Effect of Fermented Milk Containing Probiotics on Constipation Symptoms and Immune System in Subjects with Constipation

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ABSTRACT

Objective: To determine an effect of fermented milk containing probiotics *(Bifidobacterium animalis sub. lactis)* on constipation symptoms and immune system in healthy subjects with constipation.

Methods: This was a randomized triple blinded, cross-over controlled trial. Fifty healthy adult volunteers with constipation were included. A 10-week crossover study was conducted using Bifidobacterium yogurt and the regular yogurt. The subjects were divided into 2 groups and the study was divided into 4 periods i.e. pre-intake period (1 week), first intake period (3 weeks), rest period (3 weeks) and second intake period (3 weeks). The subjects were asked to keep a daily record of their defecation patterns of stool frequency, amount, shape, hardness, color, odor, straining during bowel movement, sensation of incomplete evacuation and blockage. Immune system changes were assessed using complete blood count, CD3+, CD8+, CD4+, CD19+, CD56+, and serum total IgA and IgG.

Results: Fifty constipated subjects aged 21-55 years were analyzed. Most of them were women. Baseline data on age, body mass index, dairy product, fiber or water intake, exercise, vital signs and blood tests between two groups were not significantly different. Carry-over effect and period effect were found to be significant in the present study. Therefore only the data from the first intake period were analyzed. Bifidobacterium yogurt intake increased frequency of defecation from 3 to 5.84 times/week ($p<0.001$) and 3.20 to 4.56 times/week in the regular yogurt group ($p=0.001$) at 3 weeks. Probiotics yogurt increased frequency of defecation more than that in the regular yogurt 1.48 times/week ($p=0.002$). Stool quality was improved in all aspects in probiotics yogurt group ($p=0.048$). Only total score and sensation of blockage were improved in the regular yogurt group ($p<0.001$ and 0.048). Complete blood count and all CD markers had no clinically significant changes. Total IgA and IgG were decreased after 3 weeks of yogurt intake in both groups ($p<0.001$), but the changes had no clinical significance.

Conclusion: Stool frequency and quality of the constipated subjects were significantly improved after 3 weeks of Bifidobacterium yogurt and regular yogurt intake. Probiotics yogurt had more effects on stool frequency and quality than regular yogurt. No clinically important changes in immune system and no serious adverse events were observed in both groups.

Keywords: Fermented milk, probiotics, Bifidobacterium, constipation, immunity

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INTRODUCTION

Constipation is a very common symptom in general population. The prevalence of constipation is usually 10-50% in the general population,[1,2] and almost everyone experiences constipation at some point in one's life. Most constipation symptoms are temporary and mild. Treatments of constipation include life style modifications (i.e. exercise, adequate fiber and water intake), laxative or other medications depending on severity and individual concern.

Many previous studies showed that fermented milk with or without probiotics improved constipation symptoms[3-5] and immune system[6,7] without any serious adverse effects.[3-7] However, the study results were dependent on the type of the study population, type of probiotics and dosage of fermented milk products.

The purpose of the present study was to determine the effect of fermented milk containing probiotics (*Bifidobacterium spp.*) on constipation symptoms and immune system in Thai subjects with constipation.

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MATERIALS AND METHODS

The protocol for the present study was approved by Siriraj Institutional Review Board (IRB Project Number 678/2010). The study was randomized triple blinded, cross-over controlled trial in healthy subjects with constipation and was conducted at Siriraj clinical research center. Inclusion criteria were (1) patients aged at least 18 years (2) having any two of the following symptoms for at least 12 weeks, not always consecutive, in the previous 12 months: (straining during bowel movements, lumpy or hard stool, sensation of incomplete evacuation, sensation of anorectal blockage or obstruction, and/or fewer than three bowel movements per week), according to the clinical definition of constipation recommended by National Digestive Disease Information Clearing house (NDDIC). Exclusion criteria were allergy to cow's milk, known history or symptoms compatible with lactase deficiency or lactose intolerance, other gastrointestinal diseases, pregnancy or lactation.

The flow of the study is shown in Fig 1. Subjects were randomized into 2 groups (group 1 and 2). A 10-week crossover study was conducted using *Bifidobacterium* yogurt (yogurt A: 110 g containing *Bifidobacterium animalis sub. lactis* $1.2x10^8$ cfu/g, *Streptococcus thermophilus* $2x10^7$ cfu/g and *Lactobacillus bulgalicus* $2x10^7$ cfu/g) and regular yogurt (yogurt B: 110 g containing Streptococcus thermophilus $2x10^7$ cfu/g and Lactobacillus bulgalicus $2x10^7$ cfu/g). The study was divided into 4 periods i.e. pre-intake period (1 week), first intake period (3 weeks), rest period (3 weeks) and second intake period (3 weeks). Subjects were asked to keep a daily record of their defecation patterns[8] containing stool frequency, amount, and shape (Bristol stool score[9], hardness, color, odor, straining during bowel movement, sensation of incomplete evacuation and blockage). Subjects returned to see the investigators every week to assess well being, appropriate record and adverse events. Immune outcomes were assessed by blood test i.e. complete blood count, CD3+ (T lymphocyte), CD8+ (cytotoxic T lymphocyte), CD4+ (helper T lymphocyte), CD19+ (B lymphocyte), CD56+ (natural killer cells), and serum total IgA and IgG. The primary outcome was frequency of defecation after 3 weeks of yogurt intake in each group. Cross-over analyses was done, carry-over effect and period effect were tested. Subjects, investigator and statistician were blinded to the type of yogurt taken by each subject until the analysis was completed.

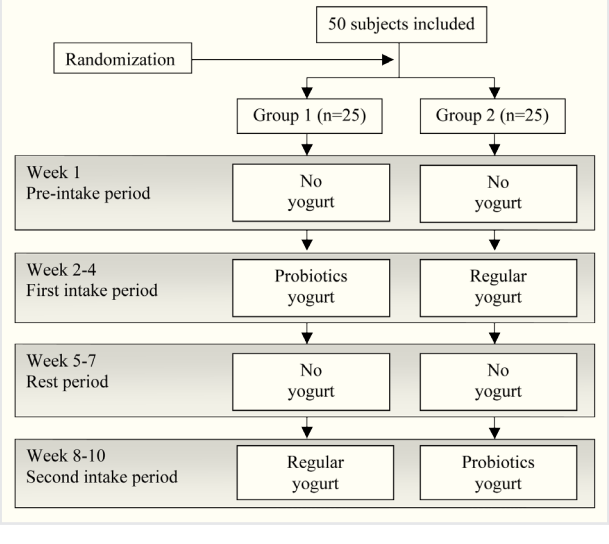


Fig 1. Flow of study.

Data were prepared and analyzed using PASW statistics 18.0 (SPSS Inc., Chicago, IL, USA) and Statistical software R version 2.12.0 (R Development Core Team 2010, Austria). Continuous data were expressed as mean and standard deviation, or median and range, as appropriate. Number and percentage were described for categorical data Student t test, Mann-Whitney U test or Wilcoxon signed ranks test, as appropriate, was used to compare continuous data between groups. Categorical data were compared using Chi-square test or Fisher's exact test. Repeated measures analysis of variance or Friedman test was used to analyze data which were measured at least 3 times consecutively. A p-value < 0.05 was considered statistically significant.

RESULTS

Fifty constipated subjects aged 21-55 years were analyzed. Most of them were women. Baseline data including age, body mass index, dairy product consumption, fiber or water intake, exercise, vital signs and blood test between two groups were not significantly different as shown in Table 1.

Analyses of the confounding factors on constipation i.e. dairy product, fiber, water intake and exercise during 10 weeks revealed that both groups had a tendency to increase water intake from baseline ($p<0.001$) without any significant difference between two groups ($p=0.352$). Subjects' body weights and vital signs were not significantly different from their baseline values (minimum $p=0.043$). Compliance to product intake of the subjects during the study was 98.86%.

The average frequency of defecation before the study was 3 times/week in both groups. The data on frequency of defecation in each consecutive week are shown in Fig 2. The carry-over effect and period effect were found to be significant ($p=0.002$ and 0.001 respectively) in frequency of defecation of the subjects during 10 weeks of cross-over study. Therefore, only the data from the first intake period were analyzed as shown in Table 2. Bifidobacterium yogurt intake increased the frequency of defecation from 3.00 to 5.84 times/week ($p<0.001$) whereas regular yogurt increased the frequency of defecation from 3.20 to 4.56 times/week ($p=0.001$) at 3 weeks. The differences of more defecation were 2.84 ± 1.68 for Bifidobacterium yogurt and 1.36 ± 1.85 for regular yogurt respectively. Probiotics yogurt increased the frequency of defecation more than regular yogurt 1.48 times/week ($p=0.002$). However, the difference in frequency of defecation increase between the two groups was only significant since the end of the second week ($p=0.007$). At the end of the first week, both groups had increased frequency of defecation 1.64 and 0.92 times/week from baseline ($p<0.001$ and 0.006), but the difference between both groups was not significant ($p=0.073$).

The stool quality was improved in all aspects in the probiotics group i.e. total score, fecal amount, characteristics, hardness, color, odor, straining during bowel movements, sensation of incomplete evacuation and sensation of anorectal blockage or obstruction ($p<0.001$, 0.048, <0.001, <0.001, <0.001, 0.007, 0.013, 0.009 and 0.037 respectively) after intake of yogurt for 3 weeks as shown in Table 3. Only total score and sensation of blockage were improved in the regular yogurt group ($p<0.001$ and 0.048). The total score, fecal characteristics, hardness and color were more significantly improved in the probiotics yogurt

TABLE 1. Baseline data of constipation subjects.

Data	Group 1 (n = 25)	Group 2 (n = 25)	p-value
Female (%)	25 (100%)	24 (96%)	1.000
Mean Age ± SD (year)	34.86 ± 8.30	33.96 ± 6.17	0.666
Mean BMI ± SD (kg/m^2)	23.17 ± 3.26	22.56 ± 3.07	0.521
Mean Body weight ± SD (kg)	57.88 ± 10.40	55.04 ± 7.96	0.285
Frequency of defecation (times/week)			0.800
Median (max, min)	3 (2,7)	3 (1,7)	
Mean ± SD	3.00 ± 1.23	3.20 ± 1.71	
Dairy product intake (%)			0.836
None	14 (56%)	14 (56%)	
1-2 days/week	7 (28%)	8 (27%)	
3-7 days/week	4 (16%)	3 (12%)	
Fiber intake (%)			0.383
Few	8 (32%)	7 (28%)	
Moderate	13 (52%)	16 (64%)	
Many	4 (16%)	2 (8%)	
Exercise (%)			0.189
None	14 (56%)	16 (64%)	
1-2 days/week	5 (20%)	6 (24%)	
3-7 days/week	6 (24%)	3 (12%)	
Water intake (ml/day)			0.697
Median (max, min)	1000 (500, 2000)	1125 (500, 2000)	
Mean ± SD	1098.00 ± 484.44	1123.00 ± 414.00	
Vital sign median (max, min)			
Pulse (beat per minute)	82.08 ± 9.34	76.84 ± 9.74	0.058
Systolic blood pressure (mmHg)	111.28 ± 13.18	109.20 ± 15.23	0.608
Diastolic blood pressure (mmHg)	67.00 ± 10.17	68.56 ± 12.05	0.623
Body temperature (degree celsius)	36.52 ± 0.32	36.40 ± 0.25	0.245

TABLE 2. Frequency of defecation.

Frequency of defecation (times/week)	Pre-intake	Week 1	Week 2	Week 3	p-value
Group 1 (n = 25)		Probiotics yogurt			
Median (min, max)	3 (2,7)	5 (2,7)	6 (2,15)	6 (3,15)	<0.001
Mean	3.00	4.64	5.80	5.84	
Group 2 (n = 25)		Regular yogurt			
Median (min, max)	3 (1,7)	4 (1,12)	5 (2,11)	4 (3,14)	<0.001
Mean	3.20	4.12	4.68	4.56	

TABLE 3. Mean Score of defecation.

Score of defecation	Probiotics yogurt (n = 25)			Regular yogurt (n=25)		
	Before	After 3 weeks	p-value	Before	After 3 weeks	p-value
Total score (8-40)	23.42	20.11	<0.001	21.94	20.66	<0.001
Fecal amount (1-6)	2.18	2.45	0.048	2.23	2.19	0.841
Fecal characteristic (1-6)	4.22	3.38	<0.001	3.88	3.59	0.125
Fecal hardness (1-6)	4.34	3.44	<0.001	3.92	3.62	0.064
Fecal color (1-6)	3.54	2.91	<0.001	3.14	3.12	0.890
Fecal odor (1-4)	2.59	2.32	0.007	2.46	2.31	0.232
Straining during bowel movements (1-4)	2.38	2.10	0.013	2.32	2.21	0.388
Sensation of incomplete evacuation (1-4)	2.17	1.81	0.009	2.10	1.90	0.187
Sensation of anorectal blockage (1-4)	1.99	1.70	0.037	1.92	1.72	0.048

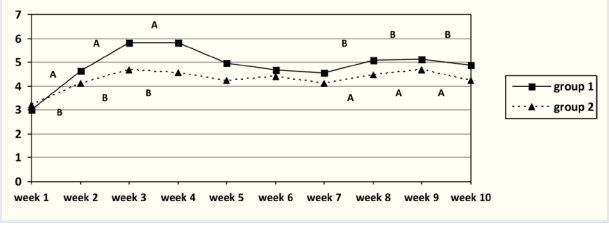
group than the regular yogurt group (p=0.047, 0.034, 0.013 and 0.005 respectively). Further analysis showed that stool quality was improved since the first week of probiotics yogurt intake compared with baseline in total score, fecal characteristics, hardness, color and sensation of anorectal blockage or obstruction (p<0.001, <0.001, <0.001, 0.002 and 0.026 respectively). No score improvement was found in the first week after taking regular yogurt (minimum p=0.095).

Complete blood count and all CD markers were

TABLE 4. Complete blood count and white blood cell subsets.

	Probiotics yogurt (n = 25)			Regular yogurt (n=25)		
	Before (mean ± SD)	After 3 weeks (mean ± SD)	p-value	Before (mean ± SD)	After 3 weeks (mean ± SD)	p-value
Hemoglobin (g/dl)	12.87 ± 1.06	12.59 ± 0.95	0.050	12.43 ± 0.88	12.40 ± 0.87	0.782
Hematocrit (%)	38.83 ± 2.77	37.95 ± 2.45	0.040	37.26 ± 2.29	37.16 ± 2.30	0.756
Platelet ($x10^3/\mu L$)	281.52 ± 75.05	287.40 ± 66.36	0.541	290.56 ± 2.23	306.80 ± 83.74	0.011
White blood cell ($x10^3/\mu L$)	7.15 ± 2.64	6.94 ± 1.66	0.607	7.36 ± 2.23	7.62 ± 1.97	0.485
Neutrophil (%)	57.68 ± 10.71	56.43 ± 7.99	0.531	58.37 ± 5.89	59.13 ± 8.85	0.643
Lymphocyte (%)	34.02 ± 9.93	34.98 ± 6.18	0.609	33.98 ± 5.49	33.80 ± 8.55	0.905
Monocyte (%)	5.15 ± 1.49	4.74 ± 1.09	0.106	4.94 ± 1.20	4.62 ± 0.90	0.164
Eosinophil (%)	2.68 ± 2.64	2.62 ± 2.43	0.668	2.24 ± 1.92	2.06 ± 1.36	0.378
Basophil (%)	0.44 ± 0.30	0.41 ± 0.24	0.369	0.47 ± 0.32	0.39 ± 0.22	0.220
CD3+cell (%)	68.62 ± 5.29	68.29 ± 5.62	0.417	68.51 ± 6.54	68.71 ± 6.52	0.757
CD3+cell (cells/μL)	1571.24 ± 451.96	1644.24 ± 464.67	0.373	1681.68 ± 450.78	1718.68 ± 492.62	0.600
CD8+cell (%)	26.18 ± 5.46	26.05 ± 6.19	0.809	25.83 ± 5.54	26.09 ± 6.19	0.413
CD8+cell (cells/μL)	601.80 ± 216.55	632.80 ± 252.59	0.442	637.12 ± 223.38	660.72 ± 246.72	0.472
CD4+cell (%)	38.82 ± 5.77	38.03 ± 6.14	0.070	38.28 ± 5.71	38.25 ± 6.00	0.959
CD4+cell (cells/μL)	883.44 ± 262.85	904.92 ± 253.04	0.580	931.24 ± 268.81	956.44 ± 318.69	0.534
CD19+cell (%)	16.61 ± 4.97	16.48 ± 4.96	0.789	16.21 ± 4.59	15.08 ± 4.19	0.335
CD19+cell (cells/μL)	371.16 ± 128.64	387.44 ± 125.55	0.291	394.52 ± 137.47	388.76 ± 111.97	0.702
CD56+cell: (%)	13.91 ± 4.69	13.92 ± 4.99	0.986	14.30 ± 5.19	14.63 ± 5.16	0.732
CD56+cell: (cells/μL)	319.96 ± 139.60	348.16 ± 177.61	0.217	346.80 ± 145.29	369.28 ± 172.29	0.548
IgA (mg/dl)	252.12 ± 69.11	229.80 ± 58.74	<0.001	266.84 ± 60.44	240.56 ± 63.24	<0.001
IgG (mg/dl)	1486.40 ± 248.86	1364.80 ± 206.88	<0.001	1582.40 ± 240.20	1403.20 ± 204.67	<0.001

Fig 2. Frequency of defecation in each week during 10 weeks cross-over study.



Friedman test: group 1 (n =25) p-value <0.001, group 2 (n=25) p-value <0.001

A = Probiotics yogurt
B = Regular yogurt

not significantly different after 3 weeks of yogurt intake in both groups as shown in Table 4. Hemoglobin and hematocrit were decreased in the probiotics yogurt group, platelets were increased in the regular yogurt group, and total IgA and IgG were decreased after 3 weeks of intake in both groups, but all such differences had no clinical importance.

Adverse events were found 9 and 11 times during probiotics or regular yogurt intake period respectively. They were upper respiratory tract infection, dyspepsia, abdominal discomfort, diarrhea, inflammation at venepuncture site, rash, urinary tract infection, dry lip, mouth ulcer, dizziness and myalgia. All of them were mild with spontaneous recovery.

DISCUSSION

Our findings revealed that the frequency and quality of defecation were improved after taking either probiotics yogurt or regular yogurt. However, probiotics yogurt was superior to regular yogurt in improving constipation symptoms. Our observations confirm previous studies.[3-5] However, improvement of immunity after probiotics yogurt intake including monocytes, neutrophils, natural killer cells and IgA level reported in previous studies were not observed in the present study. The contributing factors associated with such differences could be due to a difference in gender, race, type and amount of probiotics.

We also recognized that the carry-over effect and period effect of yogurt intake on constipation were longer than 3 weeks. Therefore, we were unable to analyze the study results as a full cross-over study and we had to analyze only the data from the first intake period. Further study on the effects of yogurts should extend the rest period to more than 3 weeks or until the baseline symptoms recur.

It should be kept in mind that almost all subjects who participated this study were women since the prevalence of constipation is more common in women. Therefore, the extent to which the study results could be applied to men is unknown. It's also worth noting that the effects of both types of yogurt used in our study had more effects on relieving constipation for the first 3 weeks of intake than the subsequent 3 weeks of intake. Therefore, it is unknown if probiotics yogurt and regular yogurt have long-term beneficial effects on relieving constipation, and further study on the long-term effects of probiotics yogurt and regular yogurt should be examined.

CONCLUSION

Stool frequency and quality of the constipated subjects were significantly improved after 3 weeks of Bifidobacterium yogurt and regular yogurt intake. Probiotics yogurt had more effects on stool frequency and quality than regular yogurt. No clinically important changes in immune system and no different adverse events were observed in both groups.

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All authors have seen and approved the manuscript and are fully conversant with its contents. All authors declare that we have no competing interests.

REFERENCES

1. Garrigues VC, Galvez CS, Ortiz VC, Ponce MT, Nos PL, Ponce JL. Prevalence of Constipation: Agreement among Several Criteria and Evaluation of the Diagnostic Accuracy of Qualifying Symptoms and self-reported Definition in a Population-based Survey in Spain. Am J Epidemiol. 2004 Mar 1;159(5):520-6.
2. Petticrew M, Rodgers M, Booth A. Effectiveness of laxatives in adult. Qual Health Care. 2001 Dec;10(4):268-73.
3. Shioya M, Nakaoka K, Iizuka N, Benno Y. Effect of fermented milk containing *Bifidobacteriumlactis* FK 120 on the fecal flora, with special reference to *Bifidium* species, the fecal properties in elderly volunteers. J Nutritional Food. 2000;3(2):33-44.
4. Nishida S, Gotou M, Akutsu S, et al. Effect of yogurt containing *Bifidobacteriumlactis* BB-12 on improvement of defecation and fecal microbioflora of healthy female adult. Milk Science. 2004;53:71-80.
5. Uchida K, Akashi K, Kusunoki A. Effect of fermented milk containing *Bifidobacteriumlactis* Bb-12 on stool frequency, defecation, fecal microbiota and safety of excessive ingestion in healthy female students. J Nutritional Food. 2005;8(1):39-51.
6. Olivares M, Ropero M, Gomez N, Lara-Villoslada F, Sierra S, Maldonado JA, et al. The consumption of two new probiotic strains, *Lactobacillusegasseri* CECT 5714 and *Lactobacillus coryniformmis* CECT 5711, boosts the immune system of healthy humans. IntMicrobiol. 2006 Mar;9(1): 47-52.
7. Amster HL, Rochat F, Saudan KY, Mignot O, Aeschlimann JM. Modulation of specific humoral immune response and changes in intestinal flora mediated through fermented milk intake. FEMS Immunol Med Microbiol. 1994 Nov;10(1):55-64.
8. Lewis SJ, Heaton KW. Stool form scale as a useful guide to intestinal transit time. Scand J Gastroenterol. 1997 Sep;32(9):920-4.
9. Bristol Stool Scale. Available from: http://en/wikipedia.org/wiki/Bristol_Stool_Scale. Accessed September 30, 2010.