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Dietary patterns and effect of consumption of probiotic powder containing indigenous bacteria *Lactobacillus plantarum* Dad-13 on *Streptococcus*, *Enterococcus*, *Escherichia coli* and *Klebsiella pneumoniae* in the gut of students at Junior High School Pangururan 1, Samosir

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Abstract

Diet is regulating the amount and type of food in order to prevent or help cure diseases. Carbohydrates, proteins, and fats are the main components in diet; the type of food will affect the variation of intestinal microbiota, and changes in dietary pattern may cause change in population structure of gut microbiota. The present work aimed to determine the dietary pattern in students at Junior High School Pangururan 1, Samosir, and investigate the consumption effect of indigenous probiotic powder *Lactobacillus plantarum* Dad-13 on bacteria (*Streptococcus, Enterococcus, Escherichia coli,* and *Klebsiella pneumoniae*) in the gut. The study was conducted for 43 d (33 d intervention) in 40 adolescent subjects aged 13 - 14 years, divided into placebo and probiotic groups. The research design used was Randomised Double-Blind-Placebo-Controlled. The results showed that the energy intakes for the placebo and probiotic group were 70 and 72.84%, respectively, of the Recommended Dietary Allowances (RDA) requirement. Results showed that the consumption of probiotic powder of *L. plantarum* Dad-13 for 33 d decreased the *Streptococcus* and increased *Enterococcus*, *E. coli*, and *K. pneumoniae*; however, the effect was not significant ($p > 0.05$). The inability of *L. plantarum* Dad-13 to affect the intestinal microbiota may be related to the high number of *Streptococcus, Enterococcus, E. coli,* and *K. pneumoniae* in the digestive tract of subjects. Adolescents in Samosir consumed a lot of fatty and high-protein foods such as pork, beef, and fish. This could be the reason of high population of Enterobacteriaceae in the intestinal microbiota of the students at Junior High School Pangururan 1, Samosir.

Introduction

Diet is a way or effort to regulate the amount and type of food by maintaining health and nutritional status in order to prevent or help cure diseases (Kemenkes, 2010). A good diet is a diet that is guided by balanced nutrition. Balanced nutrition contains more or less the same components, namely: sufficient quantity, sufficient quality, energy, protein, vitamins, and minerals needed to maintain health, and to carry out daily activities for all age groups and physical conditions (Kemenkes, 2015). Daily diet pattern has high effect on the variation of gut microbiota because food is the main energy source for gut microbiota, and change in diet pattern may result in structural change of microbiotic population.

The composition of microbiota is affected by many factors, for example dietary habits, age, and environmental factors (geographically) (De Filippo *et al.,* 2010). Carbohydrates in the digestive tract which cannot be digested by the body are used for microbiota growth nutrients; meanwhile, degraded proteins in the large intestine are suitable conditions for the growth of proteolytic bacteria; and high fat consumption will induce changes in the composition of intestinal microbiota and reduce the number of good bacteria in the digestive tract (Riaz Rajoka *et al.,* 2017).

Europeans with western diet had a high *Bacteroides* in their digestive tracts. Meanwhile rural children in Africa who consumed more plant-based foods had high *Prevotella* (De Filippo *et al.,* 2010). The population of gut microbiota among children in China (Lanzhou and Beijing), Japan (Tokyo and Fukuoka), and Taiwan (Taipei and Taichung) was dominated by the *Bifidobacterium* and *Bacteroides* group because they consumed a lot of high-protein foods. The gut

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microbiota of the subjects in Khon Kaen (Thailand) was dominated by *Prevotella* group because they consumed a lot of carbohydrates, and that in Bangkok (Thailand) was dominated by the *Bacteroides* group (Nakayama *et al.,* 2015).

Indonesia is a country that has a broad geographical area, a diversity of cultures, and different eating habits in each region which can form microbiotic variations. The population of children's intestinal microbiota in Yogyakarta and Bali was dominated by the *Prevotella* group because children consumed a lot of high-carbohydrate foods (Nakayama *et al.,* 2015). Another area that has a lot of diversity is Samosir, because it is a small island in North Sumatra which is located in the middle of Lake Toba, and food in Samosir comes from animal protein such as pork, beef, chickens, and fish caught on the lake. In addition to diet and geographical location, another factor that affects intestinal microbiota is probiotics.

Probiotics are a group of living microorganisms that provide beneficial health effect to human body if they are consumed adequately (FAO and WHO, 2011). FAO and WHO suggested that the adequate quantity is 10^6 - 10^8 CFU/g, which is expected to increase by 10^{12} CFU/g in the colon. Indonesia has several probiotic isolates of which the potentials and beneficial is *Lactobacillus plantarum* Dad-13, which is indigenous to Indonesia. It has resistance to gastric acid in low pH, and able to inhibit the growth of pathogenic bacteria (Rahayu *et al.,* 2016a). Previous study found that it could prevent the growth of *Escherichia coli* in Wistar rat faeces (Sumaryati *et al.,* 2009). *Lactobacillus plantarum* Dad-13 is able to survive in the gastrointestinal tract of healthy Indonesian subjects and reduces Enterobacteriaceae, *E. coli*, and non-*E. coli* coliform in faeces (Rahayu *et al.,* 2016a).

The composition and variation of gut microbiota in each individual are different, and one of the available methods to understand the composition and variation of microbiota is polymerase chain reaction (PCR). Real-Time PCR, or also known as qPCR, is the most sensitive PCR tool to detect and measure the quantity of mRNA, including in small-size sample. This technique is also sensitive to enable amplification to occur coincidentally and sequential quantity of nucleic acid can be understood. Not only does it have higher sensitivity, it is also a more excellent and dynamic test than conventional PCR, as well as has lower cross contamination risk and high applicability (Kubista *et al.,* 2006).

In Indonesia, the information about microbiota ecosystems in human gut and diet continue to be gathered through research. Samosir region in the province of North Sumatra was chosen because there is no previous study on the dietary pattern and human gut microbiota. The population in Samosir also has diverse dietary habits that are different from other regions in Indonesia. Based on the above-mentioned consideration, the present work was conducted to investigate the dietary pattern and the effect of the consumption of indigenous probiotics of *L. plantarum* Dad-13 powder on *E. coli, Streptococcus, K. pneumoniae,* and *Enterococcus* in the guts of adolescents in Samosir, North Sumatra. The present work used faecal samples from subjects between the ages of 13 - 14 years old, specifically in Junior High School Pangururan 1, Samosir.

Materials and methods

Location and time of research

The present work was conducted in Junior High School Pangururan 1, Pangururan, Samosir, North Sumatra from February 2018 - March 2019. Samples of faeces were analysed in Microbiological Laboratory, Universitas Gadjah Mada, Yogyakarta.

Instrument and materials

Instrument and materials used in the present work were faecal samples, appropriate growth media, bacterial cultures, skim milk, sucrose 1%, ZymoBIOMICS™ DNA Miniprep Kit (750 µL lysis solution, 1,200 µL binding buffer, 400 µL wash buffer 1, 700 µL wash buffer 2, and DNase free water), 2N NaOH, Na/K-phosphate, *p*-isopropylphenol, box faecal kit (faecal bottle, trail paper, plastic glove, masker, and ice gel), and q-PCR.

Research subjects

The present work involved 40 subjects consisting of students at Junior High School Pangururan 1, Samosir, whose age ranged from 13 to 14 years old. The exclusion criteria of the subjects' screenings were actively consuming alcohol beverages; consuming antibiotics, laxative, yoghurt, and probiotic/prebiotic food products; using drugs or having history of drug use; have gastrointestinal tract surgery (except appendectomy), and suffering from gastrointestinal disorders periodically (such as constipation and diarrhoea).

Production of probiotic powder

Probiotic powder used in the present work was packed (1 g) and stored in a refrigerator to keep the viability of 10^9 cells/g. Probiotic culture of *L. plantarum* Dad-13 was obtained in pure and freeze-dried form from Food Nutrition and Culture Collection (FNCC), Universitas Gadjah Mada. Pure culture of the probiotic

(50 µL) was incubated in halal media for 24 h at 30°C. From the incubated culture, approximately 1.5 mL was taken and incubated in 150 mL halal media for 24 h at 30°C. About 10 mL from the incubated culture was taken to be incubated in 1 L of halal media for another 24 h at 30°C. The final incubated culture was then centrifuged at 3,000 rpm for 20 - 30 min to produce supernatant and pellet. Approximately, 10% skim and 1% sucrose solution were added into the pellet. The pellet was then frozen at -40°C for 12 h, and freeze-dried at -40°C for 72 h to produce probiotic powder with viability of 109 cells. The probiotic powder was stored in a freezer at 0 - 4°C to keep the viability cells stable until packing process.

Subjects' intervention

The intervention used Randomized Double-Blind Placebo-Controlled. A total of 40 subjects were equally divided into two groups; the treatment groups that consumed probiotic power and the placebo group that consumed placebo (skim milk). The Double-Blind design was chosen to avoid bias for both the researchers and subjects. During the consumption phase, the product was given to the subjects during school break. The subjects were also given food record, medical record, and subject's diary to keep in track of the subject's daily diet, medicine intake, and defecation frequency. The present work was conducted for a total of 43 d, which consisted of 10 d for preliminary phase, and 33 d for consumption phase.

Faecal sampling

Sampling was conducted before (on the 10th day) and after consumption (on the 43rd day). The sample was collected by distributing icebox containing faecal kit for each. The faecal kit consisted of faecal tube, ice gel, trail paper, rubber gloves, and surgical mask for faecal sampling. Icebox was distributed to the subjects one day prior to the faecal sampling. An instruction on how to take the faecal sample was explained to ensure a correct way of taking sample. Faecal samples were collected in the morning and subsequently stored in the refrigerator.

Sample analysis

Analysis of diet using NutriSurvey software

Analysis of diet in adolescent was performed using NutriSurvey software. NutriSurvey is a program used to analyse nutrient content (macronutrient and micronutrients) from food based on age and sex. The selected subjects were given a form to record their food consumption every day for 33 d. The data of food type and amount consumed were acquired to count the total calorie and nutrient intake (macronutrient and micronutrient) per day as percentage (%) of the recommended requirements based on age and sex which were later compared with the RDA table. This analysis showed whether the subjects' energy and nutritional needs were fulfilled.

The analysis of the distribution of energy and nutrient intake from food ingredients was performed by dividing the food ingredients consumed based on each food group, and expressed in percent (%) to see how much (%) a food group contributed to the energy and nutrient intake of the subjects for 33 d. The food types were grouped by looking at the shopping list in NutriSurvey software, after which a diagram was drawn to describe the food intake based on their nutritional content. Data analysis was performed using SPSS 16.0 software by normalising the data using the Paired *t*-Test advanced test with significance of $p < 0.05$, and the prevalence was calculated using the Microsoft Excel program.

DNA extraction of faecal samples

Isolation of DNA was done using ZymoBIOMICSTM DNA Miniprep Kit (D4300). There were 15 samples from the subjects that were chosen for isolation. The isolation was done by following the instructions from the kit with slight modification.

Analysis of gut microbiota using quantitative-PCR (q-PCR) method

Analysis of q-PCR started with preparing 20 µL PCR mix with the composition of 10 µL SYBR Green I master mix, 7 µL distilled water, 1 µL forward primer, 1 µL reserve primer, and 1 µL DNAse free water. Then, PCR program was performed, and the results interpreted. DNA primer sequences of the specific microbiota were: *Streptococcus* F-Strep (CTWACCAGAAAGGGAC GGCT) and R-Strep (AAGGRYCYAACACCTAGC), *Enterococcus* g-Encoc-F (ATCAGAGGGGGATAACACTT) and (ACTCTCATCCTTGTTCTTCTC), *E. coli* F-Ecoli (CATGCCGCGTGTATGAAGAA) and R-Ecoli (CGGG TAACGTCAATGAGCAAA), and K. pneumoniae K. pneumoniaeF (CCTGG ATCTGACCCTGCAGTA) and K. pneumoniaeR (CCGTCGCCGTTCTGTTTC).

Results and discussion

Research subjects

The present work involved 40 subjects between 13 to 14 years old who were equally divided into placebo and probiotic groups. The inclusion criteria of the subjects screenings were not consuming alcohol beverages; not taking any antibiotic or

antimycotic medication, antidiarrheal or laxative medication, and not consuming probiotic or prebiotic foods as part of their daily diet for 30 d prior to the study; not having the history of gastrointestinal tract surgery (except appendectomy) and not suffering from gastrointestinal disorders periodically (such as constipation and diarrhoea).

The present work was conducted for 43 d consisting of 10 d period before consuming probiotic powder, and 33 d period of consuming probiotic powder. The 10 d period before consumption was used to determine the initial condition of the gastrointestinal tract before the addition of probiotic powder, and the 33 d period of consumption was used to determine the effect of consumption of probiotic powder on the diet and the effect of *L. plantarum* Dad-13 on *E. coli, Streptococcus, K. pneumoniae,* and *Enterococcus* in the gut.

Dietary patterns in Junior High School Pangururan 1, Samosir

The subjects were asked to record the data on the type and amount of foods consumed for 33 d (during the intervention period), which later were analysed using NutriSurvey software. The average percentage of energy, macronutrient, and micronutrient intake was calculated based on age and sex. Next, the differences of diet and distribution of macronutrients (carbohydrates, proteins, and fats) and micronutrients (food fibre, vitamins A, E, B^1, B^2, B^6, C, calcium, magnesium, iron, and zinc) were observed.

Eating various types of nutritious foods is beneficial for one's health, and provides energy for growth and activity. Healthy foods are rich in nutrients which are important for the body's metabolisms and functions. Distribution based on the average of supply energy and macronutrient can be seen in Table 1. The average energy intake in Table 1 of the placebo group was 1,419.1 kcal, and that in the probiotics were 1,483.3 kcal with *p*-value of 0.05. This means that the energy intake of placebo and probiotic group was comparable. The recommendation for energy needs for adolescents aged 13 - 14 years is 2,036.3 kcal, therefore, the placebo group met 70%, whereas the probiotic group 72.84% of the total recommended energy. According to Indonesia Basic Health Research (2013), a person is categorised as having malnutrition status when his/her energy intake is < 70% of the recommended. The students of Junior High School Pangururan 1, Samosir, were close to under-nutrition status. The standard recommended total energy intake by the Ministry of Health of the Republic of Indonesian (2012), for healthy people is 97.5%. Based on the data on the amount of carbohydrates, proteins, and fats, the students of Junior High School Pangururan 1, Samosir, did not meet the recommended nutritional standards.

Results showed that carbohydrates were mainly sourced from grains, tubers, nuts, and sugars. The types of processed carbohydrates were rice, noodles, rice noodles, breads, flour, and syrup. The source of proteins was divided into animal protein and vegetable protein. The sources of animal protein were milk, meat, poultry, fish, and seafood, while the sources of vegetable protein were tofu, tempeh, and beans. The types of food in Pangururan included stewed chicken, grilled pork, sautéed anchovy, cow's milk, fried/grilled chicken, fried shrimp, sautéed long beans, fried tofu, and fried tempeh. The main sources of fats were nuts, seeds, meat, chicken, cream, milk, egg yolks, and foods cooked using oil. Some examples of foods included boiled peanuts, boiled eggs, fried fish, fish soup, fried pork, beef curry, chicken curry, fried chicken, fried tofu, and fried tempeh. In fact, there were no differences in the types of food consumed by the placebo and probiotic groups, and this was the same every day.

The daily consumption and contribution of each type of food to the energy intake of adolescent among the students of Junior High School

Table 1. Distribution based on average intake and macronutrients for 33 d.

Food intake category	Average intake ± SD		Recommendation	% RDA		*p*-value
	Placebo (*n* = 20)	Probiotic (*n* = 20)		Placebo	Probiotic	
Energy (Kcal)	1,419.1 ± 43.2	1,483.3 ± 32.9	2,036.3	70.00	72.84	0.05
Carbohydrate (g)	232.4 ± 6.0	233.2 ± 5.1	290.7	79.94	80.22	0.11
Protein (g)	47.3 ± 2.4	48.0 ± 1.8	60.1	78.70	79.87	0.10
Fat (g)	31.1 ± 1.9	38.0 ± 0.8	69.1	45.01	54.99	0.03*

Data are mean ± standard deviation. * = $p < 0.05$. RDA = Recommended Dietary Allowances. Significant differences between the placebo and probiotic groups (*t*-test / Wilcoxon test), and average intake (%) of nutritional adequacy rates that are met.

Pangururan 1, Samosir, can be seen in Figure 1. Based on the percentage of the total energy as shown in Figure 1 between the placebo and probiotic groups, there was a difference in the amount of food consumed even though the difference of energy intake between placebo and probiotic group was not significant. The types of food consumed were grouped according to food groups including rice and porridge, noodles, bread and cereals, fish and seafood, chicken, meat, eggs, legumes, fruits, vegetables, snacks, drinks, and milk. Based on these data, the main intake category of the students in Junior High School Pangururan 1, Samosir, was from carbohydrates and proteins. In terms of the highest energy intake, the food consumption pattern consisted of rice, noodles, and fish. In terms of the highest percentage of macronutrient adequacy, foods belonging to carbohydrate groups were rice, noodles, and snacks; those belonging to protein groups were fish, meat, chicken; and those belonging to fat groups were fish and snacks. This result is in line with the data of the Ministry of Health of the Republic of Indonesia (2017) which reported that rice is the main energy source and is the staple food.

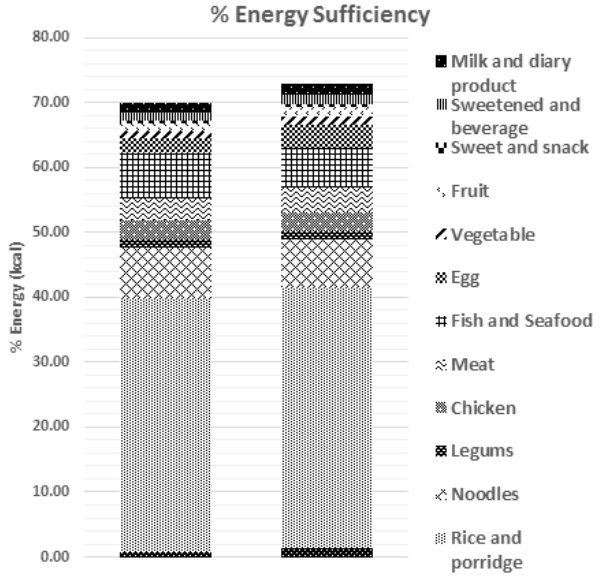

Figure 1. Daily consumption and contribution of each type of food to energy intake.

In addition to the average macronutrient intake, the present work also looked at the average micronutrient intake. Micronutrients are nutrients that the body needs in relatively small amounts, but have a very important role in hormone formation, enzyme activity, and in the regulation of the immune system function and the reproductive system. Based on Table 2, the micronutrient intake did not meet the recommended standards. In terms of vitamins A and C, calcium, and magnesium intake, the results showed p-value of < 0.05 for both the placebo and probiotic groups, which means that there was a significant difference between the results of vitamins A and C, calcium, and magnesium intake in the placebo group and the probiotic group.

Effect of probiotic consumption on gut microbiota

The population of intestinal microbiota was also analysed to determine the effects of probiotic consumption on intestinal *Streptococcus, Enterococcus, E. coli,* and *K. pneumoniae*. A study by Rahayu *et al.* (2016b) showed that *L. plantarum* Dad-13 is a local strain that has a potential as a probiotic agent. It can be used as a starter culture to produce fermented milk drinks or probiotic powder which is favoured by consumers, and can nourish the intestines as it can help to absorb macronutrients and micronutrients. *Lactobacillus plantarum* Dad-13 can survive in a bile salt up to a concentration of 3%, with an incubation time of 6 h, and an average mortality rate of 2 - 3 log cycles. The results of the effects of probiotic consumption on intestinal *Streptococcus, Enterococcus, E. coli,* and *K. pneumoniae* can be seen in Table 3.

From Table 3, it is apparent that *Enterococcus, E. coli,* and *K. pneumonia* increased after consuming *L. plantarum* Dad-13 powder for 33 d, but the increase was not significant ($p > 0.05$). From the data obtained, the number of *Enterococcus* increased from $\log^{10}$ 6.42 CFU/g (before consumption) to $\log^{10}$ 6.49 CFU/g (after consumption). The increasing trend was also observed in *E. coli* and *K. pneumoniae.* The increase in *Enterococcus* was from 0.36 to 3.60 log cycles, *E. coli* increased from 0.08 to 1.87 log cycles, and *K. pneumonia* increased from 0.02 to 1.56 log cycles.

Results indicated that the consumption of probiotic *L. plantarum* Dad-13 for 33 d could decrease the number of *Streptococcus* in subjects. *Streptococcus* population before consumption was $\log_{10}$ 6.59 CFU/g, and after consumption it became $\log_{10}$ 6.45 CFU/g, but the decrease in *Streptococcus* population was not significant ($p > 0.05$). Based on Table 3, the average of increase in *Streptococcus* was $\log_{10}$ 0.07 CFU/g, while the average of decrease was $\log_{10}$ 2.2 CFU/g. *Lactobacillus plantarum* can produce lactic acid which can reduce the pH of the substrate, thus resulting in acidic condition, which can inhibit the growth of *Streptococcus*. The effect of consuming *L. plantarum* Dad-13 powder can be seen in Figure 2.

Probiotic efficacy relies on its ability to survive in the digestive system and be able to

Table 2. Distribution based on the average dietary fibre and micronutrient intake for 33 d.

Intake category	Average intake ± SD		Recommendation	% RDA		*p*-value
	Placebo (*n* = 20)	Probiotic (*n* = 20)		Placebo	Probiotic	
Dietary fibre (g)	7.00 ± 0.38	7.60 ± 0.46	30	23.33	25.33	0.10
Vitamin A (µg)	333.90 ± 27.40	477.90 ± 69.95	1000	33.39	47.79	0.04*
Vitamin E (mg)	3.10 ± 0.38	4.00 ± 0.20	12	25.83	33.33	0.07
Vitamin B_1 (mg)	0.50 ± 0.26	0.50 ± 0.31	1.1	45.45	45.45	0.26
Vitamin B_2 (mg)	0.50 ± 0.00	0.60 ± 0.05	1.3	38.46	46.15	0.05
Vitamin B_6 (mg)	0.80 ± 0.03	0.90 ± 0.04	1.4	57.14	64.29	0.08
Vitamin C (mg)	32.30 ± 8.44	31.90 ± 8.45	100	32.30	31.90	0.04*
Calcium (mg)	543.70 ± 24.23	604.50 ± 17.58	1200	45.31	50.38	0.03*
Magnesium (mg)	268.00 ± 6.05	294.80 ± 15.24	310	86.45	95.10	0.04*
Iron (mg)	4.70 ± 0.30	5.10 ± 0.30	15	31.33	34.00	0.15
Zinc (mg)	5.00 ± 2.00	5.10 ± 2.08	7	71.43	72.85	0.19

Data are mean ± standard deviation. * = $p < 0.05$. RDA = Recommended Dietary Allowances. Significant differences between the placebo and probiotic groups (*t*-test / Wilcoxon test), and average intake (%) of nutritional adequacy rates that are met.

Table 3. Changes in the population of intestinal microbiota in the faeces of the subjects of the probiotic group analysed using qPCR.

	Streptococcus **(log_{10} cells)**	***Enterococcus*** **(log_{10} cells)**	***E. coli*** **(log_{10} cells)**	***K. pneumoniae*** **(log_{10} cells)**
Increase subject*	6 (40%)	10 (66.67%)	11 (73.33%)	8 (53.33%)
Average (min-max)*	0.07 (0.07-1.45)	0.37 (0.36-3.06)	0.12 (0.08-1.87)	0.03 (0.02-1.56)
Decrease subject*	8 (53.33%)	4 (26.67%)	2 (13.33%)	7 (46.67%)
Average (min-max)*	2.2 (0.06-2.95)	3.73 (0.38-4.39)	1.14 (0.24-0.4)	1.69 (0.03-1.81)

* = Data are mean ± SD / log CFU/g.

proliferate in the gut. In a similar study, Spanhaak *et al.* (1998) reported that the number of faecal Enterobacteriaceae in healthy volunteers after the ingestion of LcS (*Lactobacillus casei* Shirota) fermented milk was not significantly different from that of before treatment. The consumption of LcS fermented milk for two weeks had no effect on the number of *E. coli* in the faeces of healthy volunteers in the United Kingdom (Tuohy *et al.,* 2007). The level of Enterobacteriaceae in faeces of sub-optimal health state volunteers in Japan also did not change after the ingestion of LcS fermented milk for two weeks (Matsumoto *et al.,* 2010). There was a decrease in the number of Enterobacteriaceae, *E.coli*, and non-*E.coli* coliform in faeces after the ingestion of LcS for 10 d in almost half of the volunteers (Utami *et al.,* 2015). A research conducted by Sumaryati *et al.* (2009) on the effect of *E. coli* infection and administration of *L. plantarum* Dad-13 on faecal microbiota in Wistar mice showed that the *L. plantarum* Dad-13 could inhibit the growth of *E. coli* and other coliform bacteria.

Diet can affect the growth of intestinal microbiota. De Filippo (2010) mentioned that if

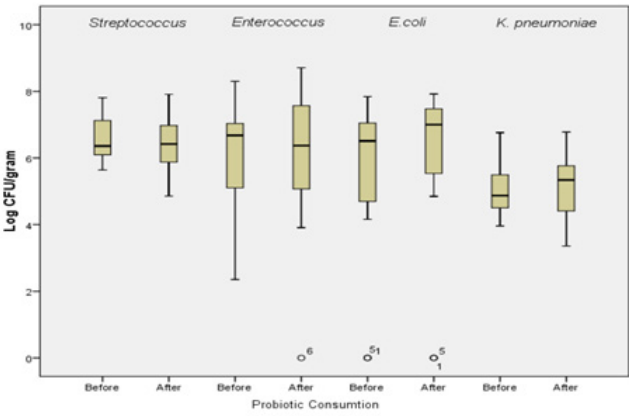

Figure 2. Effect of consumption of probiotic powder *Lactobacillus plantarum* Dad-13 on gut microbiota (*Streptococcus, Enterococcus, Escherichia coli,* and *Klebsiella pneumoniae*) for 33 d.

high-fat and animal protein-sourced foods are consumed, there is a potential in the increase in Enterobacteriaceae. In fact, adolescents in Samosir consumed a lot of fatty and high-protein foods such as pork, beef, and fish. Therefore, this could be the reason why the consumption of *L. plantarum* Dad-13 did not have a significant effect on the intestinal microbiota of the students at Junior High School Pangururan 1, Samosir.

Hasan *et al.* (2018) and Manurung *et al.* (2018) mentioned in their reports that after 33 d of intervention using probiotic, the population of *Prevotella, Bacteroides fragilis, Clostridium coccoides, Bifidobacterium, L. plantarum,* and Enterobacteriaceae also did not increase significantly ($p > 0.05$). In a similar study conducted in Yogyakarta, the population of *L. plantarum* before and after the ingestion of probiotic powder was not significantly different (Panjaitan *et al.,* 2018).

Each pathogenic bacterium has different sensitivity to antimicrobial compounds produced by *L. plantarum* Dad-13. The inability of *L. plantarum* Dad-13 to affect the intestinal microbiota in the present work may be related to the number of *Streptococcus, Enterococcus, E. coli,* and *K. pneumoniae* in the digestive tract which was naturally high. In addition, *L. plantarum* Dad-13 might also have to compete with other bacteria existing in the gastro-intestinal tract for nutrients.

Conclusion

The energy intakes of the 40 subjects from Junior High School Pangururan, Samosir, were 70 and 72% of RDA in the placebo and probiotic groups, respectively. The energy intakes of placebo and probiotic group were not significantly different. The results of the present work showed that the consumption of probiotic powder of *L. plantarum* Dad-13 for 33 d decreased the *Streptococcus* and increased *Enterococcus, E. coli,* and *K. pneumoniae;* however, the effect was not significant ($p > 0.05$). The inability of *L. plantarum* Dad-13 to affect the intestinal microbiota in the present work may be related to the high number of *Streptococcus*, *Enterococcus, E. coli,* and *K. pneumoniae* in the digestive tract of subjects. Adolescents in Samosir consumed a lot of fatty and high-protein foods such as pork, beef, and fish. This could be the reason of high population of Enterobacteriaceae in the intestinal microbiota of the students at Junior High School Pangururan 1, Samosir.

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