

Prebiotic Milk Shake and Its Health Benefits

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ABSTRACT

Study on the effect of prebiotic consumption to the fecal material volunteer was conducted at Center for Food and Nutrition Studies, Gadjah Mada University. Aim of this study was to investigate the health beneficial effect of prebiotic product directly using volunteers. Prebiotic product used in this study was StefitTM - non fat milk shake which contains dietary fiber, antioxidant (vitamin E and C), calcium, and chicory root extract (inulin) as prebiotic. Seven healthy volunteers (5 males and 2 females), 30-40 years old, were recruited for this study. During the study (6 weeks), volunteers were asked to avoid antimicrobial drugs and fermented foods containing life microbial cells. The volunteers were divided into two group, group 1 (consist of 2 persons) were asked to consumed original non-fat milk, while group 2 (5 persons) consumed non-fat milk shake prebiotics. Consumption of milk shake was done every day (2 sachets per day, morning and afternoon) during 4 weeks. Twice a week, fecal materials of volunteers were microbiologically analyzed, including a week before and after consumption. Diet of each volunteer was not controlled, they ate as their usual food every day and the menus were recorded. Consumption of milk shake prebiotic by normal healthy volunteer resulted in increased the number of fecal lactic acid producing bacteria (from about 10^6 to 10^7 CFU/g fecal material), and decreased

the population of fecal enterobacteriaceae and coliform. According to the data, fecal lactic acid producing bacteria of volunteers who consumed the original milk shake were mostly constant. Conclusion of this study is the increasing number of lactic acid bacteria induced by prebiotic inulin in the colon has the potential to improve the health and well being of the host.

Keywords : prebiotics, inulin, lactic acid bacteria, enterobacteriaceae, coliform

INTRODUCTION

Prebiotic was introduced by Gibson and Roberfroid (1995) as a non-digestible food ingredient that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, thus improving host health. This definition more or less overlaps with the definition of dietary fiber, with the exception of selectivity for certain species. This selectivity was shown for bifidobacteria and lactic acid bacteria, which may be promoted by the ingestion of substances such as fuctooligosaccharides and inulin, transgalactosylated oligosaccharides, and soybean oligosaccharide (Schrezenmeir and de Vrese, 2001). While the term of probiotic is used for a preparation of a product containing viable, defined microorganisms in sufficient numbers, which alter the microflora in the intestinal tract and by that exert beneficial health effects in this host. The term

synbiotic is used when a product contains both prebiotics and probiotics.

Prebiotics could be both naturally occurring and synthetic sugars (Bezkorovainy, 2001). The criteria used for classification of food component as a prebiotic are as follows: Resistance to digestion, hydrolysis and fermentation by colonic microflora, and most importantly, selective stimulation of growth of one or limited number of bacteria in feces (in vivo in human).

Currently, food component that seem to exert the best prebiotic effects are inulin-type fructans (Roberfroid, 2001). Inulin-type fructans are composed of α -D-fructofuranoses attached by α -2-1 linkages. The first monomer of the chain is either a α -D-glucopyranosyl or α -D-fructopyranosyl residue. They constitute a group of oligosaccharides derived from sucrose that are isolated from natural vegetables sources. Several commercialized probiotic components are as follows: Inulin is a product with a degree of polymerization (DP) from 2 to \leq 60 extracted from chicory roots (Raftiline, Orafiti, Tienen, Belgium). Oligofructose, which is produced by partial enzymatic hydrolysis of inulin, has a DP $<$ 10 (Raftilose; Orafiti) and the inulin from which the small-molecular weight oligomers have been eliminated is called high-performance inulin (Raftiline HP; Orafiti). Neosugar is synthetic probiotic, with the use of sucrose as a substrate and 1,2- α fructan 1-fructosyltransferase-catalyzed reaction, a synthetic low-molecular-weight fructan is produced that has a DP $<$ 4 (Belgium-Meiji Industries, Paris).

Based on in vitro studies, in pure culture, most species of bifidobacteria are adapted to the utilization of the non-digestible oligosaccharides but many other bacteria are also capable of metabolizing them. Clearly, these studies of pure bacteria are of limited use unless their results are supported by the result of studies using mixed cultures. Indeed, as many components of the gut microbiota as possible should be measured to indicate a true prebiotic effect. Simple stimulation of bifidobacteria is insufficient to demonstrate an effect; the effects on other gut microorganisms in vivo with human volunteers is necessary (Roberfroid, 2001).

Gut bacterial ecosystem is composed of 400-500 different types of bacteria which all interact.

Most of these bacteria are commensal, they participate in the fermentation of non-digestible food compounds, but do not directly interact with the physiological processes in the host. Some bacteria are considered to be harmful for instance *Clostridium difficile*, or certain types of *E. coli*, *Listeria*, *Salmonella*, etc. In dysbalanced intestinal conditions they can develop and produce toxins, which can cause acute or chronic illness of the host. On the other side, there are group of bacteria which considered to be useful, such as Bifidobacteria and lactic acid bacteria, whose their presence is associated with a well-functioning intestinal ecosystem of a healthy host. When well balanced, they constitute a colonisation barrier against the pathogens that in healthy individuals are suppressed down to harmless level ($<10^6$ /ml).

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MATERIALS AND METHODS

Prebiotic product used in this study was Stefit - non fat milk shake (produced by Netania) which contains dietary fiber, antioxidant (vitamin E and C), calcium, and chicory root extract (inulin) as prebiotic.

Seven healthy volunteers (5 male and 2 female), 30-40 years old, were recruited for this study. During the study (6 weeks), volunteers were asked to avoid antimicrobial drugs and fermented foods containing viable cells. The subjects were divided into two group, group 1 (consist of 2 volunteers) were asked to consumed original non-fat milk, while group 2 (5 volunteers) consumed non-fat milk shake prebiotics. Consumptions of milk shake was done every day (2 sachets per day, morning and afternoon) for 4 weeks. During treatment, fecal material of volunteers were microbiologically analyzed, twice a week, including a week before and after consumption.

Microbiological analysis for fecal materials were coliform using violet red bile agar,

enterobacteriaceae (using violet red bile glucose agar), Salmonella-Shigella using SS agar (Oxoid). Lactic acid bacteria were analyzed using PGY (peptone glucose yeast extract) + CaCO₃.

Diet of each volunteer was not controlled, they ate each day as their usual menu, however what they eat each day were recorded. Monitoring was also done each day to the general health condition for volunteer and their fecal properties (solid-liquid, smell, frequency).

RESULTS AND DISCUSSION

In this study, peptone glucose yeast extract supplemented with CaCO₃ was used for the enumeration of lactic acid producing bacteria. These bacteria were indicated by the clear zone appear after 2 days incubation. In this study the population of lactic acid producing bacteria were consist of lactic acid bacteria and bifidobacteria. Population of lactic acid producing bacteria in fecal material of volunteer before consumption of milk shake prebiotic generally were considered very low (about 10⁶ CFU/g), presented in Figure 1. During the consumption, this number increased about 1 log cycle, and tend back to normal after consumption were stopped. While population of lactic acid bacteria of two volunteers who took original milk shake (without prebiotic) considered to be constant. Increasing number of LAB could be used to strengthen the suggestion that prebiotic inulin could selectivity stimulate the beneficial bacteria in the colon.

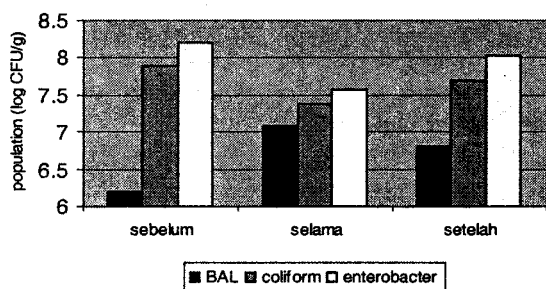


Figure 1. Bacterial counts of fecal material volunteers before, during and after consumption of Stelit™ Shake Probiotic

Figure 1 presented number of lactic acid bacteria, enterobacteriaceae and coliform. Before consumption of prebiotic product, number of enterobacteriaceae and coliform were close to 10⁸, however during the consumption these number decreased in parallel with the increased of lactic acid bacteria. When the consumption stopped, the number of enterobacteriaceae and coliform increased again, while the lactic acid bacteria decreased. In this study, group of bacteria which suggested to be pathogenic salmonella-shigella (according to the selective media used) were also analysed. Number of this group of bacteria at the beginning was very high (10⁵ CFU), during the consumption of prebiotic, this group of bacteria could be suppressed into very low number, close to 10². According to the data, several volunteer hardly detected the occurrence of this pathogenic group of bacteria. However, when the consumption was stopped, the number of this bacteria tend to increase. In this study, clear, that the increased of harmless bacteria or lactic acid producing bacteria could suppress the growth of the detrimental bacteria, and this result was similar to other result before, studies using both in vivo or in vitro.

In vitro study by Wada, 1990, using 122 different pure cultures isolated from human intestines, on a defined media with oligofructose, inulin or glucose (positive control), showing that bifidobacteria and some species of *Bacteriodes* are much more stimulated than Clostridia or *E. coli* by inulin and oligofructose. Other pure cultures results (Gibson and Wang, 1994a) showing that bifidobacteria had highest maximum specific growth rate on oligofructose, followed by inulin and an experimental branched fructan, and lowest on glucose. While for other bacterial cultures used (*E. coli* and *C. perfringens*) the fastest growth was on glucose. Gibson and Wang (1994b), the addition of the chicory fructans to the continuously running system (Using fecal slurries as inocula) caused the numbers of Bifidobacteria to increase, and the numbers of Clostridia to decrease. In other experiment Gibson and Wang (1994c) demonstrated that the growth of Clostridia was inhibited by a growing culture of Bifidobacteria.

In vivo study by Gibson, et al., 1995 (in Bezkorovainy, 2001), showed that subject receiving

15 g fructooligosaccharides or inulin per day had higher hydrogen and methane outputs in their breath than did subject fed sucrose. Their fecal bifidobacterial counts increased almost 10 fold, whereas those of bacteriodes, coliform, and cocci decreased. While fecal short-chain fatty acid concentrations (e.g., acetic, propionic, and butyric acids) did not change significantly.

In this study, enterobacteriaceae and coliform, as well as pathogenic *Salmonella-shigella* were used as indicator the effect of consumption of prebiotics. However, result showed that increasing the number of lactic acid bacteria could suppress the growth of other colonic bacteria which could be detrimental to the host.

At the beginning of treatment some volunteers had flatulency (producing gas) and stopped after several days. This is considered to be a normal condition. Hummings et al., 2001, stated that mild flatulence is frequently observed by subject being fed prebiotics; even, in a significant number of subjects it is severe enough to be unacceptable and to discourage consumption. Prebiotics are like other carbohydrates that reach the cecum, such as non-starch polysaccharides, sugar alcohol, and resistant starch, in being substrates for fermentation. They are, however, distinctive in their selective effect on the microflora and their propensity to produce flatulency.

During treatment, health conditions of volunteers were generally good. Their fecal properties were normal, including frequency (1-2 twice a day). General comments of volunteers about their bowel during consumption were considered to be normal. Hummings et al., 2001, reported that prebiotics, in addition to their selective effect on bifidobacteria and lactobacilli, influence many aspects of bowel function through fermentation. Short-chain fatty acids are a major product of prebiotic breakdown, but as yet, no characteristic pattern of fermentation acids has been identified. Through stimulation of bacterial growth and fermentation, prebiotic affect bowel habit and are mildly laxative. In this study, analysis of acids as a product of prebiotic breakdown during colonic fermentation had not been conducted.

Diet of each volunteer was not controlled, they ate each day as their usual menu, however what

they eat each day were recorded. Their menu, usually include rice, vegetables, tempe, tahu, meat, egg, fish, etc. During treatment, volunteers were asked for not eating any fermented food which contain life microbial cell. Six among 7 volunteers took meals three times a day. One volunteer who took meal twice a day seemed gave no effect to the colonic microbiota. Even though, the number of lactic acid bacteria of his fecal materials increased, however, the number of coliform and enterobacteriaceae keep constant

Conclusion of this study is the increasing number of lactic acid bacteria induced by prebiotic inulin in the colon has the potential to improve the health and well being of the host.

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