Research article

Effect of Synbiotic Supplementation on Defecation among Adults with Gastrointestinal Disorder

Nattakan Nin-on [1], Promluck Sanporkha [1], Suwimol Sapwarobol [2], Chatrapa Hudthagosol[1*]

[1] *Department of Nutrition, Faculty of Public Health, Mahidol University*

[2] *Department of Nutrition and Dietetics, Faculty of Allied Health Sciences, Chulalongkorn University*

ABSTRACT

Gastrointestinal infection can be caused by pathogenic contamination leading to an intestinal microflora imbalance and may lead to disease or other symptoms. Consequently, a modification that balances microflora in the gut is a good alternative for preventing disease or symptoms. This study is a randomized controlled trial, comparing the effect of synbiotic supplementation on stool frequency and stool consistency between intervention and placebo groups for 12 weeks. Self-reported bowel habits were made based on the Bristol Stool Scale. The statistical analysis included paired t-test to analyze data within groups, as well as independent t-test to analyze data between groups. In addition, the Chi-square test or Fisher's exact test was used to analyze the difference in stool consistency. Results showed that stool frequency remained unchanged within and between groups. However, stool consistency was significantly different between groups. Comparison between normal stools (Type 4-5) and abnormal stools (Hard stool type 1-2 and loose stool type 6-7), the synbiotic supplementation group had a significantly higher normal stools than in the placebo group. This study concludes that synbiotic supplementation for 12 weeks does not affect stool frequency when compared within and between groups, but it significantly affected a change in stool consistency to an improved normal stool type.

Key words: Synbiotic, Defecation, Gastrointestinal disorder

Received: 9 November 2020 Accepted: 12 Febuary 2021 Available online: 7 April 2021

*Corresponding author's email: chatrapa.hud@mahidol.ac.th

บทความวิจัย

ผลของผลิตภัณฑ์ซินไบโอติกต่อการขับถ่ายอุจจาระในผู้ใหญ่ที่มีปัญหาระบบทางเดินอาหาร

ณัฐกานต์ นิลอ่อน[1], พร้อมลักษณ์ สรรพ่อค้า[1], สุวิมล ทรัพย์วโรบล[2], ฉัตรภา หัตถโกศล[*1]

[1]ภาควิชาโภชนวิทยา คณะสาธารณสุขศาสตร์ มหาวิทยาลัยมหิดล
[2]ภาควิชาโภชนาการและการกำหนดอาหาร คณะสหเวชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

บทคัดย่อ

การติดเชื้อในระบบทางเดินอาหาร มีสาเหตุจากการปนเปื้อนของเชื้อก่อโรค ซึ่งทำให้จุลินทรีย์ในลำไส้เสียสมดุลอาจก่อให้เกิดโรคหรืออาการอื่นๆตามมา ดังนั้นการปรับสมดุลของจุลินทรีย์ในลำไส้จึงเป็นทางเลือกที่ดีต่อการป้องกันโรคหรืออาการ การศึกษาเป็นการทดลองแบบสุ่มโดยมีวัตถุประสงค์เพื่อเปรียบเทียบผลการได้รับซินไบโอติกต่อความถี่การขับถ่ายและลักษณะอุจจาระระหว่างกลุ่มทดลองและกลุ่มยาหลอกเป็นระยะเวลา 12 สัปดาห์ ผู้เข้าร่วมการศึกษาต้องผ่านเกณฑ์การคัดเข้าและคัดออก และได้รับแบบบันทึกการขับถ่ายอ้างอิง Bristol Stool Scale การวิเคราะห์ทางสถิติใช้ Pair t-test เพื่อวิเคราะห์ข้อมูลภายในกลุ่ม Independent t-test เพื่อวิเคราะห์ข้อมูลระหว่างกลุ่ม การทดสอบ Chi-square หรือ Fisher's exact เพื่อเปรียบเทียบลักษณะอุจจาระในกลุ่มที่ได้รับซินไบโอติกกับกลุ่มควบคุม ผลการทดลอง ไม่พบการเปลี่ยนแปลงความถี่การขับถ่ายภายในกลุ่มและระหว่างกลุ่ม แต่พบความแตกต่างของลักษณะอุจจาระจากลักษณะเหลวไปสู่ลักษณะที่เป็นก้อนแข็งขึ้น และการจัดกลุ่มเปรียบเทียบระหว่างอุจจาระปกติ (ประเภทที่ 3-5) และอุจจาระผิดปกติ (ประเภทที่ 1-2 อุจจาระแข็ง ประเภทที่ 6-7 อุจจาระเหลว) พบว่าร้อยละอุจจาระปกติในกลุ่มทดลองและกลุ่มยาหลอกมีความแตกต่างอย่างมีนัยสำคัญทางสถิติ สรุป การได้รับซินไบโอติก 12 สัปดาห์ไม่พบความแตกต่างของความถี่การขับถ่าย แต่ลักษณะอุจจาระเปลี่ยนแปลงเป็นลักษณะอุจจาระที่ดีขึ้น

คำสำคัญ: ซินไบโอติก การขับถ่าย ความผิดปกติของระบบทางเดินอาหาร

*Corresponding author's email: chatrapa.hud@mahidol.ac.th

Introduction

The rapidly changing environment, climate, and demographics have affected several pathogens, thus making them more adaptable and stronger[1]. In addition, air pollution, toxins, or pathogens are generated by contaminated air, water, and food, which can cause infectious diseases[2]. The Institute for Health Metrics and Evaluation has reported an increasing mortality rate due to diarrheal diseases[3]. Public health statistics in Thailand for 2018 revealed the mortality rate was 29.4 per 100,000 population annually[4]. Recurring diarrheal diseases may cause irritable bowel syndrome and its symptoms, such as abdominal pain related to defecation, as well as changes in stool characteristics or stool frequency[5]. It may also lead to inappropriate use of antibiotics and to microbiome dysbiosis in the gastrointestinal tract[6,7]. However, an adaptation of microflora using a synbiotic in the gut has a potential to alleviate and prevent symptoms[8].

Synbiotic is a dietary supplement mixture of probiotics and prebiotics that beneficially affects the host by improving the survival and activity of beneficial microorganisms in the gut. These two coexisting components generate a synergistic effect to enhance efficacy, working directly through probiotic and prebiotic elements[9]. By the definition, a probiotic is comprised of live microorganisms that under adequate and suitable conditions can provide a host with health benefits[10]. A prebiotic is a non-digestible fiber resistant to acid, bile salt, and enzymes that stimulates and improves the beneficial activity of microorganisms present in the colon[11,12]. This synbiotic activity induces modifications of microbiota in the gastrointestinal tract[9,11,13]. The activity increases *Lactobacilli* and *Bifidobacterial* but decreases levels of hazard microorganisms, such as *Coliforms* and *Clostridia*[11]. Those actions reduce the risk factors for several diseases and improve bowel movement, the absorption of minerals[14] and antioxidants, improves immune function[15] and reduces inflammation[16]. This study was primarily interested in the probiotic *Bacillus coagulans* and the prebiotic inulin as the synbiotic as they affect defecation among Thai adults with gastrointestinal disorders. Employing a randomized controlled trial, this study compared the effect of synbiotic supplementation on defecation in terms of stool frequency and stool consistency between intervention and placebo groups.

Materials and methods

This study's sample size was calculated based on a study by Kimmel et al.[17] in which each group had least 16 participants and adding drop-out rate. Therefore, the participants each group was 25 participants. This study's participants comprised both males and females, aged between 18 and 60 years, had a body mass index of 23.0-29.9 kg/m^2. The participants presented symptoms of discomfort or abdominal pain and/or changing stool frequency and/or consistency. They had no chronic diseases, were non-smokers and non-drinkers of alcoholic beverages, and were not taking prebiotic or probiotic supplements. Exclusion criteria consisted of illness, pregnancy or lactation status, use of antibiotics or laxatives, and compliance less than 70%. The study advertised for volunteers using

online postings and an application on Rajavithi Campus, Mahidol University. All volunteers who were enrolled in the trial adhered to the inclusion criteria. They were screened and each person was interviewed by telephone. The participants were randomized by drawing lots for placement in the synbiotic (intervention) or placebo groups. Each group had 25 participants and received five tablets of a daily supplement in the morning for 12 weeks. The intervention group received daily synbiotic tablets containing 1.4 gram of inulin and 500 million CFU of *Bacillus coagulans* GBI-30 (BALANCE Synbiotic Tablets Yoghurt Flavor). The placebo group received supplements similar in appearance and smell to the synbiotic group, but the placebo supplement tablets did not include a probiotic and a prebiotic. The participants were advised to maintain their dietary habits and physical activity throughout the study. At week 0, the data collected for defecation included daily self-reported bowel habits in terms of stool frequency and stool consistency based on the 7-point Bristol Stool Scale. This scale evaluates stool form in humans who have a gastrointestinal disorder. The classified 7-point scale includes: Type 1: the stool is hard and separates into pieces; Type 2: the stool is hard and long or in a sausage shape with a rough surface; Type 3: the stool has a hard and long shape with a cracked surface; Type 4: the stool has a soft, long shape with a smooth surface; Type 5: the stool has a very soft, droplet shape with separating clear edges; Type 6: the stool has a very soft to mushy consistency with tattered edges; and Type 7: the stool is liquid without solid adulteration[18].

Statistical analysis

Statistical analysis was conducted using SPSS for Windows Version 18. All data differences were statistically significant at *P*-value less than 0.05. Baseline characteristics were present as mean ± SEM and sex was reported as Number (%). Stool frequency was presented as Mean ± SD. Comparisons were made at two weeks and included the initial start and end of the trial. Comparisons were made between the two groups using paired t-test and independent t-test, respectively. In addition, stool consistency as presented by stool type group (hard, normal, loose stool) and classified in terms of stool type by normal (Type 3-5) and abnormal stools (Type 1-2 as hard stool, type 6-7 as loose stool)[19] were also reported as percent (%) using Chi-square test or Fisher's exact test to compare the relation of receiving the intervention to stool consistency. These tests assessed independent outcomes associated between the two variables when comparing groups[20]. This study received ethical approval by the committee on human research in the Faculty of Public Health, Mahidol University, Approval number COA. No. MUPH 2019-047.

Results

At the end of the study, 46 participants had fully completed the intervention, 22 and 24 participants in the synbiotic and the placebo group, respectively (**Figure 1)**. Baseline characteristics of the synbiotic and placebo groups in terms of sex, age, weight, body mass index, and body fat percentage were not significantly different between groups (**Table 1**). After the intervention period, comparison of

stool frequency (times/week) between groups did not differ and results compared within groups remained steady from baseline to the trials' end (p>0.05) (**Table 2**). Stool consistency was present as number and percentages (%) by type of defecation consisting of hard, normal, and loose stool. There was a significant difference between the synbiotic and the placebo groups from week 7 (p=0.007) to week 12 (p=0.044) (**Table 3**). Similarly, by recategorization of defecation to be normal stool type (Type 3-5) and abnormal stool type (Type 1-2 as hard stool, Type 6-7 as loose stool), there was a significant difference between the synbiotic group and placebo group from week 7-12. (**Table 4**) These results showed that receiving the synbiotic supplement was affected the stool consistency.

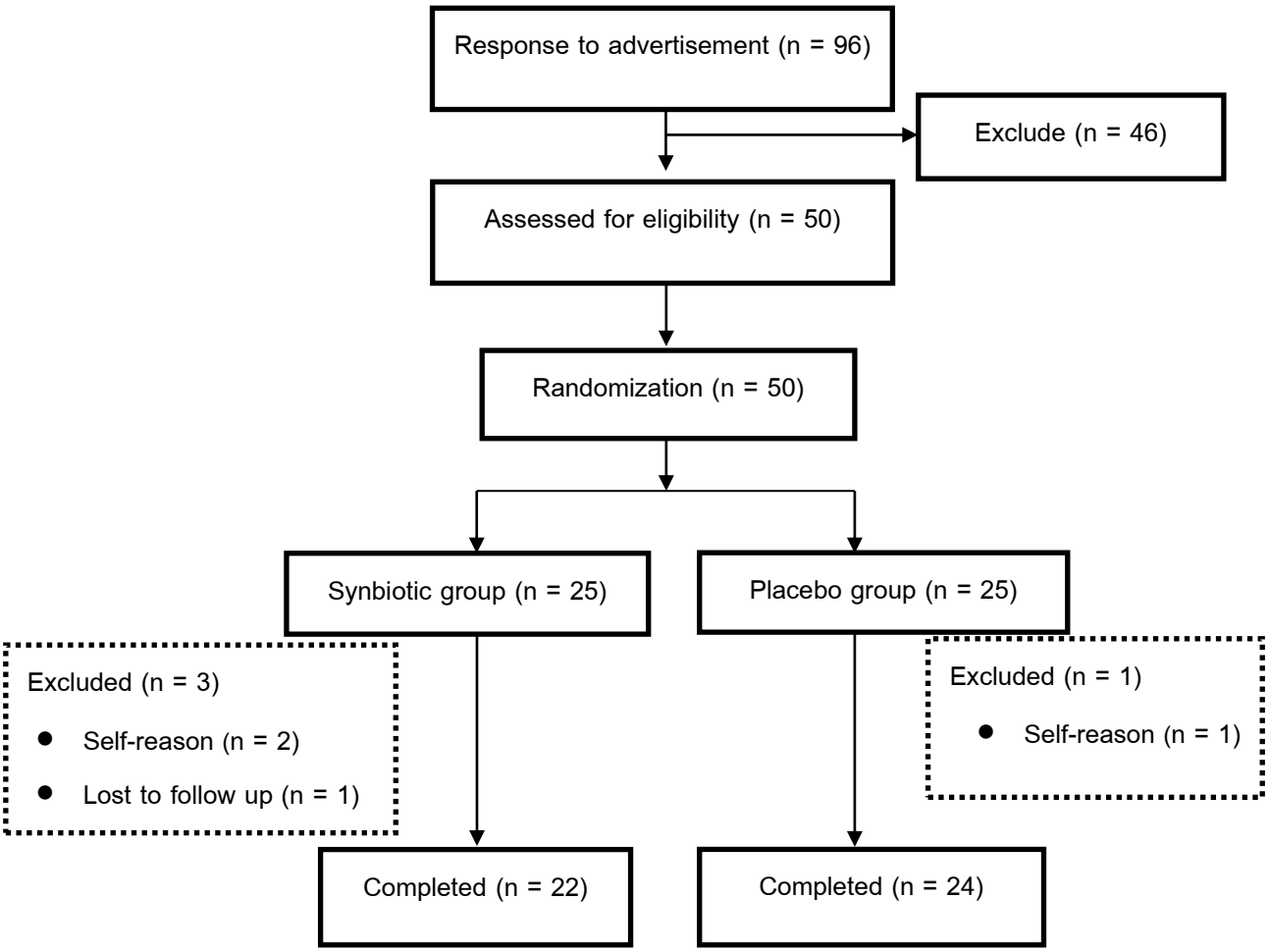

Figure 1 Details of participant recruitment and withdrawal

Table 1 Baseline characteristics of synbiotic and placebo groups

Characteristic	**Synbiotic** (n=17)	**Placebo** (n=17)	**P-value**
Female (%)	13 (76.47%)	11 (64.71%)	0.452[b]
Male (%)	4 (23.53%)	6 (35.29%)	
Age (years)	34.59 ± 2.72	35.82 ± 2.92	0.759[a]
Weight (Kg)	67.45 ± 2.76	68.68 ± 2.64	0.748[a]
BMI (Kg/m^2)	25.43 ± 0.77	27.97 ± 1.19	0.083[a]
Body fat percentage	31.85 ± 1.97	34.56 ± 2.42	0.391[a]

[a] Comparison between the intervention and the placebo groups by Independent t-test

[b] Comparison between the intervention and the placebo groups by Chi-Square Test

Table 2 Stool frequency (average times/week) of participants by treatment period

Study group	**Study period; Mean±SD**		***P-value*** [a]
	Week 0	**Week 12**	
Synbiotic	8.65 ± 3.57	8.00 ± 2.74	0.272
Placebo	7.59 ± 2.96	8.00 ± 3.20	0.248
P-value [b]	0.353	1.000	

[a] Comparing Mean ± SD within group between weeks 0 and 12 by Paired Samples T-test

[b] Comparing Mean ± SD between synbiotic and placebo groups by Independent-Samples T-test

Table 3 Number (%) of participants showing stool consistency during the study period

Study group	**Stool consistency**	**Study period; n (%)**				
		Week 0	**Week 1-3**	**Week 4-6**	**Week 7-9**	**Week 10-12**
Synbiotic	1-2 (Hard)	4 (23.5%)	3 (17.6%)	2 (11.8%)	0 (0%)	0 (0%)
	3-5 (Normal)	6 (35.3%)	10 (58.8%)	12 (70.6%)	17 (100%)	17 (100%)
	6-7 (Loose)	7 (41.2%)	4 (23.5%)	3 (17.6%)	0 (0%)	0 (0%)
Placebo	1-2 (Hard)	3 (17.6%)	2 (11.8%)	3 (17.6%)	3 (17.6%)	0 (0%)
	3-5 (Normal)	5 (29.4%)	8 (47.1%)	11 (64.7%)	10 (58.8%)	12 (70.6%)
	6-7 (Loose)	9 (52.9%)	7 (41.2%)	3 (17.6%)	4 (23.5%)	5 (29.4%)
P-value[a]		0.822	0.634	1.000	0.007	0.044

[a] Comparing number (%) between synbiotic and placebo groups using Fisher-exact test

Table 4 Number (%) of participants showing stool consistency as normal or abnormal stool type during the study period

Study groups	Stool consistency	Study period; n (%)				
		Week 0	Week 1-3	Week 4-6	Week 7-9	Week 10-12
Synbiotic	Normal	6 (35.3%)	10 (58.8%)	12 (70.6%)	17 (100%)	17 (100%)
	Abnormal	11 (64.7%)	7 (41.2%)	5 (29.4%)	0 (0.00%)	0 (0.00%)
Placebo	Normal	5 (29.4%)	8 (47.1%)	11 (64.7%)	10 (58.8%)	12 (70.6%)
	Abnormal	12 (70.6%)	9 (52.9%)	6 (35.3%)	7 (41.2%)	5 (29.4%)
P-value		0.714[a]	0.492[a]	0.714[a]	0.007[b]	0.044[b]

[a] Comparing number (%) between synbiotic and placebo groups using Chi-square test

[b] Comparing number (%) between synbiotic and placebo groups using Fisher-exact test

Discussion

This study compared the effect of synbiotic supplementation on stool frequency and stool consistency between intervention and placebo groups for 12 weeks. Results showed that the consumption of synbiotic supplementation for 12 weeks does not cause a significant difference in stool frequency between the synbiotic and placebo groups. Our findings are similar to a previous study by Vaisman et al. that reported the effect of galacto-oligosaccharide (GOS) and fructo-oligosaccharides (FOS) among children with acute diarrhea. Their results showed a decline in stool frequency but was not significantly different between groups[21]. Our results are consistent with other findings, which showed that prebiotic chicory-derived inulin-type fructan at a daily intake of inulin 8 gram for 4 weeks did not change stool frequency for patients with moderate abdominal discomfort without diarrhea[22]. In contrast, in an earlier study of a probiotic that was identical to the *Bacillus coagulans* strain, Majeed et al. reported that *Bacillus coagulans* at a daily dose of 2×10^9 CFU significantly reduced stool frequency for 90 days among patients with diarrhea-predominant irritable bowel syndromes (IBS)[23]. Nevertheless, this trial concentrated on adults who had gastrointestinal disorders with recurrent constipation and diarrhea. The participants in the present study received a synbiotic supplement containing 1.4 gram of inulin and 500 million CFU of *Bacillus coagulans* GBI-30. A previous study on gastrointestinal function confirmed that stool frequency remained unchanged and may be due to other factors during the intervention period[24]. In addition, Kelly et al. found an effective dose of at least 2.5 to 5 gram prebiotic as inulin-type might be affected by *Bifidobacterium* strains[25]. This finding demonstrated the unchanging number of stools during an intervention may be affected by several factors including food consumption, exercise, life style, and characteristics of an

individual,[24] such as age over 35 years, especially among older females who have lower stool frequency[26]. Moreover, sex hormones play a crucial role in modulating stress and gut motility associated with the neural and emotional systems[27]. A probiotic dose at 10^{10}-10^{11} CFU/day, which is the recommended dosage, has a greater effect on acute infectious diarrhea[28]. These factor may affect the appearance and progression of most common functional gastrointestinal disorders[27].

This study showed statistical difference in stool consistency between groups, a result that is similar to a previous study. Consumption of galacto-oligosaccharide (GOS) and fructo-oligosaccharides (FOS) changed stool characteristics from loose to hard[21]. Prebiotic of inulin-type can maintain the colonic microbiota as *Bifidobacteria* and *Lactobacillus*[29-30] by improved epithelial barrier function, improved host defense immunity, and inhibited translocation pathogens[31]. In addition, the probiotic *Bacillus coagulans* 2 billions CFU was found to improve stool consistency,[32] which is similar to that reported by Rogha et al. *Bacillus coagulans* (15×10^7 Spores) contained in a synbiotic decreased watery stool frequency after the intervention among adults with irritable bowel disease[33]. This situation influenced the intestinal environment and reduced toxic metabolites[34]. The possible mechanism acts as a synbiotic via probiotic and prebiotic action. *Bacillus coagulans* and inulin act by increasing fermentation in the gut that provides induced SCFAs products such as butyrate, propionate, and acetate acids[35-36]. This stimulates excretion of mucus in the intestine that supports and strengthens the epithelium layer and has the potential to affect intestinal homeostasis which increases counts of *Lactobacilli* and *Bifidobacteria* species but inhibits the number of E. *coli, Bacteroides, Clostridia* and other pathogens[36-37]. It thus affects gut mucosal barrier integrity[28]. Furthermore, gut microbiota diversity is correlated with nutrient mobility, water activity, and transit time of feces in the large intestine. These factors affect the growth rate of bacteria in a selective manner and is strongly related to microbiota composition, hence, might also improve stool consistency characteristics[38].

This study's results regarding stool consistency might be due to increasing microbial diversity by synbiotic action that affects stool characteristics. The proportion of normal stools that were soft and sausage like in the synbiotic group was greater than that in the placebo group. One limitation of the trial was that participants did not record information about the stool charactersitics in the daily report after defecating, in spite of being reminded to do so immediately on the day. This could affect the accuracy of the stool information. Additionally, the prebiotic in this study is 1.4 gram which may reduce gastrointestinal disorder, it is not adequate for improvement. Hence, a future study should entail an increasing intake of the prebiotic dose to better determine treatment efficacy.

Conclusions

Results on the consumption of a synbiotic supplement on defecation among adults with a gastrointestinal disorder for 12 consecutive weeks showed that the stool frequency did not significantly differ between

baseline to the end of the trial. Comparison between intervention and placebo groups in terms of stool consistency, however, indicated significant stool characteristics wherein hard stool (Type 1-2) and loose stool (Type 6-7) types changed to the normal stool (Type 3-5) type from week 7 onwards in the intervention group. Likewise, significantly difference was evident in terms of changes from abnormal stools to normal stools when compared between the synbiotic and placebo groups.

References

1. Wilcox BA, Colwell RR. Emerging and reemerging infectious diseases: bio-complexity as an interdisciplinary paradigm. Ecohealth. 2005; 2:244-57.
2. Prüss-Ustün A, Wolf J, Corvalán C, Bos R, Neira M. Preventing disease through healthy environments: a global assessment of the burden of disease from environmental risks [Internet]. USA: World Health Organization; 2016 [cited 2018 Sep 10]. Available from: https://www.who.int/quantifying_ehimpacts/publications/preventing-disease/en/.
3. Dadonaite B, Ritchie H, Roser M. Diarrheal diseases [Internet]. USA: Institute for Health Metrics and Evaluation; 2019 [cited 2020 Oct 5]. Available from: https://ourworldindata.org/diarrheal-diseases.
4. กลุ่มข้อมูลข่าวสารสุขภาพ. สถิติสาธารณสุข พ.ศ. 2561. นนทบุรี: กระทรวงสาธารณสุข; 2561.
5. Pimentel M. Evidence-based management of irritable bowel syndrome with diarrhea. Am J Manag Care. 2018; 24(3 Suppl):S35-S46.
6. Lacy BE. Diagnosis and treatment of diarrhea-predominant irritable bowel syndrome. Int J Gen Med. 2016; 9:7-17.
7. Barbut F, Meynard JL. Managing antibiotic associated diarrhoea. BMJ. 2002; 324(7350):1345-6.
8. Bengmark S. Pre-, pro- and synbiotics. Curr Opin Clin Nutr Metab Care. 2001; 4:571-9.
9. Markowiak P, Śliżewska K. Effects of probiotics, prebiotics, and synbiotics on human health. Nutrients. 2017; 9(9):1021.
10. Binns N. Probiotics, prebiotics and the gut microbiota. Belgium: ILSI Europe publishes; 2013.
11. Abhari K, Shekarforoush SS, Sajedianfard J, Hosseinzadeh S, Nazifi S. The effects of probiotic, prebiotic and synbiotic diets containing *Bacillus coagulans* and inulin on rat intestinal microbiota. Iranian J Vet Res. 2015; 16(3):267-73.
12. Abhari K, Shekarforoush SS, Hosseinzadeh S, Nazifi S, Sajedianfard J, Eskandari MH. The effects of orally administered *Bacillus coagulans* and inulin on prevention and progression of rheumatoid arthritis in rats. Food Nutr Res. 2016; 60:10.
13. Pandey KR, Naik SR, Vakil BV. Probiotics, prebiotics and synbiotics a review. J Food Sci Technol. 2015; 52(12):7577-87.
14. Abrams SA, Griffin IJ, Hawthorne KM, Liang L, Gunn SK, Darlington G, et al. A combination of prebiotic short- and long-chain inulin-type fructans enhances calcium absorption and bone mineralization in young adolescents. Am J Clin Nutr. 2005; 82(2):471-6.

15. Baron M. A patented strain of *Bacillus coagulans* increased immune response to viral challenge J Postgrad Med. 2009; 121(2):114-8.
16. Asemi Z, Khorrami-Rad A, Alizadeh SA, Shakeri H, Esmaillzadeh A. Effects of synbiotic food consumption on metabolic status of diabetic patients: a double-blind randomized cross-over controlled clinical trial. Clin Nutr Res. 2014; 33(2):198-203.
17. Kimmel M, Keller D, Farmer S, Warrino DE. A controlled clinical trial to evaluate the effect of GanedenBC(30) on immunological markers. Methods Find Exp Clin Pharmacol. 2010; 32(2):129-32.
18. Lewis SJ, Heaton KW. Stool form scale as a useful guide to intestinal transit time. Scand J Gastroenterol. 1997; 32(9):920-4.
19. Blake MR, Raker JM, Whelan K. Validity and reliability of the Bristol Stool Form Scale in healthy adults and patients with diarrhoea-predominant irritable bowel syndrome. Aliment Pharmacol Ther. 2016; 44(7):693-703.
20. Nowacki A. Chi-square and Fisher's exact tests. Cleve Clin J Med. 2017; 84(9 Suppl 2):e20-e5.
21. Vaisman N, Press J, Leibovitz E, Boehm G, Barak V. Short-term effect of prebiotics administration on stool characteristics and serum cytokines dynamics in very young children with acute diarrhea. Nutrients. 2010; 2:683-92.
22. Azpiroz F, Molne L, Mendez S, Nieto A, Manichanh C, Mego M, et al. Effect of chicory-derived inulin on abdominal sensations and bowel motor function. J Clin Gastroenterol. 2017; 51(7):619-25.
23. Majeed M, Nagabhushanam K, Natarajan S, Sivakumar A, Ali F, Pande A, et al. *Bacillus coagulans* MTCC 5856 supplementation in the management of diarrhea predominant irritable bowel syndrome: a double blind randomized placebo controlled pilot clinical study. Int J Nutr. 2016; 15(1):21.
24. Vermorken AJM, Andrès E, Cui Y. Bowel movement frequency, oxidative stress and disease prevention. Mol Clin Oncol. 2016; 5(4):339-42.
25. Kelly G. Inulin-type prebiotics--a review: part 1. Alternative medicine review: Altern Med Rev. 2008; 13(4)(Pt 1):315-29.
26. Panigrahi M, Kar S, Singh SP, Ghoshal U. Defecation frequency and stool form in a coastal eastern indian population. J Neurogastroenterol Motil. 2013; 19:374-80.
27. Kim YS, Kim N. Sex-gender differences in irritable bowel syndrome. J Neurogastroenterol Motil. 2018; 24(4):544-58.
28. Allen SJ, Martinez EG, Gregorio GV, Dans LF. Probiotics for treating acute infectious diarrhoea. Cochrane Database Syst Rev. 2010; 2010(11):Cd003048.
29. Micka A, Siepelmeyer A, Holz A, Theis S, Schön C. Effect of consumption of chicory inulin on bowel function in healthy subjects with constipation: a randomized, double-blind, placebo-controlled trial. Int J Food Sci Nutr. 2017; 68(1):82-9.
30. Collado Yurrita L, San Mauro Martín I, Ciudad-Cabañas MJ, Calle-Purón ME, Hernández Cabria M. Effectiveness of inulin intake on indicators of chronic constipation; a meta-analysis of controlled

randomized clinical trials. Nutr Hosp. 2014; 30(2):244-52.

31. Akram W, Garud N, Joshi R. Role of inulin as prebiotics on inflammatory bowel disease. Drug Discov Ther. 2019; 13(1):1-8.
32. Sudha RM, Bhonagiri S. Efficacy of *Bacillus coagulans* strain unique IS-2 in the treatment of patients with acute diarrhea. Int J Probiotics Prebiotics. 2012; 7:33-7.
33. Rogha M, Esfahani MZ, Zargarzadeh AH. The efficacy of a synbiotic containing *Bacillus Coagulans* in treatment of irritable bowel syndrome: a randomized placebo-controlled trial. Gastroenterol Hepatol Bed Bench. 2014; 7(3):156-63.
34. Cao J, Yu Z, Liu W, Zhao J, Zhang H, Zhai Q, et al. Probiotic characteristics of *Bacillus coagulans* and associated implications for human health and diseases. J Funct Foods. 2020; 64:103643.
35. Honda H, Gibson GR, Farmer S, Keller D, McCartney AL. Use of a continuous culture fermentation system to investigate the effect of GanedenBC30 (*Bacillus coagulans* GBI-30, 6086) supplementation on pathogen survival in the human gut microbiota. Anaerobe. 2011; 17(1):36-42.
36. Rueangwatcharin U, Wichienchot S. Digestibility and fermentation of tuna products added inulin by colonic microflora. Food Res Int. 2015; 22(5):2068-77.
37. Lin SY, Hung A, Lu JJ. Effects of supplement with different level of *Bacillus coagulans* as probiotics on growth performance and intestinal microflora populations of broiler chickens. Int J Anim Vet Adv. 2011; 10(1):111-4.
38. Vandeputte D, Falony G, Vieira-Silva S, Tito RY, Joossens M, Raes J. Stool consistency is strongly associated with gut microbiota richness and composition, enterotypes and bacterial growth rates. Gut. 2016; 65(1): 57-62.