Effect of Synbiotic Yogurt Made with Indigenous Probiotic *Lactobacillus plantarum* Mut7 and Sweet Potato Fiber (*Ipomoea batatas*) in Healthy Children

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ABSTRACT

The objectives of this study were to investigate the effect of synbiotic yogurt on the immune responses and microbiota profile of the feces. A double-blinded randomized controlled trial with cross over design was used in this study. Twenty children aged 8-12 years were recruited and assigned into 2 groups i.e. control yogurt and synbiotic yogurt. They received yogurt 50g/day for 14 days. The food consumption was recalled with 4x24 hours food recall. At the end of experiment, the saliva and feces were collected and analyzed for sIgA. The microbiota profile of the feces was enumerated in selective medium. The results showed that consumption of synbiotic yogurt resulted in a higher concentration of sIgA in saliva and feces compared to control yogurt. The microbiota profile of the synbiotic group was not significantly different with control group. This indicated that the new synbiotic yogurt has similar health benefit as the regular yogurt.

Keywords: Synbiotic yogurt, probiotic, *Lactobacillus plantarum* Mut7, sweet potato fiber, sIgA, microbiota.

INTRODUCTION

A probiotic has been defined as “live microorganisms which when administered in adequate amounts confer a health benefit on the host” (Reid *et al.,* 2003). Prebiotic was defined as “nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one, or a limited number of bacteria in the colon that can improve the host health” (Gibson and Roberfroid, 1995). This definition was updated in 2004 and prebiotics are defined as “selectively fermented ingredients that allow specific changes, both in the composition and/or activity in the gastrointestinal microbiota that confers benefits upon host well-being and health” (Gibson *et al.,* 2004).The term synbiotic is used when a product contains both probiotics and prebiotics (Schrezenmeir and de Vrese, 2001).

The microbiota in the large intestine influences health and well-being. The gut bacteria play a role in host pathogenesis. Certain microbial components can have beneficial effects on gastroenteritis resistance, blood lipids, antitumor properties, lactose tolerance, and gastrointestinal immunity. Modification of the human intestinal microbiota has currently become an important issue. This goal can be reached by either to include a significant proportion of beneficial bacteria in the diet, mainly *Bifidobacterium* and *Lactobacillus* species, with the expectation that they will be able to colonize the intestinal tract (probiotics); or to give non-digestible carbohydrates, like fructo-oligosaccharides, which have shown an ability to promote the growth of desirable bacteria (prebiotics).

Food and Nutrition Culture Collection (FNCC), Gadjah Mada University had isolated and identified several numbers of probiotic bacteria from traditional fermented food. *Lactobacillus plantarum* Mut7 was one of the indigenous probiotic bacteria that had been isolated from fermented cassava. *Lactobacillus plantarum* Mut7 possess probiotic criteria such as survival under acidic condition and bile acid, antagonism against pathogenic bacteria such as *Salmonella choleraecius* and *Shigella*

flexneri (Lestari *et al.*, 2008). In another study, heat-killed *L. plantarum* Mut7 has immunomodulatory effect in human HB4C5 cell line and the capability for increasing the production of IgM was the highest among other indigenous probiotic (Lestari *et al.,* 2010).

Sweet potato is one of the local crops commonly consumed by Indonesian people. The total fiber in sweet potato (Bestak variety) was 11.36% consisted of 9.23% insoluble fiber and 2.13% soluble fiber (Astuti *et al*., 2005). After extraction with ethanol to reduce sugar and starch, the total fiber could increase up to 20.57%, consisted of 14.60% insoluble fiber and 5.97% soluble fiber. Among other varieties such as Genjah Rante and Cilembu, Bestak variety has higher total fiber (Astuti and Harmayani, 2005). The fiber could not be digested in gastrointestinal tract, so that it could be fermented by colonic microbiota and improving the health of the host. In our previous study, sweet potato fiber extract could increase the lactobacilli population and prevent the *Salmonella typhimurium* diarrhea (Astuti *et al*., 2005; Astuti and Harmayani, 2005).

The aims of this study were to investigate the effect of synbiotic yogurt on the immune responses and fecal microbiota profile of the healthy children.

MATERIAL AND METHODS

Subjects

The subjects were recruited from the orphanage in Yogyakarta City area. Inclusion criteria were healthy children aged 8-12 years. The number of the subject was 20 children from 2 orphanages. The health criteria were not suffering from gastrointestinal problem and chronically diseases. All subjects signed the informed consent before the supplementation.

Experimental design

This was a double blinded, randomized controlled trial with cross over design study. The subjects were divided into 2 groups. First group received control yogurt that contains 10^8 CFU/g *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, whereas second group received synbiotic yogurt that contains 10^8 CFU/ *Lactobacillus plantarum* Mut7 and 2% sweet potato fiber supplementation. During 2 weeks of yogurt supplementation period, the subject had to consume 50 g of yogurt per day. All subjects were prohibited to consume any other probiotic product during the experiment. The food consumption was recalled with 4 x 24 hours food recall.

Production of synbiotic yogurt and control yogurt

The yogurt was made in our laboratory. The control yogurt was made from Ultra High Temperature (UHT) milk with addition of 10% sugar, 2% skim milk, and 10% starter (*L. bulgaricus* and *S. thermophilus* with ratio of 1:1). The mixture was incubated at 43 ^{O}C for 6-7 hours. The synbiotic yogurt was made from UHT milk with addition of 10% sugar, 2% skim milk, 2% sweet potato fiber, and 10% starter (*L. plantarum* Mut7). The mixture was incubated at 37 ^{O}C for 18-20 hours. Following incubation, the yogurts were refrigerated at 4 ^{o}C until consumption. The yogurt was made once a week.

Sample collection

Saliva samples were collected from each subject in sterile 15 ml conical tubes at the end of supplementation. The saliva samples were immediately frozen at – 20 ^{O}C. Fecal samples were collected from each subject in sterile tubes and immediately frozen at – 20 ^{O}C.

Bacterial and sIgA quantification

The fecal sample was diluted with serial number of dilution (10^1, 10^2, 10^3, 10^4, 10^5, 10^6, and 10^7). 1 ml of the appropriate dilution was pour plated into selective media and incubated at appropriate temperature described in the manual. All selective medium were obtained from Oxoid. *E. coli* was quantified with TBx agar. *Lactobacilli* were quantified with Rogosa agar. *Bifidobacteria* was quantified with Columbia agar. *Clostridia* were quantified with OPSP Perfringens agar with supplement A and B. *Salmonella* was quantified with SS agar. Before sIgA analysis, the saliva was centrifuged at 3.000 rpm for 5 minutes. The supernatant was diluted 2.000 times before the assay. sIgA from saliva was quantified with ELISA kits from Immundiagnostic, Netherland. Fecal sampel was weighed as much as 1g, suspended in sterile PBS, mixed and then centrifuged at 3.000 rpm for 5 minutes. The supernatant was collected and diluted 12.500 times before the sIgA analysis.

Anthropometry and Food Recall

The weight and height of the subjects were measured. The nutritional status of the subject was determined using Body Mass Index (BMI). The food consumption was recalled with 4 x 24 hours food recall. The intake of energy, carbohydrate, protein, fat, and fiber was calculated using Nutrisurvey software.

Statistical Analysis

The effect of yogurt synbiotic consumption was compared with control yogurt and analyzed using paired t-test with

Table 1. Characteristic of the Subjects

Characteristics	Value (mean + SD)
Age (years)	10.61 ± 2.17
Weight (kg)	31.33 ± 8.43
Height (cm)	137.23 ± 10.24
BMI (kg/m$^{2)}$	16.30 + 2.53

Table 2. The Food Intake of the Subjects

Parameter	Groups		
	Control Yogurt	Synbiotic Yogurt	P value
Energy (Kcal)	1171 ± 476	1248 ± 485	0.38
Carbohydrate (g)	156 ± 55	176 ± 73	0.11
Fat (g)	39 ± 21	39 ± 19	0.57
Protein (g)	38 ± 15	39 ± 15	0.94
Dietary Fiber (g)	5 + 3	5 + 3	0.70

Table 3. sIgA Concentration in Saliva and Feces of the Subject

Group	sIgA Concentration (mg/mL)	
	Saliva	Feces
Control Yogurt	599,52 ± 222,11[a]	383,93 ± 193,04[a]
Synbiotic Yogurt	642,61 ± 348,59[a]	466,56 ± 417,59[a]

Notes: [a] the same superscript in the same column showed that there were no significant differences

SPSS software. The data distribution was analyzed for its normality using Kolmogorov-Smirnov test. Differences were considered significant at $P < 0.05$.

Ethical Clearance

This study has already approved by Medical and Health Research Ethics Committee (MHREC), Faculty of Medicine, Gadjah Mada University, Indonesia.

RESULT

Characteristic of the Subjects and the Food Intake

Twenty subjects were included in this study. However, 2 of them were dropped out because they were no longer available for testing. Subjects were aged between 8 and 12 years and the average of age was 10.61 ± 2.17 years. The average of BMI was 16.30 ± 2.53 kg/m^2. Based on the average BMI value, we assumed that the nutritional status of the subject was underweight. It could be understand because we recruited the subject from orphanage. However 26% of the subject had a good nutritional status, whereas 74% had an underweight nutritional status. The characteristic of the subjects could be seen in Table 1.

The food consumption was recalled with 4 x 24 hours food recall. The intake of energy, carbohydrate, protein, fat, and fiber between 2 groups were not significantly different ($P>0.05$). Based on the Dietary Reference Intake (DRI), we concluded that the intake of carbohydrate and dietary fiber were lower than the recommended intake. However the intake of protein and fat were slightly higher than the recommended intake. In short, there are no confounding factors that came from the diet. The food intake of the subjects could be seen in Table 2.

Secretory IgA Concentration in Saliva and Feces

The sIgA concentration in saliva and feces showed that there were no significantly different between control yogurt and synbiotic yogurt (Table 3). However, consumption of synbiotic yogurt resulted in a higher sIgA concentration compared to control yogurt. This indicated that the synbiotic yogurt had a similar immunomodulatory effect as control yogurt.

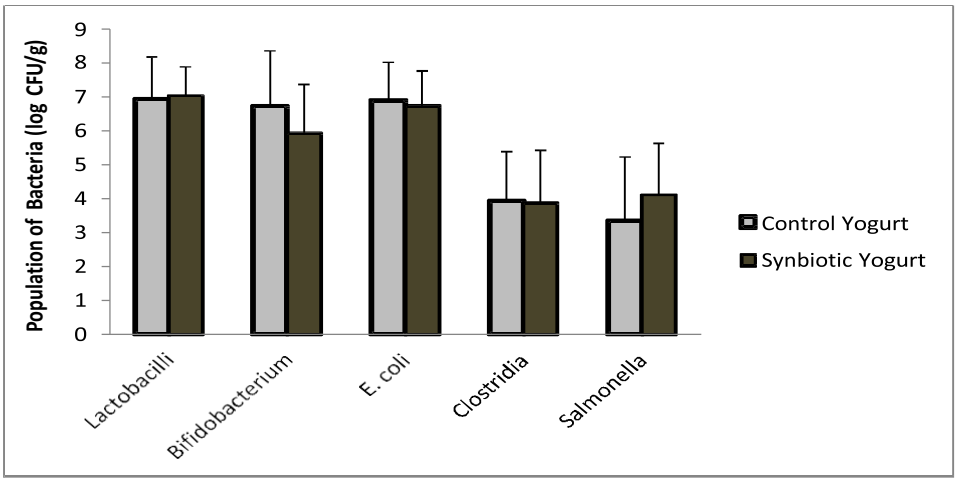

Figure 1. The population of Selected Bacteria in the Fecal Samples (n=18)
Notes: The value are expressed in Log10 CFU/g of feces

Microbiota Profile in the Feces of Subjects

Figure 1 showed that total lactobacilli in the synbiotic group were slightly higher than control group, whereas the total *Bifidobacteria* was lower. The number of *E. coli, Clostridia,* and *Salmonella* was not significantly different. This indicated that our synbiotic yogurt has a similar effect as good as control yogurt.

DISCUSSION

Secretory IgA is the most abundantly produced immunoglobulin at the surface of mucous membranes. Production of sIgA in the gastrointestinal tract was depend on the antigen sampling by M cell, processing by APC (antigen-presenting cell), and B-cell switch in the Peyer's patch. IgA switch and production was affected by certain cytokines such as TGF-β (Corthesy *et al.,* 2007). IgA production involves interaction of immune cell in the gastrointestinal tract (Weiner, 1997). The mechanisms were through M cell from PP, through the epithelial cell with processing and presentation or not of the antigen, and interaction with the epithelial cells and elimination of the antigen by portal circulation or by inducing a local immune response activated by the release of cytokines.

The results of this study are also in line with other research such as yogurt consumption could increase cytokine production in PBMCs (*peripheral blood mononuclear cell*) (Miettinen *et al.,* 1996, Aattouri and Lemonnier, 1997), phagocytic activity (Schiffrin *et al.,* 1997, Schiffrin *et al.,* 1995), specific humoral immune responses (Link-Amster *et al.,* 1994), CD4+ and CD8+ function (Aattouri and Lemonnier, 1997) and NK cell activity (De Simone *et al.,* 1989).

Probiotic bacteria play important role in the maintaining the body's immune responses. Probiotic cell or its component such as peptidoglycan activates the immune cell especially in the Gut Associated Lymphoid Tissue (GALT). Perdigon *et al.* (1988) has studied the effect of yogurt consumption on the systemic immune responses. They found that the mixture of *L. acidophilus* and *L. casei* were more effective than yogurt that fermented by *L. debrueckii ssp. bulgaricus* and *Streptococcus thermophilus.* Our previous study showed that *L. plantarum* Mut7 has immunomodulatory effect in vitro and in vivo. Heat-killed *L. plantarum* Mut7 could increase the production of IgM in HB4C5 cell line (Lestari *et al.,* 2010). The HB4C5 cell line is the fusion product of human B lymphocytes from a lung cancer patient and human fusion partner NAT-30 cells (Sugahara *et al.,* 2006). *L. plantarum* Mut7 could increase the native and adaptive immune responses in Balb/c mice. *L. plantarum* Mut7 could increase the phagocytic activity of peritoneum macrophages, serum IgM and IgG, and sIgA in the intestinal fluid (unpublished study).

The synbiotic yogurt contains dietary fiber from sweet potato. The sweet potato fiber (SPF) contains non digestible oligosaccharide such as fructooligosaccharide (FOS), inulin, and raffinose. Our previous study showed that SPF could increase the IgM production of HB4C5 cell line. SPF could also increase the sIgA concentration in the intestinal fluid, but could not increase the serum IgM and IgG. The proposed mechanisms underlying the immunomodulating effects of dietary fibres that change the gut microflora were 1) direct contact of lactic acid bacteria or bacterial products with immune cells in the intestine, 2) production of short-chain fatty acids (SCFA) from fiber fermentation, and 3) modulation of mucin production (Schley and Field, 2002).

There is strong evidence that consumption of probiotic and prebiotic component such as FOS, inulin, another dietary fiber increase the beneficial lactic acid bacteria in the human colon (Gibson *et al.,* 1995; Schley and Field, 2002). Our previous in vitro study showed that SPF has a prebiotic activity score that was not significantly different with inulin and FOS. We assume that *L. plantarum* Mut7 could utilize and ferment SPF as carbon source.

The consumption of probiotic products containing beneficial bacteria, such as *L. plantarum* Mut7 could maintain the equilibrium and stability of the enteric microbiota, which aids gastrointestinal functions and gastrointestinal immune activity (Delcenserie *et al.,* 2008). The balance of the microbial community can be achieved through increased levels of good bacteria (lactobacilli and bifidobacteria) and decreased levels of pathogenic bacteria (*E. coli, Clostridia,* and *Salmonella).* Consumption of probiotic and prebiotic could change the composition of microbiota in the gastrointestinal tract. The changes in the intestinal microbiota result in induction of sIgA production in the GI tract.

CONCLUSIONS

Consumption of synbiotic yogurt resulted in a higher concentration of secretory IgA in saliva and feces, although it was not significantly different with control yogurt. The microbiota profile between those two groups was not significantly different. This indicated that the synbiotic yogurt has a similar health benefit as regular yogurt in modulating the immune responses and the microbiota in gastrointestinal tract.

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