Randomized Double-blind Controlled Trial: Benefits of *Lactobacillus reuteri* in Chronic Functional Constipation Patients

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ABSTRACT

Background: *Chronic functional constipation is a common problem that affects between 15-25% of the population and cause symptoms and disorders that creates discomfort, morbidity, and high costs for health care. Recently, the consumption of probiotics in treating chronic constipation in adults have been investigated. However, there are still limited and controversial evidences available from controlled trials. The aim of this study was to evaluate the effects of Lactobacillus reuteri (L. reuteri) in improving the Agachan constipation score, the number of L. reuteri in the feces and the fecal pH in the patients with chronic functional constipation.*

Method: *A double-blind, placebo randomized controlled trial (RCT) was conducted in 40 adults (12 males/28 females with mean age 45.95 ± 16 years) affected by chronic functional constipation according to Rome III criteria. Patients were randomly assigned to receive a supplementation of L. reuteri or placebo for 4 weeks.*

Results: *At week 4, the decrease in Agachan constipation score was from 17.00 to 8.00 with $p < 0.001$, the increase number of L. reuteri was from 6.80 x 107 to 2.12 x 108 with $p < 0.001$ and the decrease of pH feces was from 5.44 (SD 0.70) to 4.78 (SD 0.56) with $p < 0.001$ in the L. reuteri group, otherwise in the placebo group there were no significant results in Agachan constipation score, the number of L. reuteri and fecal pH assessed.*

Conclusion: *L. reuteri is more effective than the placebo group in improving the Agachan constipation score, increasing the number of L. reuteri in the feces and decreasing the fecal pH in adult with chronic functional constipation.*

Keywords: *Agachan constipation score, constipation, Lactobacillus reuteri (L. reuteri), fecal pH, polymerase chain reaction (PCR).*

ABSTRAK

Latar belakang: *Konstipasi fungsional kronik adalah masalah yang sering ditemukan di masyarakat dengan prevalensi sekitar 15-25%. Konstipasi menimbulkan berbagai gejala, meningkatkan angka kesakitan dan biaya kesehatan. Saat ini, penggunaan probiotik untuk pengobatan konstipasi kronik pada dewasa telah diteliti, namun,*

dari berbagai penelitian yang telah dilakukan hasil yang diperoleh masih terbatas dan menimbulkan kontroversi. Tujuan penelitian ini adalah untuk menilai manfaat Lactobacillus reuteri (L. reuteri) dalam memperbaiki skor konstipasi Agachan, jumlah L. reuteri feses dan pH feses pada pasien konstipasi fungsional kronik.

Metode: *Uji acak tersamar ganda dilakukan pada 40 pasien dewasa (12 laki-laki/28 perempuan), rerata usia 45,95 ± 16 tahun, yang menderita konstipasi fungsional kronik sesuai kriteria Rome III, selanjutnya dilakukan randomisasi dan diberikan L. reuteri atau plasebo selama 4 minggu.*

Hasil: *Pada minggu ke-4, setelah pemberian L. reuteri terjadi perbaikan gejala konstipasi, yang dinilai dari penurunan skor konstipasi Agachan dari 17 menjadi 8 dengan p < 0,001. Terjadi peningkatan jumlah L. reuteri feses dari 6,80 x 107 menjadi 2,12 x 108 dengan p < 0,001 dan penurunan pH feses dari 5,44 (SB 0,70) menjadi 4,78 (SB 0,56) dengan p < 0,001 pada kelompok L. reuteri, sedangkan pada kelompok plasebo tidak didapatkan hasil yang bermakna pada perbaikan skor konstipasi Agachan, jumlah L. reuteri feses dan pH feses.*

Simpulan: *L. reuteri lebih efektif dibandingkan plasebo dalam memperbaiki konstipasi, meningkatkan jumlah L. reuteri feses dan menurunkan pH feses pada pasien konstipasi fungsional kronik dewasa.*

Kata kunci: *skor konstipasi Agachan, konstipasi, Lactobacillus reuteri (L. reuteri), pH feses, polymerase chain reaction (PCR).*

INTRODUCTION

Chronic functional constipation is a gastrointestinal complain in form of bloating, constipation, abdominal pain that occured at least 12 weeks, in 6 months, without any organic or biochemistry disorder.[1,2] Chronic functional constipation have an impact on activity and give the sense of discomfort that will decrease the patients' quality of life, as well as give high-risk of hemorrhoids, anal fissures, and colorectal cancer occurance.[1,3] Prevalence of chronic functional constipation is about 15-25% of the population.[3] In Cipto Mangunkusumo Hospital Jakarta, from patients undergone colonoscopy procedure, 9% of them are constipation patients.[4] Treatment of chronic constipation which consists of long-term use of laxatives can lead to decreased gastrocolic and duodenocolic reflex, therefore caused dependence.[5] Therefore, it was necessary for a long-term alternative theraphy options which are beneficial and safe, one of them is by utilize the microbiota in the intestine. Human intestine contains various commensal and pathogenic microbiota. If the commensal and pathogenic microbiota in intestine balance is impaired intestinal pathogens, will cause gastrointestinal motility disorders and hardening of the stool that will cause constipation.[3,6,7,8,9]

Probiotics are live microorganisms when consumed in adequate amounts will provide health benefits, including for the treatment of constipation.[3,10] The use of probiotics for the treatment of chronic functional constipation recently received much attention. The most common used probiotics are *Lactobacillus* and *Bifidobacterium* which are short-chain fatty acids producing bacteria. Short-chain fatty acids lower pH of feces that will trigger increased intestinal motility and the frequency of defecation, deconjugation intestinal bile acids and increased water and minerals absorption, that will have effect on softening of stool. Several studies have reported that probiotics administration will increase intestinal motility and the frequency of defecation.[11,12,13,14] Futhermore, probiotics also can accelerate transit time of feces in the intestine, thus improving symptoms of constipation, which lead to improvement in the Agachan scores, as well as safe for use in patients with chronic constipation. *Lactobacillus reuteri* is one of commonly used probiotics.[3,15,16,17]

However, several studies report that probiotics do not give good results in patients with constipation. Coccurullo et al, Tabbers et al, and An et al reported that there is no significant differences in the frequency and consistency of defecation in patients taking probiotics.[16,18,19] This research was conducted because of the differences in the reported results of various studies on the use of *L. reuteri* in chronic constipation patients, and the lack of research on chronic constipation in adult patients which were assessed based on various aspects of constipation.

METHOD

This study is an experimental study with double-blind randomized trials. Subjects were divided into two groups, which are group A who are given *L. reuteri* and group B who are given placebo. This study was conducted in Cipto Mangunkusumo Hospital Jakarta, Bogor Medical Center Hospital, and Bina Husada Hospital Cibinong from April 2015 to May

Table 1. Characteristics of sujects before treatment

Characteristic	Group		p
	Placebo (n = 20)	*L. reuteri* (n = 20)	
Sex			
Male	6 (50)	6 (50)	1.000
Female	14 (50)	14 (50)	
Age (years old), mean (SD)	39.45 (14.86)	45.95 (16.74)	0.202
Feces pH before treatment, mean (SD)	5.39 (0.75)	5.44 (0.70)	0.831
Agachan score before treatment, range (min-max)	16.0 (15-19)	17.0 (16-20)	0.050
Numbers of L. reuteri/gram feces before treatment, range (min-max)	$1.34x10^8$ ($1.07x10^6$ – $1.43x10^9$)	$6.80x10^7$ ($3.23x10^7$ – $9.85x10^9$)	0.298

2015. Sampling was done by consecutive sampling technique with simple random sampling method. The sample size was calculated using the formula sample size for analytical research with numerical unpaired data, so that it was found that there must be 20 patients in every group. The inclusion criteria for this study are patients with age more than 18 years old, meet the Rome III criteria for constipation, increased stool consistency, unsatisfied defecation, sensation of anorectal obstruction, use of fingers to help defecation, defecate less than three times per week, not suffering from depression, absent of organic disorder in the colon which proven by colonoscopy, and provide writing consent to participate in this study. The exclusion criteria for this study are patients with age more than 65 years old, post-stroke patients, pregnant patients, suspected organic constipation, depression, patients with alarm symptoms, consumption of laxative drugs, patients who given antibiotics theraphy, presence of neurological disorders in the spinal cord of S2–S4, rectum disorders, and refused to give consent to participate in the study.

Forty constipation outpatients from Cipto Mangunkusumo Hospital (Jakarta), Bogor Medical Center Hospital, and Bina Husada Hospital (Cibinong), who meet the inclusion criteria are randomized. Patient history was taken for demographic data, medical history, drug history. Physical examination and cosntipation assessment with Agachan constipation score are done. Patients were given a sterile tube for stool specimen collection. Stool samples collection procedure described in the appendix. Stool samples were sent to the microbiology laboratory of Faculty of Medicine, University of Indonesia/Cipto Mangunkusumo Hospital using a special container with temperatures cooler to check *Lactobacillus reuteri* bacteria and feces pH. The examination procedure of *Lactobacillus reuteri* using the polymerase chain reaction (PCR) method described in the appendix. Then, the patient were given treatment with drugs with code A or drugs with code B which containing *Lactobacillus reuteri* 2 x 108 colony-forming unit (CFU) or placebo respectively, according to the results of randomization. Drugs taken 2 times a day for 4 weeks.

The data were processed using SPSS version 20. Variables with normal distribution were tested using unpaired t-test while variables with abnormal distribution were tested using Wilcoxon test. The differences were considered statistically significant if the value of $p < 0.05$ was obtained. This study was approved by the health research ethics committee of Faculty of Medicine, University of Indonesia.

RESULTS

During the study, it was found 40 subjects of chronic functional constipation patients, of which 6 subjects are from Gastroenterology Clinical Cipto Mangunkusumo Hospital (Jakarta), 25 subjects are from Bogor Medical Center Hospital, and 9 subjects from Bina Husada Hospital (Cibinong). Subjects were divided into two groups, 20 subjects (50%) were given placebo and 20 subjects (50%) were given *L. reuteri*, and there was no drop out subject. Ranges of Agachan scores before treatment in placebo and *L. reuteri* group are 16.00 (15-19), dan 17.00 (16-20) respectively. Ranges of numbers of *L. reuteri* before treatment in placebo and *L. reuteri* group are 1.34×10^8 (1.07×10^6-1.43×10^9)/gram feces and 6.80×10^7 (3.23×10^7- 9.85×10^9)/gram feces respectively. Means of feces pH before treatment are 5.39 ($\pm$ 0,75) in placebo group and 5.44 ($\pm$ 0.70) in *L. reuteri* group. Characteristics of subjects before treatment can be seen in Table 1.

There is a significant difference in Agachan scores in *Lactobacillus reuteri* group before and after treatment, while there is no significant difference in placebo group, as shown by Table 2.

Table 2. Agachan scores in placebo and *Lactobacillus reuteri* group before and after treatment

Group	Agachan score		p*
	Before	After (4 weeks)	
Placebo	16.00 (15-19)	16.0 (15-19)	0,132
Lactobacillus reuteri	17.00 (15-20)	8.00 (4-13)	< 0,001

*Wilcoxon test

Improvement of Agachan score's components in placebo and *Lactobacillus reuteri* groups are shown by Table 3.

Table 3. Changes in components of Agachan scores in placebo and *Lactobacillus reuteri* group before and after treatment

Variables of Agachan score	Placebo		*L. reuteri*	
	Before	After (4 weeks)	Before	After (4 weeks)
Frequency of defecation	2 (1-3)	2 (1-3)	2 (1-3)	1 (0-1)
Difficulty of defecation	3 (2-3)	3 (2-3)	3 (2-4)	1 (0-3)
Uncompleteness of defecation	3 (2-4)	3 (2-4)	3 (3-4)	2 (0-3)
Abdominal pain	3 (2-4)	3 (2-4)	3 (2-4)	1 (0-3)
Time in the toilet	3 (2-4)	3 (2-4)	2 (1-3)	2 (1-3)
Assistance needed for defation	1 (1-2)	1 (1-2)	1 (1-2)	1 (1-2)
Failure of defecation	2 (1-3)	2 (1-3)	1 (1-2)	0 (0-1)
Duration of costipation	1 (0-3)	1 (0-3)	1 (0-4)	1 (0-4)

Numbers of *Lactobacillus reuteri* in both groups are not balanced, so that Wilcoxon test was used. There is a significant difference in numbers of *Lactobacillus reuteri* in *Lactobacillus reuteri* group before and after treatment, while there is no significant difference in placebo group, as shown by Table 4.

Table 4. Numbers of *Lactobacillus reuteri* in placebo and *Lactobacillus reuteri* before and after treatment

Group	Numbers of feses *L. reuteri* (PCR)/ gram feces		p*
	Before	After	
Placebo	$1.34x10^8$ ($1.07x10^6$ – $1.43x10^9$)	$1.35x10^8$ ($1.11x10^8$ – $1.41x10^8$)	0.955
Lacto-bacillus reuteri	$6.80x10^7$ ($3.23x10^7$ – $9.85x10^9$)	$2.12x10^9$ ($2.26x10^7$ – $5.07x10^{11}$)	< 0.001

*Wilcoxon test

Measurement of feces pH in two groups given normally distributed data, therefore unpaired t-test was used. There is a significant difference in feces pH in *Lactobacillus reuteri* group before and after treatment, while there is no significant difference in placebo group, as shown by Table 5.

Table 5. Feces pH feses in placebo and *Lactobacillus reuteri* group before and after treatment

Group	Feces pH		p*
	Before	After	
Plasebo	5.39 (SD 0.75)	5.45 (SD 0.62)	0.588
Lactobacillus reuteri	5.44 (SD 0.70)	4.78 (SD 0.56)	< 0.001

*unpaired t-test

There are significant differences of delta median of Agachan score, numbers of *Lactobacillus reuteri* and feces pH between placebo and *Lactobacillus reuteri* group before and after treatment ($p < 0.001$). These results can be seen in Table 6.

Table 6. Differences of delta mean of feces pH, delta median of Agachan score and median numbers of *Lactobacillus reuteri* between placebo and *Lactobacillus reuteri* group

Variable	Group		p*
	Placebo	*L. reuteri*	
Delta feces pH, mean (SD)	0.05 (SD 0.42)	-0.66 (SD 0.46)	< 0,.001
Delta Agachan score, median (range)	0.0 (-1.0 – 1.0)	-10.0 (-14-(-3.0))	< 0.001*
Delta numbers of *Lactobacillus reuteri*, median (range)	8.0×10^4 (-7.09×10^7 – 3.4×10^7)	2.02×10^9 (-7.09×10^7 – 3.4×10^7)	< 0,001*

*unpaired t-test

DISCUSSION

In this study, female subjects are more than male subjects. Study by Yang et al reported that constipation are occured more in female than in male with ratio 4:1. This difference is caused by females who are more often exposured to anxiety, work fatigue, volatile emotions, and inactivity.[11,20]

In this study, it was found that there is significant difference in Agachan score in *Lactobacillus reuteri* group before and after treatment compared with placebo group. Improvement in defecation pattern occured in components of defecation frequency (100%), difficulty in defecation (85%), uncompleteness of defecation (90%), abdominal pain (85%), time in toilet (50%), assistance in defecation (100%), failure in defecation (100%) and duration of constipation (0%). This result is according to report of Waitzberg et al which found that improvement in Agachan score in patients which are given synbiotic.[15] Several studies in use of probiotic in constipation reported that there are significant improvements in frequency of defecation, intestinal motility, and stool consistency. Ojetti et al, Coccorullo et al, and Indrio et al reported that there are significant improvement in defecation frequency in the fourth week after *Lactobacillus reuteri* administration. Yang et al, Guera et al, Guyonnet et al, and Piano et al reported that there are increased in defecation frequency and improvement in stool consistency with *Bifidobacterium* administration.[3,6,14,11,21,22,23] From several studies which already been conducted, assessment only done in several components of constipation, which are defecation frequency, stool consistency and abdominal pain, however in this sudy, assessment was done in many components with significant result.

In the study by Tabbers et al, there is no siginificant improvement in defecation frequency in children whose given *Bifidobacterium*, whereas the study by Bekkali et al reported that there is significant improvement in

defecation requency, but there is no improvement in stool consistency.[18,24] Difference in results found by two studies mentioned above is becaused the sudies conducted in children in which there are differences in constipation in children and adults in terms of onset, etiology, symptoms, treatment and prognosis. The study by An et al reported that there is increased in defecation frequency in elderly patients whose given *L. reuteri*, but it was unsignificant, this caused by high risk of imobility, polypharmacy, and other chronic diseased owned by the elderly patients. This difference with this study is the age ranges of patients in this study are 39.45 tahun (± 14.86) for female patients and 45.95 tahun (± 16.74) for male patients.[19]

In this study, numbers of *L. reuteri* in feces increased significantly in *L. reuteri* group in fourth week after the treatment, but there is no significant difference in placebo group. This result is according to the study by Wolf et al which reported that there is significant increased in numbers of *L. reuteri* in feces in patients whose given *L. reuteri* in seventh, fourteenth, and twenty first day.[17] The same result also reported by Lidbeck et al and An et al.[25] Study by Spanhaak et al reported that administration of 10^9 CFU *Lactobacillus* in healthy adults can increased the numbers of *Lactobacillus* as much as 10^7/gram feses and decreased the numbers of *Clostridium* bacteria. This result showed that there is balance between comensal and pathogenic bacteria in intestine.[26] Bu et al reported that there is increased of *Lactobacillus* in feces after *Lactobacillus casei rhamnosus* administration, but it was not related to defecation frequency.[27] The difference in the results are probably by different strain of *Lactobacillus* which used in each study.

Numbers of consumpted *L. reuteri* can affect numbers of *L. reuteri* in feces. Bu et al reported that *L. reuteri* must be given in dose of 10^9-10^{10} CFU per day in order to be detected in feces. In this study, the patients were given 2 x 10^8 CFU *Lactobacillus reuteri* and the numbers of *L. reuteri* in feces increased significantly.[27] Study by Sinkiewicz et al reported that women in Japan have higher prevalence of *L. reuteri* colonization compared to women in other countries, which probably cuased by consumption of functional foods, probiotics and various fermented foods in Japanese diet so that it can be concluded that the types of foods with high fiber and low fat may affect the numbers of *L. reuteri* in intestine.[28,29]

Studies by Lee et al and Stewart et al reported that consumption of glutathione and inulin can increased numbers of *L. reuteri* in intestine. In our study, the assessment in patients' diet was not done, therefore effect of diet to numbers of *L. reuteri* cannot be determined. However, in our study, both groups of subjects already informed not to consume foods that contain probiotic, fermented foods and several herbal medicines that will affect the study's result.[30,31] Feces pH in this study was significantly decreased after the treatment compared to placebo group. This result is according to result of Koebnick et al which reported *Lactobacillus casei* administration in children patients with chronic cosntipation can decreased feces pH.[32] The decreased feces pH caused by production of acid forming bacteria. However, Spanhaak et al reported that there is no difference of feces pH in probiotic administraion so that the effect on intestinal motility is doubted.[26]

There is no side effect of *L. reuteri* administration in this study. The result is correspond with studies by Bekkali et al and Jones et al which reported that there is no side effect found in administration of 2.9 x 10^9 *L. reuteri* for 9 weeks in constipation patients.[24,26] Futhermore, Bu et al reported that there is no side effect of *Lactobacillus casei rhamnosus* administration in children patients.[24,26,27] *L. reuteri* can be considered as a treatment option for chronic functional constipation. Examination of feces *L. reuteri* feses can be used to assess number of feces *L. reuteri* in chronic functional constipation patients which given treatment of *L. reuteri*. Feces pH feses also can be used as examination to monitor *L. reuteri* administration in chronic functional constipation patients.

CONCLUSION

After *L. reuteri* administration for 4 weeks, it can be concluded that there are improvement in defecation pattern in chronic functional constipation patients, increased number of *L. reuteri* feces in chronic functional constipation patients, and pH reduction of feces in chronic functional constipation patients.

REFERENCES

1. Jayasimhan S, Yap NY, Roest Y, Rajandram R, Chin KF. Efficacy of microbial cell preparation in improving chronic constipation: a randomized, double-blind, placebo-controlled trial. Clin Nutr 2013;32:928-34.
2. Thompson WG, Longstreth GF, Drossman DA. Functional bowel disorders and functional abdominal pain. Gut 1999;45:II43-II47.
3. Ojetti V, Ianiro G, Tortora A, D'Angelo G. The effect of *Lactobacillus reuteri* supplementation in adults with chronic functional constipation: a randomised, double-blind, placebo-controlled trial. J Gastrointestin Liver Dis 2014;23:387-91.

4. Perkumpulan Gastroenterologi Indonesia (PGI). Konsensus Nasional Penatalaksanaan Konstipasi di Indonesia. In: Simadibrata M, Makmun D, Abdullah M, Syah A, Fauzi A, eds. Jakarta: Interna Publ 2010.
5. Lacy BE, Levenick J, Crowell M. Recent advances in the management of difficult constipation. Curr Gastroenterol Rep 2012;14:306-12.
6. Coccorullo P, Strisciuglio C, Martinelli M. *Lactobacillus reuteri* (DSM 17938) in infants with functional chronic constipation: a double-blind, randomized, placebo-controlled study. J Pediatr 2010;157:598-602.
7. Probiotics-friendly bacteria with a host of benefits. Dairy Council of California [serial online] 2000 [cited June 25 2015]. Available from:URL:http://www.dairycouncilofca.org/p dfs/probiotics.pdf.
8. Chmielewska A, Szajewska H. Systematic review of randomised controlled trials: probiotics for functional constipation. World J Gastroenterol 2010;16:69-75.
9. Tazoe H, Kaji YO, Tanaka R, Karaki SI, Kuwahara A. Roles of short-chain fatty acids receptors, GPR41 and GPR43 on colonic functions. J Physiol Pharmacol 2008;59:251-62.
10. Weichselbaum E. Probiotics and health: a review of the evidence. Nutrition Bulletin 2009;34:340-73.
11. Yang YX, He M, Hu G, Wei J, Pages P, Yang XH, et al. Effect of a fermented milk containing *Bifidobacterium lactis* DN-173010 on Chinese constipated women. World J Gastroenterol 2008;14:6237-43.
12. Koebnick C, Wagner I, Leitzmann P, Stern U, Zunft HF. Probiotic beverage containing *Lactobacillus casei shirota* improves gastrointestinal symptoms in patients with chronic constipation. Can J Gastroenterol 2003;17:655-59.
13. Bu LN, Chang MH, Ni YH, Chen HL, Cheng CC. *Lactobacillus casei rhamnosus* Lcr35 in children with chronic constipation. Pediatrics International 2007;49:485-90.
14. Indrio F, Mauro AD, Riezzo G, Civardi E, Intini C, Corvaglia L, et al. Prophylactic use of a probiotic in the prevention of colic, regurgitation, and functional constipation: a randomized clinical trial. JAMA Pediatr 2014;168:228-33.
15. Waitzberg DL, Logullo LC, Bittencourt AF, Torrinhas RS, Shiroma GM, Paulino NP, et al. Effect on synbiotic in constipated adult women-a randomized, double-blind, placebo-controlled study of clinical response. Clin Nutr 2013;32:27-33.
16. Jones ML, Martoni CJ, Pietro ED, Simon RR, Prakash S. Evaluation of clinical safety and tolerance of a *Lactobacillus reuteri* NCIMB 30242 supplement capsule: a randomized control trial. Regulatory Toxicology and Pharmacology 2012;63:313-20.
17. Wolf BW, Wheeler KB, Ataya DG, Garleb KA. Safety and tolerance of *Lactobacillus reuteri* supplementation to a population infected with the human immunodeficiency virus. Food Chem Toxicol 1998;36:1085-94.
18. Tabbers MM, Chmielewska A, Roseboom MG, Crastes N, Perrin c, Reitsma JB, et al. Fermented milk containing *Bifidobacterium lactis* DN-173010 in childhoood constipation: a randomized double-blind, controlled trial. American Academic Paediatric 2015;1392-99.
19. An HM, Baek EH, Jang S. Efficacy of lactic acid bacteria (LAB) supplement in management of constipation among nursing home residents. Nutr J 2010;9:5.
20. World Gastroenterology Organisation Practise Guidelines: Constipation World Gastroenterology Organisation 2007;1-10.
21. Guerra PVP, Lima LN, Souza TC, Mazochi V, Penna FJ, Silva AM, et al. Pediatric functional constipation treatment with Bifidobacterium-containing yogurt: a crossover, double-blind, controlled trial. World J Gastroenterol 2011;17:3916-21.
22. Guyonnet D, Chassany O, Ducrotte P, Picard C, Mourets M, Mercier CH, et al. Effect of a fermented milk containing *Bifidobacterium animalis* DN-173 010 on the health-related quality of life and symptoms in irritable bowel syndrome in adults in primary care: a multicentre, randomized, double-blind, controlled trial. Aliment Pharmacol Ther 2007:26:475-86.
23. Piano MD, Carmagnola S, Anderloni A, Andorno S, Ballare M, Balzarini M, et al. The use of probiotics in healthy volunteers with evacuation disorders and hard stools. J Clin Gastroenterol 2010;44:S30-4.
24. Bekkali NL, Bongers ME, Berg MM, Liem O, Benninga MA. The role of a probiotics mixture in the treatment of childhood constipation: a pilot study. Nutr J 2007;6:17.
25. Lidbeck A, Gustafsson JA, Nord CE. Impact of *Lactobacillus acidophilus* supplements on the human oropharyngeal and intestinal microflora. Scand J Infect Dis 1987;19:531-7.
26. Spanhaak S, Havenaar R, Schaafsma G. The effect of consumption of milk fermented by *Lactobacillus casei* strain shirota on the intestinal microflora and immune parameters in human . Eur J Clin Nutr 1998;52:899-907.
27. Bu LN, Chang MH, Ni YH, Chen HL, Cheng CC. *Lactobacillus casei rhamnosus* Lcr35 in children with chronic constipation. Pediatr Int 2007;49:485-90.
28. Sinkiewicz G, Ljunggren L. Occurrence of *Lactobacillus reuteri* in human breast milk. Microbial Ecology in Health and Disease 2008;20:122-6.
29. Ray B, Bhunia A. Fundamental food microbiology. 5th ed. Florida: CRC Press Book 2013.p.196.
30. Lee K, Kim HJ, Rho BS. Effect of glutathione on growth of the probiotic bacterium *Lactobacillus reuteri*. Biochemistry 2011;76:423-6.
31. Stewart ML, Savarino V, Slavin JL. Assessment of dietary fiber fermentation: effect of *Lactobacillus reuteri* and reproducibility of short-chain fatty acid concentrations. Mol Nutr Food Res 2009;53:S114-20.
32. Koebnick C, Wagner I, Leitzmann P, Stern U, Zunft HF. Probiotic beverage containing *Lactobacillus casei Shirota* improves gastrointestinal symptoms in patients with chronic constipation. Can J Gastroenterol 2003;17:655-9.