic powder consumption did not affect the number of *Bifidobacterium*. This result is also supported by other research that reported the same result (Marta *et al.*, 2018). The research stated that no significant changes in *Bifidobacterium* numbers were shown in adolescents in Yogyakarta who had consumed *L. plantarum* Dad-13 for 2 months.

The dosage of probiotics given to subjects can be an influential factor. Research by Sepp *et al*. (2018) and Fitrianingthias *et al.* (2018) showed that the consumption of *L. fermentum* ME-3 probiotics (8×10^9 CFU per ml) and *L. plantarum* Mut-7 (2×10^9 CFU per g) for 4 weeks and 30 days respectively, did not have any effect on *Bifidobacterium* population. Whereas consumption of *L. plantarum* P-8 high dose (6×10^{10} CFU) for 4 weeks can increase the number of *Bifidobacterium* significantly (Wang *et al*., 2014). Other research reported that consumption of a symbiotic containing *L. casei* Shirota (3×10^{10} CFU) and Transgalactosylated Oligosaccharides (GOS) (2.5 g) per 80 ml (per day for 2 weeks) can increase the number of Bifidobacterium significantly (Shioiri *et al*., 2006).

Populations of *Enterobacteriaceae* before and after consumption were 5.13 ± 0.53 and 4.73 ± 0.21 Log_{10} cells/g fecal matter, respectively (Figure 6). There were no significant changes to *Enterobacteriaceae* before and after consumption of probiotic powder *L. plantarum* Dad-13. Sepp *et al.* (2018) reported that consuming a probiotic containing *L. fermentum* ME-3 for 4 weeks did not significantly change *Enterobacteriaceae* numbers. Utami *et al.* (2015) also stated that probiotic consumption of *L. casei* Shirota for 10 days did not significantly change the numbers of *Enterobacteriaceae* in healthy adults' fecal matter.

Populations of *Enterobacteriaceae* increased on 4 subjects, with an average increase of 0.34 Log_{10} cells/g fecal matter. As for subjects that displayed a decrease, 6 subjects had an average decline of 0.93 Log_{10} cells/g fecal matter. Therefore, more subjects showed a decline than showed an increase in populations of *Enterobacteriaceae.* However, 5 subjects did not show any change in *Enterobacteriaceae* populations. It is possible that insignificant changes in bacteria populations can occur due to the condition of the subjects' health. Guerin-Danan *et al.* (1998) reported that total numbers of aerobic bacteria, *Bifidobacteria*, and *Enterobacteriaceae*, in the feces of healthy babies did not show significant changes because the normal and stable condition of healthy subjects stabilizes colonic gut microbiota. Rahayu *et al.* (2016) stated that consumption of *L. plantarum* Dad-13 for 20 days reduced the *Enterobacteriaceae* population. Therefore, more subjects showed a decline rather than an

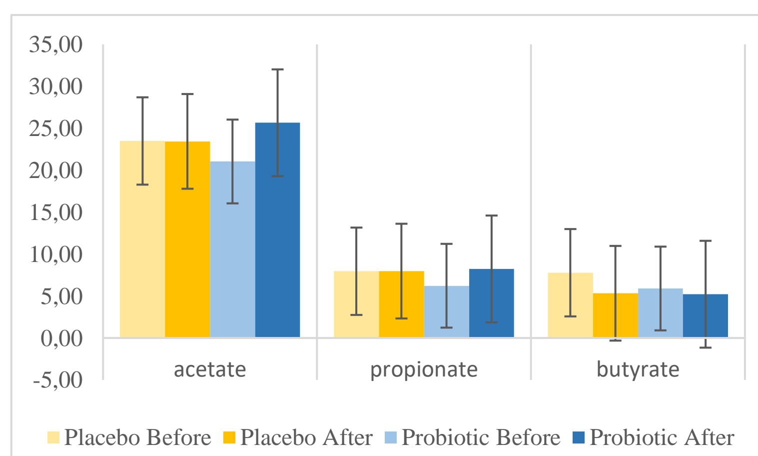

Figure 3. Concentration of Short Chain Fatty Acid (SCFA)

increase in the populations of *Enterobacteriaceae* in 19 out of 30 subjects (average decrease of 0.71 Log_{10} CFU/g fecal matter). To re-iterate, these results showed that *L. plantarum* Dad-13 has the ability to lower the population numbers of *Enterobacteriaceae.*

The Effects of Consumption of Probiotic Powder Containing L. plantarum Dad-13 on Short Chain Fatty Acid (SCFA) Concentrations

The values of acetic acid, propionate acid, and butyrate acid in the placebo group before consumption were 23.48 ± 16.62, 9.69 ± 9.51, and 7.78 ± 9.54, respectively (Figure 3).

The values of SCFA in the placebo group after consumption were 23.43 ± 19.65, 7.98 ± 9.45, and 5.33 ± 6.62 for acetic acid, propionate acid, and butyrate acid, respectively. For the probiotic group, the values of acetic acid, propionate acid, and butyrate acid before consumption were 21.03 ± 18.25, 6.22 ± 6.72, and 5.90 ± 6.39, respectively. The values of SCFA after consumption by the probiotic group were 25.65 ± 10.99, 8.23 ± 6.32, and 5.23 ± 3.41 for acetic acid, propionate acid, and butyrate acid (Figure 7). From the data, it can be concluded that there were no significant changes in both placebo and probiotic groups after consumption of the research products.

The high standard deviation was caused by the spread range of SCFA concentration between subjects being far from the mean of total SCFA concentrations. Some papers also reported a high standard deviation in the SCFA concentrations. Matsumoto *et al.* (2006) said that concentrations of acetate, propionate, and butyrate acids were 52 ± 22; 22 ± 8; 16 ± 9 for the placebo group and 49 ± 19; 19 ± 9; 14 ± 10 for the probiotic group, respectively. Matsumoto *et al.* (2010) said that concentrations of acetate, propionate, and butyrate acids were 143 ± 41; 62 ± 55; 19 ± 15 for the placebo group and 140 ± 39; 45 ± 25; 22 ± 12 for the probiotic group, respectively. Shioiri *et al.* (2006) added that concentrations of acetate, propionate, and butyrate acid were 49 ± 16; 19 ± 7; 1.8 ± 1.0 for the placebo group and 68 ± 14; 20 ± 6; 1.7 ± 0.9 for the probiotic group, respectively.

Even though no significant changes in concentrations of SCFA were displayed, more of the subjects in the probiotic group showed increases in all SCFA concentrations than those who showed decreases. The insignificant changes in the probiotic group could have been due to the insignificant increases of Bifidobacterium and *Lactobacillus plantarum* as the bacteria that produce SCFA in subjects and the short (1 month) consumption period of the probiotics. Research by Marta *et al*. (2018) on probiotic powder consumption of *L. plantarum* Dad-13 by adolescents in Yogyakarta for 2 months showed significant increases in SCFA concentrations, especially the propionate acid, which had a value of 17.15 µmol/g fecal matter, rising to 25.05 µmol/g fecal matter. The insignificant changes in SCFA concentrations could well be due to the healthy condition of the subjects.

Characteristics of Fecal Analysis

The result of the fecal characteristic analysis between the placebo and treatment groups after consuming probiotic powder containing *L. plantarum* Dad-13 is shown in Table 3.

The pH values for the placebo group registered before and after the consumption period showed no significant changes (6.22 ± 0.40 to 6.21 ± 0.53). The same results were also displayed in the probiotic group, with the pH

Table 3. Fecal characteristics data

Item	Placebo (n=20)		Treatment (n=20)	
	Before Consumption Mean ± SD	After Consumption Mean ± SD	Before Consumption Mean ± SD	After Consumption Mean ± SD
pH	6.22 ± 0.40	6.21 ± 0.53	6.20 ± 0.50	6.14 ± 0.55
Consistency (1-7)	4.05 ± 0.51	3.94 ± 0.50	3.88 ± 0.39	3.86 ± 0.43
Color (1-4)	2.18 ± 0.65	2.31 ± 0.46	2.01 ± 0.70	2.01 ± 0.54
Frequency of defecated (number/10 days)	8.06 ± 3.38	7.49 ± 2.86	7.05 ± 2.70	6.50 ± 2.42
Day of defecated (day/10 days)	7.41 ±2.65	7.20 ± 2.63	6.50 ± 2.41	7.06 ± 2.41

value before consumption being 6.20 ± 0.50 and after consumption 6.14 ± 0.55. The insignificance of the changes could have been due to the healthy condition of the subjects and the fact that the research lasted for only 1 month. According to (2003), an investigation into 24 healthy subjects who had consumed *L. plantarum* 299 for 4 weeks found no effects on the pH of feces. Another reason why the changes were not significant was due to the insignificant changes in SCFA concentrations. Shioiri *et al*. (2006) reported that a pH reduction in the colon is caused by organic acids (butyrate acid, propionate acid, and lactic acid) produced by acid-producing bacteria that trigger motility in the colon. Therefore, the pH change in feces was also influenced by quantity and the function of microorganisms present the colon that produce acid, ammonia, or phenols, such as *Enterobacteriaceae*, *Clostridium*, and *Staphylococcus*.

No significant changes were detected in either the probiotic group or the placebo group in the characteristics of fecal consistency, fecal color, defecation frequency within 10 days, and total days of defecation in 10 days. This insignificant change in both groups could be due to the healthy condition of the subjects. Goossen *et al*. (2003) stated that there was no evidence of changes in fecal consistency and defecation frequency in 24 subjects who had consumed *L. plantarum* 299 probiotics for 4 weeks.

CONCLUSIONS

This study indicates that the intake of indigenous probiotic powder containing *L. plantarum* Dad-13 for 33 days can lead to an increase in height, weight, and body mass index (BMI) in adolescents at SMPN 1 Pangururan, Samosir. However, the population of gut microbiota, SCFA, and fecal characteristics did not change after the consumption period. However, more subjects in the probiotic group had an increase in SCFA concentrations than in the placebo group. This could have been caused by the healthy condition of the subjects and the research period.

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