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Effect of Inulin Supplemented UHT Milk Consumption on Faecal *Bifidobacterium* sp. and *Lactobacillus* sp. of Healthy Children in Depok, Indonesia

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ABSTRACT

Introduction: This study investigated the effect of inulin-supplemented ultra-high temperature (UHT) milk consumption on the number of *Lactobacillus* sp. *and Bifidobacterium* sp. in the faeces of healthy Indonesian children. **Methods:** This study used a double-blind, randomised, placebo-controlled pre-post designed clinical trial of 153 healthy participants (4-12 years old children) who received UHT milk supplemented by 1 mg (n = 50), or 1.5 mg of inulin (n = 48), or placebo (n = 55). They received two servings per day over a course of 21 days with a 3-day wash-out period. Children assisted by parents were asked to complete a three-day food record prior to the course and to fill-in a FAQ over the 21-day period of this study. The faecal samples of the subjects were grown on calcium carbonate-MRS agar medium**.** The colonies which showed a clear zone were isolated and tested for *Lactobacillus* sp. and *Bifidobacterium* sp. using catalase test, Gram staining, and RapID™ ANA II. **Results:** No significant difference was found on colony counts before treatment (p = 0.986) and on inulin dosage given to all three groups (p = 0.453). Post-treatment, showed significant difference in colony counts between treatment groups (p=0.046 for 2 g/day group and p=0.049 for 3 g/day group) and placebo, but no significant difference was found between the two treatment groups. **Conclusion:** This study demonstrated that inulin-supplemented UHT milk could help increase the amount of faecal *Lactobacillus* sp. and *Bifidobacterium* sp. regardless of the dosage. There was no difference in the amount of faecal *Lactobacillus* sp. and *Bifidobacterium* sp between the two dosage regimes (2 g and 3 g inulin per day).

Key words: *Bifidobacterium* sp., Inulin, *Lactobacillus* sp., school children, UHT milk

INTRODUCTION

The human body is the host for many other living creatures and the human digestive system is a natural habitat for microorganisms. A symbiotic mutualism occurs between digestive bacteria and humans, where humans provide a nutrient rich habitat for the bacteria while the bacteria provide many benefits to their host (Hooper, Midtvedt & Gordon, 2002). Bacteria can help in fermenting substrates that are naturally indigestible to the human

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digestion system. Through bacterial assistance, humans can enjoy an increased supply of short chain fatty acids (SCFA), amino acids, vitamins and ions. Important bacteria in the human body that can improve health include the *Lactobacillus* sp. and *Bifidobacterium* sp. These bacteria can help activate the human body's immune system (Roberfroid, 2007).

Prebiotics are food components that cannot be hydrolysed in the upper digestive tract. They are fermented by intestinal microflora in the large intestine, and in turn, increase the amount of intestinal microflora. Research suggests that prebiotics can improve the symbiosis between the host and bacteria by stimulating the activity or by supporting the growth of bacteria in the gut (Roberfroid, 2007).

Inulin (β-(2,1)-fructans) is a reserve carbohydrate in many plants and is generally consumed in moderation in people's daily diet. Inulin is classified as a non-digestible oligosaccharide because it has the β-configuration at C anomer$_2$ contained in fructose monomers that do not get hydrolysed by the digestive enzymes that are specific to α-glycoside bonds (e.g., α-glucosidase, maltase, isomaltase, sucrose, etc.) contained in the small intestine. Inulin is forwarded to the large intestine/colon (Roberfroid, 2007). However, generally about 12-14% of the inulin is lost due to digestion in the small intestine. The cause of this loss is fermentation by the microbial population growing in the ileum (Kaur & Gupta, 2002).

Inulin is also known to have a prebiotic effect because it can stimulate the growth/activity of several types of digestive bacteria found in the colon, thus playing a role in improving digestive health (Kaur & Gupta, 2002). Inulin cannot be hydrolysed by enzymes in the gut and pancreas, and is selectively fermented by bacteria living in the gut ecosystem. Inulin can be found in a wide variety of plants, including vegetables and fruits. In addition, inulin is also known as a functional food ingredient and is with a component of various types of beverages, yogurts, biscuits, and other dietary supplements.

Inulin works to promote the growth of bacteria in the colon. Based on *in vivo* and *in vitro* studies performed by Gibson & Wang (1994), the increase in colonic fermentation of inulin may enhance the growth of bifidobacteria population. In addition, the prebiotic inulin-type also affects the fraction of lactobacilli, or other species of bacteria such as *Clostridium* and *Eubacterium rectale coccoides*. Research conducted by Bouhnik *et al*. (2007) showed that the consumption of 5 g of inulin per day (taken in two servings) provides a prebiotic effect by stimulating the growth of intestinal microflora. However, these findings were limited to an adult population using only one dose (single dose), i.e. 5 g in two feeds. In addition, physiological factors and racial differences might have also affected the results. This study is designed to determine the optimal levels of inulin supplementation in UHT milk to support the growth of fecal *Bifidobacterium* sp. and *Lactobacillus* sp. among school children in Depok City.

METHODS

This study of healthy children was a randomised, placebo-controlled, double-blind study with three parallel arms. The whole study was performed from September 2013 to January 2014.

The sample number was determined using the *software Sample Size Determination in Health Studies Version 2.0.21* (Lun, Chiam & Aaron,1998) to test one-way hypotheses on mean difference, with a significance level of 0.01 and 99% power. The minimum number of children required for the sample was 44 children.

Eligible participants were kindergarten and elementary school-children aged 4 to 12 years old. They must have fulfilled the

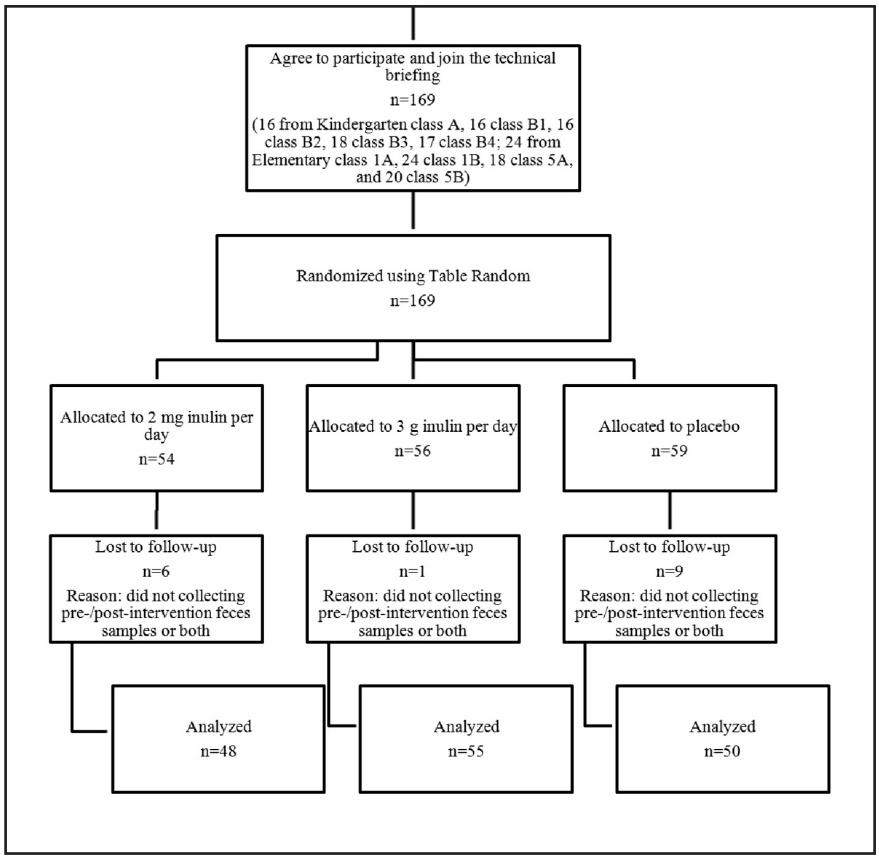

Figure 1. Study flow diagram

following exclusion criteria: no history of gastro-intestinal infections for at least one month prior to the study; not allergic to milk; and not having consumed antibiotics at least one month prior to the study. One hundred and eighty-nine children aged 4 to 12 years from one local kindergaten and several elementary schools at Depok City were included in this study. Prior to the intervention, a technical briefing was given to the parents about the three-day wash-out period. The intervention was carried out for twenty-one days**.** Routine visits were conducted on each of the twenty-one days of the intervention period by the trained investigation team.

Sixteen subjects were excluded from the analysis due to poor compliance. Hence, at the end of this study, 153 subjects, consisting of 73 kindergarten/pre-school children, and 80 elementary school children remained for analysis (Figure 1). Each child received regular UHT milk twice per day (i.e., in the morning and during the day) for 21 days. Inulin preparation was procured by PT Indolakto and added to the UHT milk product as a supplement. The experiment was double-blind, in which the investigator and the subjects did not know what the individual subject received. Product codes were stamped in the production unit (factory). An independent statistician created the randomisation code prior to the experiments to ensure that everyone had an equal chance to be placed in any intervention group.

The 153 children involved in this study were randomly divided into three groups: 50 children in the 0 g inulin dose group; 48 children in the 2 g dose group; and 55 children in the 3 g dose group. The dosage used in this study was similar to that of other studies (Moro *et al*., 2002; Kim, Lee & Meyer, 2007). Servings of UHT milk had to be consumed twice per day (at 08.00 am and 01.00 pm). Each serving was provided in a non-labelled carton-package containing 100 ml UHT milk. The investigation team consisted of undergraduate students from the Faculty of Public Health, Universitas Indonesia who were trained in the study procedures prior to initiating the experiments. They assisted and ensured that all procedures were followed by the subjects during the period of this study. The subjects' compliance was determined by weighing the milk box after each intervention. Moreover, subjects were assigned to a contact person who actively reminded them of their follow-up assessments.

This study was approved by the Committee of Experts for Research and Research Ethics FKM UI No. 70/H2.F10/PPM.00/2013 and the work described has been carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). Permission was also received from the National Unity and Politics Board (*KesBangPol*) Depok, Health Office of Depok City, and the Department of Education of Depok City. Parents or caregivers were asked to sign consent forms after a thorough explanation about the study including procedures to be followed and possible health consequences.

Food intake and assessment of clinical history

Children assisted by parents or caregivers were asked to complete a three-day food record using the FAQ collected during the study period twice a week (at days 4, 7, 11, 14, 17, and 21) and report the history of gastrointestinal disease and antibiotic consumption in the last month. Foods included in the FAQ form were milk, banana, shallots, garlic; yogurt, and cakes with milk as one of the main ingredients.

Outcome measures

The outcome measured in this study was the growth of *Lactobacillus* sp. and *Bifidobacteria* sp. colonies in the faeces of children. Stool was collected at the beginning and at the end of the treatment period (twenty-one days) and the number of microflora colonies were counted to compare the differences between data before and after the administration of inulin. To control the outcome measurement, other measurements were implemented including food intake before and during treatment using FAQ as explained above, frequency of defecation, and the condition of the faeces in terms of consistency and weight. Parents or caregivers were asked to assist their child in the measurement.

Possible source of error that might have affected the validity of this study include contamination or changes in faeces during collection, storage, and transport from school to laboratory; changes in inulin consumption during the study period; and inaccuracy in recording food intake. To minimise the error and bias caused by the above sources, several measures were implemented. Firstly, faeces handling (i.e., collection, storage, and transport) was designed to maintain zero contamination and minimise exposure to the environment. Faeces were placed in tightly closed plastic tubes, and delivered to the laboratory within six hours of collection. Faeces condition was checked to ensure that no changes occurred before they were stored in a freezer prior to analysis.

Inulin consumption during the study period was controlled through the wash-out period before the experiment was started to make sure that all subjects had a similar level of inulin intake. Inulin

intake was also monitored throughout the study period using three-day food records. To assist accuracy of the records, a list of inulin-rich food and beverage was provided with the record form.

Materials

Materials used in this study were children's stool samples. The following materials were also used: distilled water; sterile saline pro-analytic; nutrient agar (NA) (oxoid); Rogosa order; deMan Rogosa Sharpe (MRS) agar (Oxoid), $CaCO_3$, 1 set of Gram staining (crystal violet, Lugol's solution, alcohol 96%, and safranin); immersion oil; a solution of hydrogen peroxide (H_2O_2); rubbing alcohol; 70% alcohol; anaerogen indicator; RapID ANA II Panel; RapID inoculation fluid; and RapID reagent spot. The tools used were as follows: incubators; a refrigerator 4°C; laminar air flow generator; micropipette; anaerojar; microcentrifuge tube; colony counters; spirit burner; inoculum needle; L rods; 15 ml falcon tube; 1 ml microtube; disposable petri dish; object glass; microscope falcon rack; rack microtube; autoclave; Erlenmeyer flask; hotplate stirrer; analytical balance; and PC Software ERIC.

Analysis of *Lactobacillus* sp. and *Bifidobacterium* sp.

Sample preparation

Stool samples were collected at baseline (pre) and at the end (post) of the intervention phase. Fresh faecal samples were collected in the morning and were put in sterilised faecal containers. Faecal containers were then put in a cool box and transported to the laboratory, maximum within 6 hours after collection. In the laboratory, faecal samples were put into test tubes and dissolved in 0.9% physiological saline solution, and then stored in a refrigerator to be analysed within two days).

Preparation of materials and tools

An autoclave was used to prepare and sterilise NA and MRS media prior to the analyses. The autoclave was maintained at 121°C for 15 min. A total of 18.6 g of MRS agar was put into Erlenmeyer flasks. Then, 3 g of $CaCO_3$ was added and dissolved in 300 ml of distilled water, before being heated and homogenised until dissolved.

Standard analysis of Lactobacillus acidophilus ATCC 4356 and Bifidobacterium bifidum ATCC 11863

Sterile $CaCO_3$-MRS media were poured into petri dishes. Once tepid, bacteria *Lactobacillus acidophilus* ATCC 4356 and *Bifidobacterium bifidum* ATCC 11863 were scratched onto the media using a loop. Subsequently, the bacteria were incubated for 48 h at 42^0C under anaerobic conditions using an anaerobic jar.

Analysis of faeces

The subjects were asked to provide a fecal sample, within six hours the fecal sample was transported to the laboratory. Upon delivery to the laboratory, 1 gram of the sample was weighed, put into a falcon tube containing 9 ml of sterile physiological NaCl 0.9% and homogenised to get equal suspension. The solution was gradually diluted from 10^0 to 10^7 (for pre-test sample). Due to the large amount of colonies in post-test samples, the dilution level was increased to 10^8. Then 0.1 ml of the suspension was distributed on a petri disc containing $CaCO_3$-MRS. The disc was then reversed, incubated at 37°C for 48 h in an anaerobic condition using an anaerobic jar. Colonies with appropriate morphology providing a clear zone in agar media were counted using a colony counter.

Identification of bacterial isolates using Gram staining

Gram staining can be used to classify bacteria phenotypically based on the

cell wall thickness and composition of peptidoglycan and lipoproteins. The peptidoglycan layer of Gram-positive bacteria is 10-15 times thicker than Gram-negative bacteria.

Identification of bacterial isolates using catalase test

The isolate was taken out of the petri dish, and then placed on a glass object. One to two drops of H_2O_2 3% were added to the isolate and covered with a glass plate. If bubbles appeared, the isolate was categorised as catalase positive bacteria, and in the absence of bubbles, as catalase-negative bacteria.

Identification of bacterial isolates using the RapID ™ ANA II Panel

The analysis was done using RaPID isolate colonies, which were then rejuvenated in the $CaCO_3$-MRS medium, and inoculated in the inoculation RapID fluid until they reached an appropriate level of turbidity of McFarland 3. The suspension was put in a 10-well test panel, incubated for 4 to 6 h at 3°C and identified using RapID kits at 37°C. The result was observed and compared to the Color Guide. Two drops of the reagent from the RapID ANA II system were put into wells number 3 to number 9, whilst two drops of Indole Spot reagent were put into well number 10. Results were observed after a period of 30 sec to less than 2 min. Observations were recorded and compared to the Color Guide. The obtained codes were checked using RapID software (http*://www.remel.com/ERIC/).*

The RapID ANA II system was used to identify the bacteria. According to manufacturer specifications, the system was accurate for the intended identification of *Lactobacillus* sp. and *Bifidobacteria* sp. The new (second) generation of the system used in this study was improved in terms of an updated database and an option for refrigerated storage before reagent addition (Marler *et al*., 1992).

Statistical analysis

To detect statistical significance in mean differences between and within groups, paired and non-paired *t*-tests were performed. Paired *t*-tests were employed to compare means of colony counts before and after the intervention for each intervention group. The null hypothesis was that there is no difference in mean of colony count before and after the interventions. The non-paired or independent *t*-tests were conducted to detect differences in the mean between different groups of interventions. In both tests, significance level was set at α=0.05.

RESULTS

Children's daily consumption of inulin

Average consumption of inulin for each of the groups was 7.61 g/day (0 g dose group), 7.43 g/day (2 g dose group), and 8.45 g/day (3 g dose group). Statistical test results showed no significant difference in the children's daily consumption of inulin by dose (Table 2). Food sources of inulin were milk, banana, shallots, garlic, onion leaf, yogurt, prebiotic drinks, bread, noodles, biscuits, and cakes.

Colonies of *Lactobacillus* sp. and *Bifidobacterium* sp. were observed to have the following characteristics: round-shaped colonies with edges like wool, milk or cream white in colour, measuring 2-5 mm and convex. Colonies that had similar morphology to *Lactobacillus* sp. and *Bifidobacterium* sp., and also showed a clear zone were counted. Gram staining results for *Lactobacillus* sp. showed a cell shape which was elongated, nearly spherical or forming short chains, measuring 0.5 to 1.2 by 1.0-10.0 µm. *Bifidobacterium* sp. cells were observed in pairs forming a 'V' or 'Y' shape.

This study used the total plate count method based on dispersive techniques (spread plate). The dispersive technique was chosen because it produced a clear zone

Table 1. Baseline characteristics of participants

Characteristics of participants		*Number (n)*	*Percentage (%)*	*Total*	
				(N)	*(%)*
Sex	Male	72	47.0	153	100
	Female	81	53.0		
Age	4-6 Years	97	63.4	153	100
	7-12 Years	56	36.6		
Milk code	A (379)	50	32.7	153	100
	B (851)	48	31.4		
	C (462)	55	35.6		
Study class	Kindergarten	73	47.7	153	100
	Elementary School	80	52.3		

Table 2. Distribution of participants by inulin dosage group

		Number of participants by inulin dosage		
		0 g/day	*2 g/day*	*3 g/day*
Gender	Boy	28	15	29
	Girl	22	33	26
Class level	Kindergarten Class A	4	4	4
	Kindergarten Class B1	5	3	5
	Kindergarten Class B2	3	6	5
	Kindergarten Class B3	5	7	5
	Kindergarten B4	5	6	6
	Elementary 1A	8	8	6
	Elementary 1B	5	5	11
	Elementary 5A	6	5	7
	Elementary 5B	9	4	6
	Total	50	48	55

more readily and efficiently. In addition, the dispersive technique facilitated the subsequent identification of bacteria. To ensure the validity of results, catalase tests were conducted on samples of the colony; the tests gave negative results. Colonies were also identified using the RapID ANA II kit which gave results similar to those of the dispersive technique. Results from the RapID™ ANA II Panel showed positive *Bifidobacterium* sp. (86 to 99.9%) or *Lactobacillus* sp. (99.9%).

Comparison of the number of colonies before treatment

Significant differences were not observed in the number of colonies of *Bifidobacterium* sp. and *Lactobacillus* sp. in all three groups before treatment (p value = 0.986). In the 0 g inulin dose group, the average pre-treatment of intestinal bacteria colony was 50.88 colony-forming units (CFUs) per gram of faeces and after twenty-one days of treatment, it increased by 108.87% to 159.75 CFUs per gram. In the 2 g of inulin

Table 3. Participants' daily Inulin consumption by dosage group

Inulin dosage group	*Mean daily inulin intake (g/day)*	*SD*	*p-value*
0 g (n = 50)	7.61	4.03	
2 g (n = 48)	7.43	3.84	
3 g (n = 55)	8.45	5.16	0,453

Table 4. Total plate count of *Bifidobacterium* and *Lactobacillus sp.* pre and post intervention

Dosage group	*Mean pre TPC (x109CFU/g of faeces)*	*Post Mean TPC (x109CFU/g of faeces)*	*Mean Delta TPC (x109 CFU/g of faeces)*
0 g (n = 50)	50.88	159.75	108.87 *
2 g (n = 48)	57.08	214.84	157.76 *
3 g (n = 55)	44.24	199.66	155.42 *

TPC: Total Plate Count; CFU: Colony Forming Unit; *Significant at α = 0.05 level

dose group, the average intestinal bacterial colonies before treatment were 57.08 CFUs per gram of faeces and after twenty-one days of treatment, it increased by 157.76% to 214.84 CFUs per gram. In the 3 g inulin dose group, the average intestinal bacterial colonies before treatment were 44.24 CFUs per gram of faeces and after twenty-one days of treatment, it increased by 155.42% to 199.66 CFUs per gram. The 'before' and 'after bacterial' counts are shown in Table 4.

Increase in intestinal bacteria colonies based on inulin dosage

Statistical test results showed significant differences in intestinal bacterial colonies increase between 0 g and 2 g groups and between 0 g and 3 g groups. However, there was no difference between 2 g and 3 g groups, as shown in Table 5. Therefore, the optimal dosage was 2 grams of inulin per day since there was no difference found between the 2 g group and 3 g group in terms of bacteria colony counts.

DISCUSSION

The status of intestinal microflora in children who were the subjects of this study can be considered good as indicated by the 44.24-50.88 range of colonies of *Lactobacillus* sp. and *Bifidobacterium* sp. per gram of faeces. There was no significant difference in the number of colonies of *Lactobacillus* sp. and *Bifidobacterium* sp. before treatment in all three groups. The rate of change of the intestinal microflora (*Lactobacillus* sp. and *Bifidobacterium* sp.) within 21 days was 3.76 times for the 2 g dose group and 4.51 times for the 3 g dose group. Compared with the increase in the placebo group in 21 days the 2 g group increased 1.44 times more and 3 g group 1.43 times more. The results of this study are similar to those of Kolida *et al.* (2007) which showed that consumption of chocolate drinks containing 5 g inulin and 8 g inulin per day for 2 weeks increased the *Bifidobacterium* sp. level in adult faeces; they too found no significant difference between the dosage amounts.

Table 5. Differences in the increase of intestinal bacterial colonies

Dose group	*Mean Delta TPC (X109CFU / g of feces)*	*SD*	*p-value*
0 g (n = 50)	108.87	122.94	0,046 *
2 g (n = 48)	157.76	116.00	
Dose group	Mean Delta TPC (X109CFU / g of feces)	SD	p-value
0 g (n = 50)	108.87	122.94	0,049 *
3 g (n = 55)	155.42	116.62	

TPC: Total Plate Count; CFU: Colony Forming Unit; * Significant at α = 0.05 level

There was a significant increase in the number of colonies of intestinal microflora before and after treatment in all dosage groups. A significant increase also occurred in the placebo group indicating the presence of a placebo effect which is a phenomenon that is commonly encountered in clinical experimental research. The placebo effect is usually attributed to any additional treatment given to the participants (Kienle & Kiene 1997). In this study, it was the provision of milk to the placebo group. This suggests the possibility that the even the consumption of milk which does not contain inulin can improve the intestinal microflora count. For example, this could occur by elimination of harmful bacteria, making the environment more conducive to the growth of the intestinal microflora. Addition of inulin accelerates the improvement of intestinal microflora count (Guarner & Malagelada, 2003).

The analyses showed significant differences in increase in intestinal bacterial colonies between the 0 g dosage group and the 2 g dosage group (*p*-value = 0.046) as well as between the 0 g dosage group and the 3 g dosage group (*p*-value = 0.049). This is in accordance with a study by Tokunaga *et al*. (1993) which found that 3 g/day consumption of pre-biotics can increase the count of *Lactobacillus* sp. and *Bifidobacterium* sp. significantly in adults. Kim *et al*. (2007) had similar results in a study on infants; inulin consumption of 1 to 2 g/day was found to improve *Lactobacillus* sp. and *Bifidobacterium* sp. counts. According to Roberfroid (2007), increased *Lactobacillus* sp. and *Bifidobacterium* sp. in the guts of children aged 4 to 12 years old resulting from inulin consumption is due to the prebiotic characteristics of inulin. Inulin is resistant to gastric acid and can neither be hydrolysed by the digestive enzymes nor absorbed gastro-intestinally.

Inulin is also fermented by intestinal microflora and selectively stimulates the growth and activity of intestinal bacteria associated with health and well-being of the host (Gibson & Wang, 1994). Increased *Lactobacillus* sp. and *Bifidobacterium* sp. can also be affected by the consumption of inulin contained in everyday foods, such as banana, onion, garlic and wheat (Loo & Coussement, 1995; Niness, 1999).

This study determined the effects of daily inulin consumption on *Lactobacillus* sp. and *Bifidobacterium* sp. counts in the faeces of children. The analysis showed no significant difference in *Lactobacillus* sp. and *Bifidobacterium* sp counts by inulin dose (*p*-value = 0.453). Based on this result, it can be concluded that the increase in *Lactobacillus* sp. and *Bifidobacterium* sp.

in the intestines of children aged 4 to 12 years in this study was purely due to the consumption of inulin supplemented milk over the 21-day period.

Only two complaints appeared in this study: vomiting and abdominal sounds despite not being hungry (borborigmy) in participants after consuming inulin-supplemented UHT milk. Literature shows that inulin can be safely consumed, even by infants. Inulin is harmless, does not cause toxicity in target organs and reproductive organs or death or abnormality in the body, and is not carcinogenic. Several *in vitro* studies have shown that inulin does not have the potential to cause genetic mutations (Carabin & Flamm, 1999; Coussement, 1999, Clevenger *et al*., 1988) . In this study, there were no complaints of hypersensitivity to the provision of inulin in UHT milk products.

As inulin is found in everyday foods, the public has been exposed to inulin for several centuries and the consumption of foods containing inulin has been shown to be safe. Physiologically, carbohydrate mal-absorption can occur in the upper gastrointestinal tract, causing body intolerance to this substance. The fermentation process of complex carbohydrates may produce gas as a by-product (flatus). When the amount of carbohydrates exceeds the capacity of the bacterial fermentation, diarrhoea can occur (Carabin & Flamm, 1999). Gastrointestinal symptoms that may occur include flatus, flatulence, increased frequency of bowel movements, diarrhoea, and nausea (Bonnema *et al*., 2010). Changes in stool consistency (diarrhoea) may occur due to the effect of inulin on the osmotic pressure in the colon which causes an increase in water content. Generally, these symptoms will increase with increasing inulin dose.

This study found an inulin dose of 2 g per day safe and beneficial. This is far less than the dose in the study of Bonnema *et al*. (2010) which found an inulin dose of up to 10 g/day to be quite safe. Tolerance evaluation was done by observing symptoms such as abdominal bloating, nausea, flatus, abdominal pain, diarrhoea, constipation, and borborygmi. The frequency and severity of these symptoms were observed. The most commonly reported symptoms were abdominal bloating, flatus, abdominal pain, and borborygmi with mild severity. Symptoms appeared to be related to the physiological condition of each individual. It is concluded that consumption of inulin, in reasonable doses, is harmless and can be tolerated by the human digestive tract. Symptoms that occurred within fair use were mild and harmless, which was in line with previous results reported by Bonnema *et al*. (2010).

The strengths of this study included the procedures to ensure compliance especially procedure of intervention provision where children drink milk in front of the researcher. The milk product was provided in non-labelled carton-packages, so the influence of brand bias was not a concern. The product was coded and stamped in the factory's production unit. An independent statistician created the randomisation code prior to the study to ensure that everyone had an equal chance of inclusion in any intervention group. The drop-out rate was approximately 10% mainly due to non-compliance in faecal collection. The natural inulin content in daily food consumption was measured using the FAQ to prevent bias from baseline inulin status. In addition, analytical methods were verified prior to sample analysis to ensure valid results. However, there are some limitations. First, our study results cannot be generalised as the study population was relatively homogeneous given that they were from the same school. The outcomes could not be measured molecularly (RapID ANA is a micro-method employing chromogenic

substrates to confirm the *Bifidobacterium* sp. and *Lactobacillus* sp. isolated from faeces).

CONCLUSION

In conclusion, this study showed that inulin-supplemented UHT milk could help increase the amount of faecal *Lactobacillus* sp. and *Bifidobacterium* sp. in school children at Depok. This study did not find any significant difference in the bacterial counts between the 2 g and 3 g inulin per day doses.

Conflict of Interest

This study was supported by a grant from PT Indolakto. Although a financial conflict of interest was identified, we declare that there was no intervention from PT Indolakto in study design and results.

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