Journal of Functional Foods 107 (2023) 105668

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

TBG-136, a Schizophyllum commune-derived β-glucan benefits gut microbiota and intestinal health: A randomized, double-blind, and placebo-controlled clinical trial

Hui-Yeon Jang [a], Su-Jin Jung [a,b], Eun-Ock Park [a], Soo-Dong Kim [c], Je-Kyoung Kim [c], Soo-Wan Chae [a,b], Youn–Soo Cha [d,*], Seung-Ok Lee [a,b,e,*]

[a] Clinical Trial Center for Functional Foods, Jeonbuk National University Hospital, Jeonbuk 54907, Republic of Korea
[b] Biomedical Research Institute of Jeonbuk National University Hospital, Jeonbuk 54907, Republic of Korea
[c] Quegen Biotech. Co., Ltd, Siheung, 15112, Republic of Korea
[d] Department of Food Science and Human Nutrition & K-Food Research Center, Jeonbuk National University, Jeonbuk 54896, Republic of Korea
[e] Department of Gastroenterology and Hepatology, Jeonbuk National University, Medical School, Jeonbuk 54896, Republic of Korea

ARTICLE INFO

Keywords:
Schizophyllum commune
β-glucan
Gut bacteria
Intestinal health
Constipation

ABSTRACT

β-glucan exerts beneficial biological activities such as immunoregulatory, anticancer, antioxidative, antiaging, and wound healing. However, the effects of *β*-glucan on constipation have not been thoroughly studied. Thus, the present study aimed to investigate the effects of *β*-glucan-derived from *Schizophyllum commune* supplementation (TBG-136) on gut microbiota and intestinal health in constipated subjects. As a result, *Lactobacillus* was significantly increased in the TBG-136 group compared to the placebo group ($P < 0.05$) after 8 weeks of TBG-136 supplementation. Additionally, TBG-136 group significantly improved the rectosigmoid CTT and the number of defecations compared to the placebo group ($P < 0.05$). Thus, *β*-glucan derived from *Schizophyllum commune* helps individuals' health and quality of life by increasing the beneficial bacteria and improving symptoms of constipation.

1. Introduction

Gastrointestinal (GI) diseases such as chronic constipation and irritable bowel syndrome (IBS) have been rising due to ever-changing lifestyles influencing stress levels, dietary habits, food intolerance, and physical activity. These factors have a negative impact on colon functions, intestinal motility, intestinal mucosal inflammation, and dysbiosis resulting in abdominal pain, diarrhea, and difficulty in defecation. These factors are potential consequences of constipation, ulceration, diverticulitis, ulcerative colitis, and diarrhea. The intestinal microflora is critical for several aspects of GI health, and it is predicted that it will drive future biomedical technologies that regulate intestinal flora to treat GI disorders. Thus, interest in understanding the relationship between the gut microbiome and human health is on the rise. Specifically, natural foods are in focus due to their functional value and safety aspects. Prebiotics and probiotics are used to improve the gut microbiome and have been used to relieve clinical symptoms, including colitis, diarrhea, and constipation associated with IBS (Arpaia et al., 2013; Cani et al., 2007; Carabotti, Scirocco, Maselli, & Severi, 2015; Heijtz et al., 2011; Turnbaugh et al., 2006). Nevertheless, probiotics are not entirely safe due to the chance of infection from live bacteria and the difficulty in determining individual sensitivity against specific live probiotics. Mushrooms are a good source of dietary fiber, *β*-glucan (BG), trehalose, mannitol, and arabinitol. Among these, *β*-glucan is an interesting compound detected in microorganisms and fungi cell walls. *β*-glucan is a soluble fiber and a polysaccharide-based biopolymer known to have multiple physiological properties, including immunoregulatory, anticancer, antioxidant, antiaging, antidiabetic, antiobesity, and skin regeneration effects (Cheung, 2008; Deng et al., 2012; Du, Lin, Bian, & Xu, 2015; Ng & Yap, 2002). These beneficial effects could be attributed to its ability to alter the gut microbiota (Jayachandran, Chen, Chung, & Xu, 2018). Lentinan, grifolan, shizophylan, and krestin are the representative active ingredients in shiitake mushrooms, while flammulin is from enoki mushrooms. In this study, *β*-glucan derived from *Schizophyllum commune* was used as an investigational drug compound. The extracted *β*-glucan has *β*-1,3 bonds combined with *β*-1,6. *β*-glucan is suggested to have

* Corresponding authors at: Clinical Trial Center for Functional Foods, Jeonbuk National University Hospital, Jeonju, Republic of Korea.
E-mail addresses: cha8@jbnu.ac.kr (Y. Cha), solee@jbnu.ac.kr (S.-O. Lee).

https://doi.org/10.1016/j.jff.2023.105668
Received 2 January 2023; Received in revised form 24 June 2023; Accepted 1 July 2023
Available online 7 July 2023

positive roles in inhibiting breast cancer cell growth (J.-S. Lee et al., 2011), skin regeneration (Du et al. 2014), and immune promotion (J.-W. Lee, 2014). Recent preclinical investigations (in vivo) have indicated that the β-glucan from *S. commune* improves colonic motility, intestinal atrophy, and gut microbiota (Muthuramalingam et al., 2020; Vu, Muthuramalingam, Singh, Choi, et al., 2022). Previously, β-glucan derived from yeast and mushrooms focused on evaluating immune function and cancer risk (Akramienė, Kondrotas, Didžiapetrienė, & Kėvelaitis, 2007; Tao, Zhang, & Cheung, 2006). However, the effects of β-glucan from S. commune on constipation have not been thoroughly studied, and clinical research on constipation is limited. Therefore, the present study investigated the effects of β-glucan-derived from *S. commune* (TBG-136) supplementation on gut microbiota and intestinal health in constipated individuals.

2. Material and methods

2.1. Test supplements

The test compound TBG-136 was provided by Quegen Biotech Co. Ltd (Siheung, Republic of Korea). TBG-136 is produced by fermenting, filtering, and purifying *S. commune* to obtain β-glucan. The purified soluble β-1,6 branching β −1,3 glucan has a molecular weight of 1.78 ∼ 1.79 × 10(6) Da. To ensure β-glucan contents in TBG-136, provided TBG-136 compound was analyzed using UV/VIS spectrophotometer. Previous human studies indicated that a daily dose of 100–3000 mg β-glucan is safe and beneficial for various disorders (Vetvicka, Vannucci, Sima, & Richter, 2019). Therefore, a modest dose of 80 mg/day was used for the trial. TBG-136 is a colorless transparent liquid with a unique flavor of *S. Commune*. A 10 mL TBG-136 stick has 40 mg of β-glucan. Ingredients in TBG-136 and placebo compounds are listed in Table 1.

2.2. Participants

The study was conducted in accordance with the International Conference on Harmonization's Good Clinical Practice (ICH GCP). The study was reviewed and approved by the Institutional Review Board (IRB) of Jeonbuk National University Hospital (JUH IRB). The investigation was implemented from October 23, 2019 to November 12, 2020 at the Clinical Trial Center for Functional Foods (CTCF2) of Jeonbuk National University Hospital (IRB No. 2019–08-010, September/04/2019), and the trial was registered at Clinical Research Information Service (CRIS; KCT0006657). The inclusion criteria were as follows: (1) Male or female aged from 19 to 70 years old, (2) Subjects who meet the diagnostic criteria for functional constipation in Roman IV criteria (This applies to 2 or more of the following 6 items) (Mearin et al., 2016), (3) Subjects who voluntarily participated in this application test and agreed in writing to follow the guidelines after hearing and fully understanding the detailed description. The exclusion criteria were as follows: (1) Subjects who meet the diagnostic criteria for irritable bowel syndrome in Roman IV criteria (This applies to 2 or more of the following 3 items) (Moayyedi et al., 2017), (2) history of GI diseases (e.g., Crohn's disease) or GI surgery (except for simple appendectomy or hernia surgery) (3) Subjects who has received antipsychotic drug treatment within 2 months (4) Subjects who took specialty drugs related to intestinal health within 1 month from the date of the screening test (5) Subjects who have taken herbal medicines or healthy functional foods related to intestinal health within 2 weeks from the date of screening test (6) Subjects who participated in other clinical trials within 3 months from the date of the screening test (7) Subjects who show the following results in the diagnostic laboratory medical examination (AST, ALT $>$ 3 times the upper limit of the normal range, serum creatinine $>$ 2.0 mg/dL) (8) Pregnant or lactating women.

Table 1
Composition of TBG-136 and placebo products (10 mL each).

Ingredients	TBG-136 (%)/ml	Placebo (%)/ml
β-glucan	0.4	–
Saline	–	0.05
Sodium benzoate	0.3	0.3
Mineral	0.1	–
Moisture	99.2	99.65
Total	100	100

TBG-136, β-glucan derived from *Schizophyllum commune*.

2.3. Study design

This randomized, double-blind, placebo-controlled clinical trial was conducted for 8 weeks. A total of 80 subjects with constipation were recruited for the trial. Participants who fulfilled the screening process were randomly assigned to one of two groups (1:1) (TBG-136 or placebo). The selected participants visited the CTCF2 at JBUH on 4th and 8th week of the trial for efficacy assessment, vital signs, and adverse events. During the intervention period, subjects were instructed to take 80 mg of the TBG-136 or placebo product orally twice daily (one stick each time) after breakfast (TBG-136: 40 mg, Placebo: 0 mg of β-glucan) and dinner (TBG-136: 40 mg, Placebo: 0 mg of β-glucan) for 8 weeks. All the subjects were instructed to maintain their routine lifestyle, including dietary habits and physical activity, and not to consume any other functional foods, probiotics, or dietary supplements during the intervention.

2.4. Outcome measurements

All the study participants visited CTCF2 and were tested for validity and safety assessment on the first visit (baseline, 0 week) and the third visit (8th week).

2.4.1. Primary outcomes

Gut microbiota was assessed by collecting fecal samples on the first visit (Baseline) and third visit (8th week). Specifically, beneficial intestinal microorganisms like *Bifidobacterium* and *Lactobacillus* were analyzed to determine the effect of the test product on their population. The collected fecal samples were stored at −70 °C, and genomic DNA was isolated and purified using QIAamp DNA Stool Kit as per the manufacturer guidelines. The sample was lysed by adding ASL buffer and reacted at 70 °C for 5 min, then centrifugation was performed after adding inhibitEX Tablet. Afterward, proteinase K and AL buffer were added to the centrifuged sample and the membrane was washed with AW1 buffer and AW2 buffer, followed by incubation with AE buffer to extract DNA. The final extracted DNA was quantitatively analyzed using Real-Time PCR (De Gregoris, Aldred, Clare, & Burgess, 2011; Endo, Futagawa-Endo, & Dicks, 2009). Participants with an increased number of specified microorganisms were considered responders, and those with a decreased or unchanged number of microorganisms were non-responders. Contrastingly, participants with a decreased harmful bacterium (*Clostridium, Staphylococcus*) were responders, while participants with an increased or unchanged number of harmful bacteria were non-responders.

2.4.2. Secondary outcomes

Colon transit time (CTT) is the primary tool required to assess and manage patients with constipation. Thus, CTT was measured to analyze the impact of TBG-136 on transit time. CTT was evaluated at baseline and third visit (8th week). CTT was evaluated using radio-opaque markers and sequential abdominal X-rays. CTT was calculated as described previously (Jung, Oh, Park, & Chae, 2020; Metcalf et al., 1987). Additionally, at each visit, constipation symptoms were assessed with Patient Assessment of Constipation Symptom (PAC-SYM) (Frank, Kleinman, Farup, Taylor, & Miner, 1999) and Patient Assessment of Constipation Quality of Life (PAC-QOL) (Marquis, De La Loge, Dubois, McDermott, & Chassany, 2005) questionnaires. The PAC-SYM is a 12-

point questionnaire subdivided into three symptom subscales: abdominal (4), rectal (3), and stool (5). Symptoms are assessed on a five-point Likert scale, with scores 0–4, where 0 indicates no symptoms, 1 indicates mild, 2 indicates moderate, 3 indicates severe, and 4 indicates very severe symptoms. On the other hand, PAC-QOL consists of a total of 28 items under 4 subcategories. Subcategories like physical discomfort (4), psychosocial discomfort (8), worries and concerns (11), and satisfaction (5) has scores ranging from 0 to 4. Quality of life satisfaction is more positive with higher scores.

Bowel habit-related questionnaire was used to assess defecation for 2 weeks before each visit. The condition of the stool was evaluated using an image of the Bristol stool chart (BSS) (Riegler & Esposito, 2001), where type 1 and type 2 suggest constipation, types 3, 4, and 5 depict normal, and types 6, 7 represent diarrhea. Moreover, participants were asked to weigh the stool at the beginning of the trial (baseline) and 8th week. Also, a small amount of stool was frozen for further biochemical and microbiological analysis. Moreover, β-glucuronidase (Fluorometric assay) and Short-Chain Fatty Acids (SCFAs) were measured using the collected stool samples. SCFAs were analyzed described in previously (Ahn et al., 2012). Gas chromatography was used for the quantitative analysis of SCFAs. Briefly, phosphoric acid was added to stools to prepare a 10% (w/v) mixture and centrifuged to collect the supernatant. The supernatant was quantified using gas chromatography (Hewlett Packard Model 5890 Series II, USA) equipped with HP-FFAP (0.25 m, 25 m * 0.2 mm) column. Additionally, calprotectin, a protein biomarker representing intestinal inflammation, was measured with an Enzyme-linked immunosorbent assay (ELISA). pH was measured with a BDH pH strip (Merck Millipore Co. Inc. Seoul, Korea).

2.4.3. Safety measurements

To address the adverse events, the individual's clinical condition and vital signs were assessed prior to baseline and after 8 weeks of investigation. As a part of safety assessment, general parameters like WBC, RBC, Hemoglobin, Hematocrit, Platelets count, total bilirubin, ALP, gamma-GT, AST, ALT, total cholesterol, triglyceride, blood glucose, total protein, albumin, BUN, creatinine, CK, LD, Na, K, CI, Ca, P, high-sensitivity C-reactive protein (hs-CRP), urinalysis were performed. Additionally, electrocardiogram, vital signs (SBP, DBP, Pulse), and anthropometrics (height, weight, Body mass index) were recorded. All biochemical parameters were performed at the department of clinical pathology, Jeonbuk National University Hospital.

2.5. Diet and physical activity measurements

The study participants were asked to record their dietary routine 3 days before each visit. Using the software Can Pro 4.0, calorie and nutrient intake were assessed based on the dietary data obtained (The Korean Nutrition Society, Seoul, Republic of Korea). Physical activity was evaluated as per the metabolic equivalent task (MET) assessment using the global physical activity questionnaire (Armstrong & Bull, 2006).

2.6. Sample size and statistical analysis

The sample size was determined based on a previous study that observed changes in gut bacteria with β-glucan (Mitsou, Panopoulou, Turunen, Spiliotis, & Kyriacou, 2010). Each group required 40 people to obtain 80 percent statistical power at a 5% significance level (two-sided test). Thus, 80 participants were enrolled in the study, assuming a dropout rate of approximately 20%. The statistical analyses were performed using SAS v9.4 (SAS Institute, Cary, North Carolina, USA). The statistical significance level was set to $P < 0.05$. In this study, Full Analysis Set (FAS) was adopted, where participants were randomly allocated to a treatment group with at least one efficacy assessment. The chi-square test or Fisher's exact tests were used to assess the categorical variables. Further, homogeneous items were treated as covariates and analyzed with repeated measures ANCOVA (RM-ANCOVA). RM-ANOVA was used to assess various factors at each visit, including constipation-related symptoms and vital signs.

3. Results

3.1. Demographics characteristics of subjects

A total of 80 subjects were randomly divided into TBG-136 (n = 40) and placebo (n = 40) groups. A total of 74 subjects completed the study (36 in the TBG-136 group and 38 in the placebo group). The screening process of the study participants is presented in Fig. 1. The general demographic characteristics of participants at baseline are presented in Table 2. The baseline time of defecation in the TBG-136 and placebo groups is significantly different ($P = 0.038$). All other variables did not show a significant difference. Thus, statistical analyses of bowel movements, duration of defecation, type of feces, and CTT were adjusted to account for the possibility that defecation time affects these variables.

3.2. Dietary intake and physical activity

Diet is the critical factor that affects nutrient and calorie intake. Hence, the diet of participants was analyzed for potential influence on the study (Dukas, Willett, & Giovannucci, 2003). The nutrient intake of the subjects in different groups during the 8-week intervention period is presented in (Supplementary Table 1). The observations indicate no significant differences between the groups' daily intake of calories, carbohydrates, proteins, fats, fibers, and sodium ($P > 0.05$) at baseline and during the investigation. Diet is the critical factor that affects nutrient and calorie intake. Hence, the diet of participants was analyzed for potential influence on the study. However, there were no significant differences between the group's nutrient and calorie intake. Moreover, the degree of physical activity possibly influences constipation-related symptoms. Thus, metabolic equivalents (MET) analysis was performed. MET analysis demonstrated that physical activity in the placebo group was increased while it decreased in the TBG-136 group. However, there were no significant differences between the groups.

3.3. Efficacy valuation

3.3.1. Primary outcome

In this investigation, the gut microbial population was calculated as colony-forming units (CFU) per gram. After 8 weeks of TBG-136 supplementation, *Lactobacillus* in TBG-136 increased significantly than the placebo group ($P = 0.045$) (Table 3). Clostridium, a potentially harmful intestinal bacterium, decreased significantly in the placebo group after 8 weeks of intervention ($P = 0.014$). However, there was no significant difference between the TBG-136 and placebo groups ($P > 0.05$). The change in beneficial and harmful bacterial populations in TBG-136 and placebo groups were presented in Fig. 2. If *Lactobacillus* is considered, 26 responders (70.3%) were in the TBG-136 group after 8 weeks of intervention. In contrast, the placebo group showed 17 responders (47.2%). However, there was no significant difference between the TBG-136 and placebo groups in other bacterial populations.

3.3.2. Secondary outcome

The impact of TBG-136 on CTT, Constipation-related symptoms, and intestinal environment in subjects during the 8 weeks intervention period are presented in Table 4. The changes in colon transit time after 48 hours in the TBG-136 and placebo groups are presented in Fig. 3. TBG-136 and placebo groups showed a significant difference in rectosigmoid CTT ($P = 0.011$). However, there were no significant differences in the CTT values on the right and left colons ($P > 0.05$). Also, no significant differences in the CTT values of the total colon. Further, the number of defecation and satisfaction aspects of the PAC-QOL questionnaire significantly increased in the TBG-136 group ($P = 0.001$, $P <$

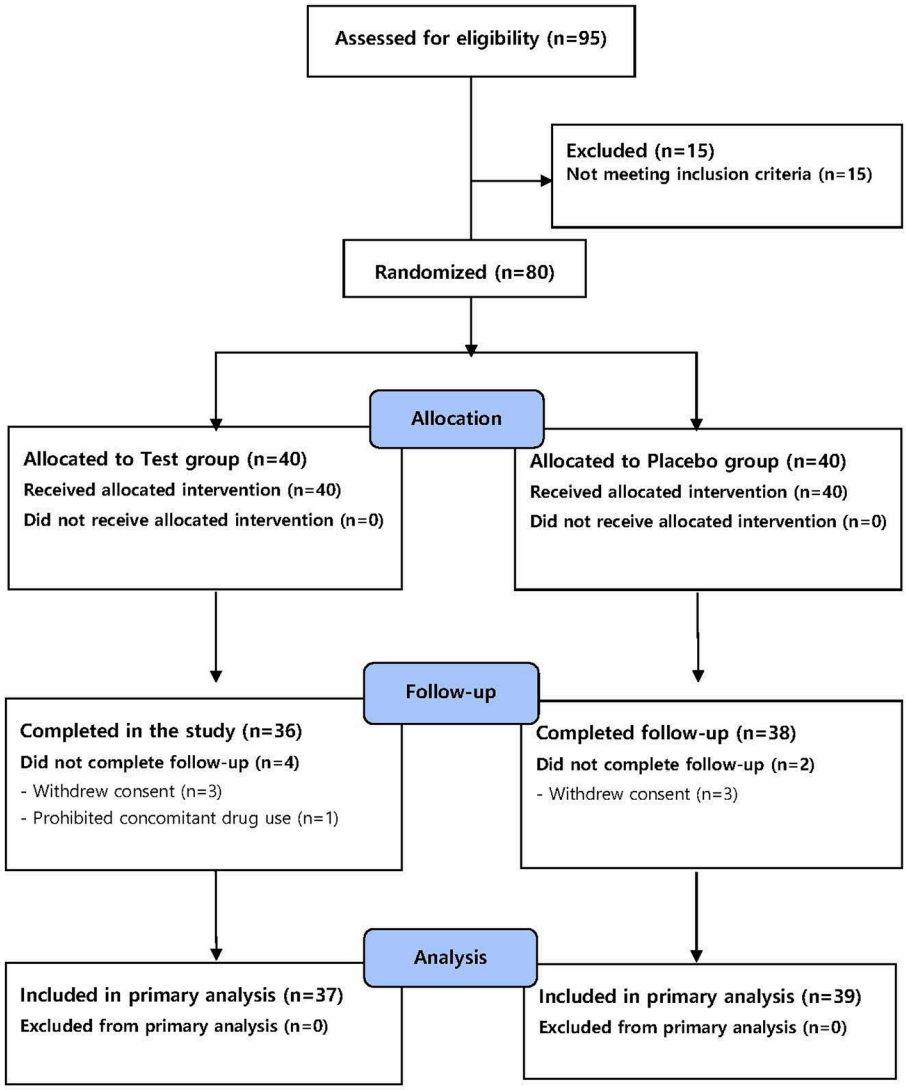

Fig. 1. The screening process of the study participants. A total of 80 subjects with constipation were recruited for the trial. Among 80 subjects, 74 completed the study (36 in the TBG-136 group and 38 in the placebo group). TBG-136, *β*-glucan derived from *Schizophyllum commune*.

0.0001) and PAC-QOL scores differ significantly between TBG-136 and placebo ($P = 0.038$, $P = 0.025$). Moreover, living with constipation index scores significantly decreased in both TBG-136 and placebo groups ($P < 0.0001$, $P < 0.0001$). Also, living with constipation index scores differ significantly between TBG-136 and placebo after 8 weeks of intervention ($P = 0.043$). Besides, fecal calprotectin levels decreased in both the TBG-136 and placebo groups, whereas β-glucuronidase activity remained similar ($P > 0.05$). The changes in fecal calprotectin, pH, and β-glucuronidase activity are listed in Table 4.

3.4. Safety and adverse events

Three subjects showed mild adverse events (AE) during the intervention period. However, there was no difference in the occurrence of adverse events between the groups. During the intervention, one instance of indigestion, abdominal pain, and irregular menstrual bleeding was observed ($P > 0.05$). However, analysis of AE revealed no immediate relation between the AE and TBG-136. The participant's vital signs, electrocardiogram, anthropometric, and biochemical tests (hematological, blood biochemistry, and urine) were within the normal range (Supplementary Table 2). Hence, the results of these tests had no clinical significance.

4. Discussion

Constipation is not life-threatening but living with it can be uncomfortable and depressing. The quality of life of constipated patients is low as intestinal contractions cause abdominal discomfort (Drossman, 2006). Hence, it is necessary to minimize constipation for a higher quality of life. In this study, TBG-136, a purified *β*-glucan from S. commune, was evaluated to assess its impact on intestinal health. Expectedly, intake of TBG-136 was effective in regulating functional constipation. The study observations indicated improved bowel movement, constipation-related symptoms, and enhanced beneficial bacterial population in the gut upon consumption of TBG-136. Notably, the increased *Lactobacillus* population in TBG-136 indicated its potential to improve intestinal health. Together, these findings exhibited the potential applications of TBG-136 in improving intestinal health. Previously, in a high-fat diet-induced constipation animal model, β-glucan from S. commune increased *Bifidobacterium*, a prebiotic bacterium, and Anaerostipes, bacteria that enhance the production of SCFA's in the intestine. These beneficial bacterial populations exert anti-inflammatory properties (Muthuramalingam et al., 2020; Vu, Muthuramalingam, Singh, Choi, et al., 2022). Similar findings were observed in murine model studies utilizing *β*-glucan derived from cereals (Bai et al., 2021;

Table 2
Demographic characteristics of the study participants.

Variables	TBG-136 group(n = 40)	Placebo group(n = 40)	*P*-value[1)]
Age (years)	39.98 ± 12.55	41.40 ± 10.05	0.577
Sex (M/F) (n, %)	2(5)/38(95)	3(7.5)/37(92.5)	>.999[2)]
Height (cm)	160.55 ± 6.08	162.73 ± 7.39	0.155
Weight (kg)	58.45 ± 10.99	61.98 ± 12.27	0.180
Body mass index (kg/m^2)	22.62 ± 3.39	23.26 ± 3.35	0.402
Alcohol (n, %)	19(47.5)	19(47.5)	>.999[3)]
Alcohol (units/week)	2.65 ± 2.78	2.97 ± 3.83	0.765
Smoking (n, %)	2(5.0)	8(20.0)	0.087[2)]
Smoking (pieces/day)	8.50 ± 2.12	7.50 ± 3.93	0.745
SBP (mmHg)	118.48 ± 12.81	114.33 ± 13.68	0.165
DBP (mmHg)	70.88 ± 9.07	69.43 ± 10.71	0.515
Pulse (BPM)	79.53 ± 10.60	79.63 ± 11.07	0.967
Gut bacteria (log_{10} CFU/g)*			
Bifidobacterium	9.76 ± 0.63	9.74 ± 0.64	0.930
Lactobacillus†	8.46 ± 0.61	8.63 ± 0.74	0.266
Clostridium	9.91 ± 1.00	9.94 ± 0.81	0.888
Staphylococcus	2.26 ± 2.66	2.78 ± 2.98	0.427
Colon transit time (CTT)**			
Right colon	7.93 ± 10.52	10.61 ± 12.17	0.320
Left colon	17.00 ± 15.89	17.38 ± 13.05	0.910
Rectosigmoid	10.70 ± 11.48	6.26 ± 8.88	0.068
Total colon	35.63 ± 23.22	34.25 ± 20.13	0.786
Constipation related symptoms			
Number ofDefecation (time/week)	4.23 ± 1.69	4.17 ± 1.75	0.874
Time of defecation (min)	6.98 ± 4.49	9.52 ± 5.87	**0.038**
BSS (score)	3.50 ± 0.94	3.30 ± 0.85	0.331
PAC-SYM (score)	15.59 ± 7.92	16.28 ± 7.54	0.700
PAC-QOL (score)			
Constipation Strength	3.81 ± 1.51	4.15 ± 1.65	0.347
Daily life impact	11.84 ± 6.46	13.54 ± 5.79	0.230
Constipation emotion (1)	7.84 ± 3.95	9.44 ± 3.48	0.065
Constipation emotion (2)	4.76 ± 2.68	4.77 ± 2.50	0.983
Living with constipation	5.70 ± 2.30	5.59 ± 2.17	0.826
Satisfaction	4.05 ± 2.87	4.51 ± 2.78	0.826
Parameters of intestinal environment			
β-glucuronidase (pmol/min/g)	4.39 ± 2.21	4.05 ± 1.73	0.461
pH	7.05 ± 0.29	7.04 ± 0.26	0.911
Calprotectin((ng/g)	36.78 ± 55.79	39.37 ± 48.73	0.832

Values are presented as mean ± SD or number1) Analyzed by independent *t*-test, compared between groups.2) Analyzed by chi-square test, compared between groups.3) Analyzed by Fisher's exact test, compared between groups.

* Sample not detection was excluded (R53, R58).
** Capsule intake and x-ray schedule mismatch was excluded (R22, R58, R74).
† Outlier was excluded (R69). Abbreviations: BSS, Bristol Tool Scale, PAC-SYM, Patient Assessment of Constipation Symptom.

Mio, Otake, Nakashima, Matsuoka, & Aoe, 2021). A recent investigation reported that *Lactobacillus* and *Bifidobacterium* have a direct relationship with a prebiotic derived from S. commune (Singh et al., 2021). Interestingly, when healthy adults consumed a cake containing barley β-glucan for 30 days, the number of *bifidobacteria* in the colon increased (Mitsou et al., 2010). Besides, β-glucan proved to improve the adhesion of *Lactobacillus* to human intestinal epithelial cells (Russo et al., 2012). Thus, we can expect improved beneficial bacterial populations upon *β*-glucan supplementation. In this investigation, the *Lactobacillus* population significantly increased upon TBG-136 supplementation. This observation could be explained by the ability of β-glucan to enhance *Lactobacillus* adherence to intestinal epithelial cells. Also, undigested polysaccharides are believed to be a source of nutrition and energy for gut microbes, especially beneficial microorganisms. Further, at a molecular level, *β*-glucan with *β*-(1,3/1,6) bond is known to bind to the dectin-1 receptor stimulating macrophage and cytokine secretion that enhances intestinal immunity and inflammation by inhibiting endotoxins (Raa, 2015; Tsukada et al., 2003). Additionally, several preclinical investigations show that administration of β-glucan derived from *S. commune* reduced NO production and inflammatory markers such as IL-6, TNF-α, and COX-2 by activating macrophages (J.-W. Lee, 2014; Muthuramalingam et al., 2020; Vu, Muthuramalingam, Singh, Hyun, et al., 2022). In this study, TBG-136 supplementation appears to reduce calprotectin levels, although the change is not statistically significant. Also, β-glucuronidase activity appeared to be decreasing, whereas it was increasing in the placebo group. These observations indicate reduced colorectal cancer risks as β-glucuronidase is known to increase the risk of colorectal cancer by generating carcinogens or mutants (Goldin, 1990). Moreover, BG is fermented by colonic microbiota, resulting in the production of SCFAs, which serve as important mediators in linking nutrition, gut microbiota, physiology, and pathology. For instance, SCFAs promote intestinal mucus production, which influences the thickness of the mucosal layer (Sun et al., 2021). Importantly, SCFAs are produced during fermentation, which mechanically acidifies the intestinal environment and reduces harmful bacteria that inhibit cell regeneration in the damaged large intestine and protect the intestinal wall (Den Besten et al., 2013; Kumar, Sinha, Makkar, De Boeck, & Becker, 2012; Wong, De Souza, Kendall, Emam, & Jenkins, 2006). Unfortunately, SCFAs were not detected in TBG-136 and placebo groups. Thus, future clinical trials on these issues must include a comprehensive analysis of fecal samples, as well as the exploration of other experiments to address the issue from several perspectives.

Furthermore, TBG-136 supplementation significantly reduced the rectosigmoid CTT and number of bowel movements than in the placebo group. Similarly, constipation-related discomfort was reduced significantly upon TBG-136 supplementation resulting in improved health-related quality of life.

These findings demonstrated a strong correlation with positive outcomes, including enhanced stool weight, mucin distribution in the mucosa, and colonic motility, in a 12-week animal model study involving the administration of *β*-glucan (Vu, Muthuramalingam, Singh, Choi, et al., 2022). The application of *β*-glucan stimulated goblet cell proliferation, leading to increased mucin production and a thicker mucosal layer (Singh et al., 2021; Vu, Muthuramalingam, Singh, Choi, et al., 2022). Another investigation revealed that *β*-glucan derived from oats also promoted increased stool weight and accelerated intestinal transit (Korczak & Slavin, 2013). *β*-glucan, a polysaccharide present in yeast, mushrooms, barley, oats, and other sources, exhibits diverse biological functions influenced by factors such as molecular structure, molecular weight, solubility, and viscosity. Notably, *β*-glucan derived from Schizophyllum has a higher molecular weight compared to oat-derived *β*-glucan, imparting increased viscosity and emulsion stability (Du, Meenu, Liu, & Xu, 2019). Consequently, *β*-glucan derived from

Table 3
Gut bacterial population in subjects during intervention period.

Gut bacteria	TBG-136 group(n = 36)		*P*-value[1)]	Placebo group(n = 38)		*P*-value[1)]	*P*-value[2)]
	Baseline	8 weeks		Baseline	8 weeks		
Bifidobacterium(log_{10} CFU/g)	9.76 ± 0.63	9.94 ± 0.39	0.113	9.74 ± 0.64	9.70 ± 0.65	0.806	0.264
Lactobacillus†(log_{10} CFU/g)	8.46 ± 0.61	8.83 ± 0.64	**0.029**	8.63 ± 0.74	8.55 ± 0.70	0.595	**0.045**
Clostridium(log_{10} CFU/g)	9.91 ± 1.00	9.83 ± 0.77	0.697	9.94 ± 0.81	9.57 ± 0.84	**0.014**	0.251
Staphylococcus(log_{10} CFU/g)	2.26 ± 2.66	2.52 ± 2.73	0.641	2.78 ± 2.98	2.19 ± 2.52	0.367	0.321

Values are presented as mean ± SDLog transformed for comparisons to their natural units for presentationSample not detected was excluded (R53, R58)† Outlier was excluded (R69)[1)] Analyzed by paired *t*-test, compared within the group.[2)] Analyzed by independent *t*-test, compared between groups.

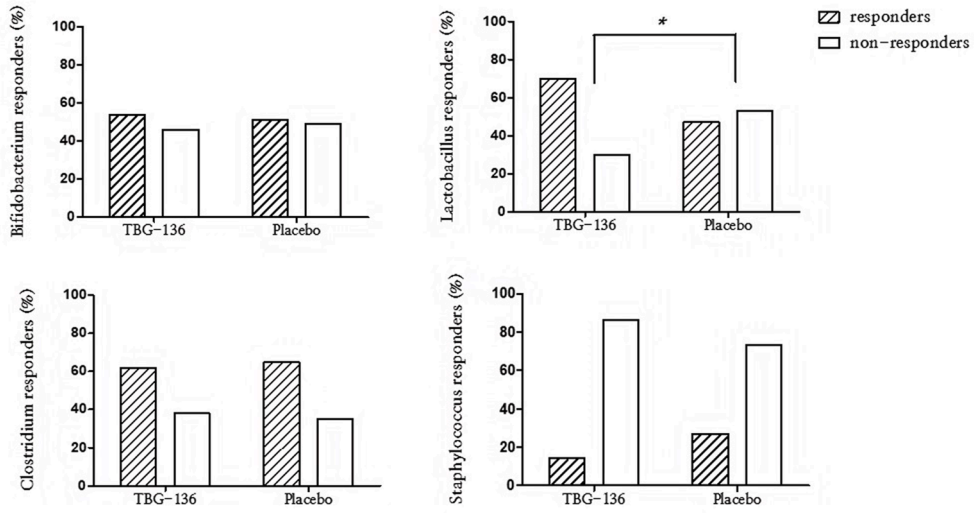

Fig. 2. Bar graphs showing responders and non-responders to specified microorganisms. Participants with an increased number of beneficial microorganisms (*Bifidobacterium, Lactobacillus*) were considered responders, and those with a decreased or unchanged number of microorganisms were non-responders. Similarly, participants with a decreased number of harmful bacteria (*Clostridium, Staphylococcus*) were defined as responders, while participants with an increased or unchanged number of harmful bacteria were defined as non-responders. (A) Responders and non-responders in Bifidobacterium (B) Responders and non-responders in Lactobacillus (C) Responders and non-responders in Clostridium (D) Responders and non-responders in Staphylococcus. Data are expressed as mean ± SD. P < 0.05 (*) was considered statistically significant.

Table 4
Colon transit time (CTT), constipation-related symptoms, intestinal environment in subjects during intervention period.

	TBG-136 group (n = 36)		*P*-value[1)]	Placebo group (n = 38)		*P*-value[1)]	*P*-value[2)]
	Baseline	8 weeks		Baseline	8 weeks		
Colon transit time *							
Right colon	7.93 ± 10.52	7.83 ± 7.89	0.958	10.61 ± 12.17	8.30 ± 8.46	0.282	0.4370.311[3)]
Left colon	17.00 ± 15.89	19.05 ± 14.69	0.486	17.38 ± 13.05	14.72 ± 11.12	0.243	0.2030.349[3)]
Rectosigmoid	10.70 ± 11.48	7.98 ± 7.97	0.147	6.26 ± 8.88	8.89 ± 12.84	0.184	**0.0490.011**[3)]
Total colon	35.63 ± 23.22	34.92 ± 22.32	0.861	34.25 ± 20.13	32.08 ± 21.76	0.543	0.7870.909[3)]
Constipation related symptoms							
Number of bowel movements (time/week)	4.23 ± 1.69	5.28 ± 1.80	0.001	4.17 ± 1.75	4.55 ± 2.11	0.058	0.068**0.038**[3)]
Time ofDefecation (min)	6.98 ± 4.49	6.06 ± 3.72	0.086	9.52 ± 5.87	8.31 ± 5.87	0.140	0.7600.456[3)]
BSS score	3.50 ± 0.94	3.87 ± 1.10	0.043	3.30 ± 0.85	3.49 ± 0.83	0.116	0.4160.436[3)]
PAC-SYM score	15.59 ± 7.92	8.26 ± 7.64	<0.0001	16.28 ± 7.54	9.38 ± 6.26	<0.0001	0.811
Constipation Strength	3.81 ± 1.51	2.30 ± 1.68	<0.0001	4.15 ± 1.65	2.36 ± 1.83	<0.0001	0.444
Daily life impact	11.84 ± 6.46	5.43 ± 6.34	<0.0001	13.54 ± 5.79	6.86 ± 6.66	<0.0001	0.851
Constipationemotion (1)	7.84 ± 3.95	3.65 ± 3.74	<0.0001	9.44 ± 3.48	5.73 ± 4.76	<0.0001	0.632
Constipationemotion (2)	4.76 ± 2.68	2.07 ± 2.43	<0.0001	4.77 ± 2.50	2.73 ± 2.57	<0.0001	0.286
Living with constipation	5.70 ± 2.30	2.38 ± 2.38	<0.0001	5.59 ± 2.17	3.51 ± 2.64	<0.0001	**0.043**
Satisfy improvement	4.05 ± 2.87	7.92 ± 4.44	<0.0001	4.51 ± 2.78	5.92 ± 4.11	0.064	**0.025**
Improvement of intestinal environment							
β-glucuronidase (pmol/min/g)	4.39 ± 2.21	4.11 ± 2.00	0.526	4.05 ± 1.73	4.93 ± 3.00	0.106	0.095
pH	7.05 ± 0.29	6.84 ± 0.29	0.010	7.04 ± 0.26	6.80 ± 0.30	0.002	0.690
Calprotectin ((ng/g)	36.78 ± 55.79	28.07 ± 26.32	0.257	39.37 ± 48.73	20.91 ± 10.61	0.024	0.373

Values are presented as mean ± SD.
Abbreviations: BSS, Bristol Tool Scale; CTT, Colon transit time; PAC-SYM, Patient Assessment of Constipation Symptom.
* Capsule intake and x-ray schedule mismatch was excluded (Test: R22, Placebo: R58, R74).
1) Analyzed by paired *t*-test, compared within the group.
2) Analyzed by independent *t*-test, compared between groups.
3) Analyzed by ANCOVA, which were adjusted for time of defecation baseline.

Schizophyllum commune is capable of elevating gastrointestinal viscosity, thereby producing a more pronounced effect in alleviating constipation. Furthermore, the TBG-136 group exhibited reduced rectosigmoid colon transit time (CTT) due to its ability to form viscous solutions within the gastrointestinal tract. This viscous solution acts as a lubricant, facilitating efficient fecal excretion. Specifically, the lubricating effect of TBG-136 improves obstructed defecation in constipated patients (Muthuramalingam et al., 2020).These functional properties and observations of TBG-136 are evident in significantly higher satisfaction scores within the TBG-136 group compared to the placebo group". Furthermore, similar clinical trials on constipation were evaluated based on the questionnaire, where patients' responses were analyzed to determine the impact of the test product (Ballou & Lembo, 2020; Quigley, Vandeplassche, Kerstens, & Ausma, 2009). However, we used experimental observations and a questionnaire for appropriate interpretation. Also, participants' dietary and physical habits were carefully examined to reduce the influence of confounding variables on bowel function. Collectively, our study confirms the influence of TBG-136 on bowel movement, constipation-related symptoms, and quality of life in patients with functional constipation. Nevertheless, study findings cannot be generalized due to the limited sample size. In addition, the study could not accurately confirm the TBG-136 fermentation process in the gut environment. Further, SCFAs were not detected in both TBG-136 and placebo groups as the collected sample volume was too low. To overcome these limitations, it is important to perform extensive research with a large number of volunteers and further studies to assess the fermentation process in the gut environment. Still, key observations of the study confirm the positive influence of TBG-136 on constipation-related symptoms.

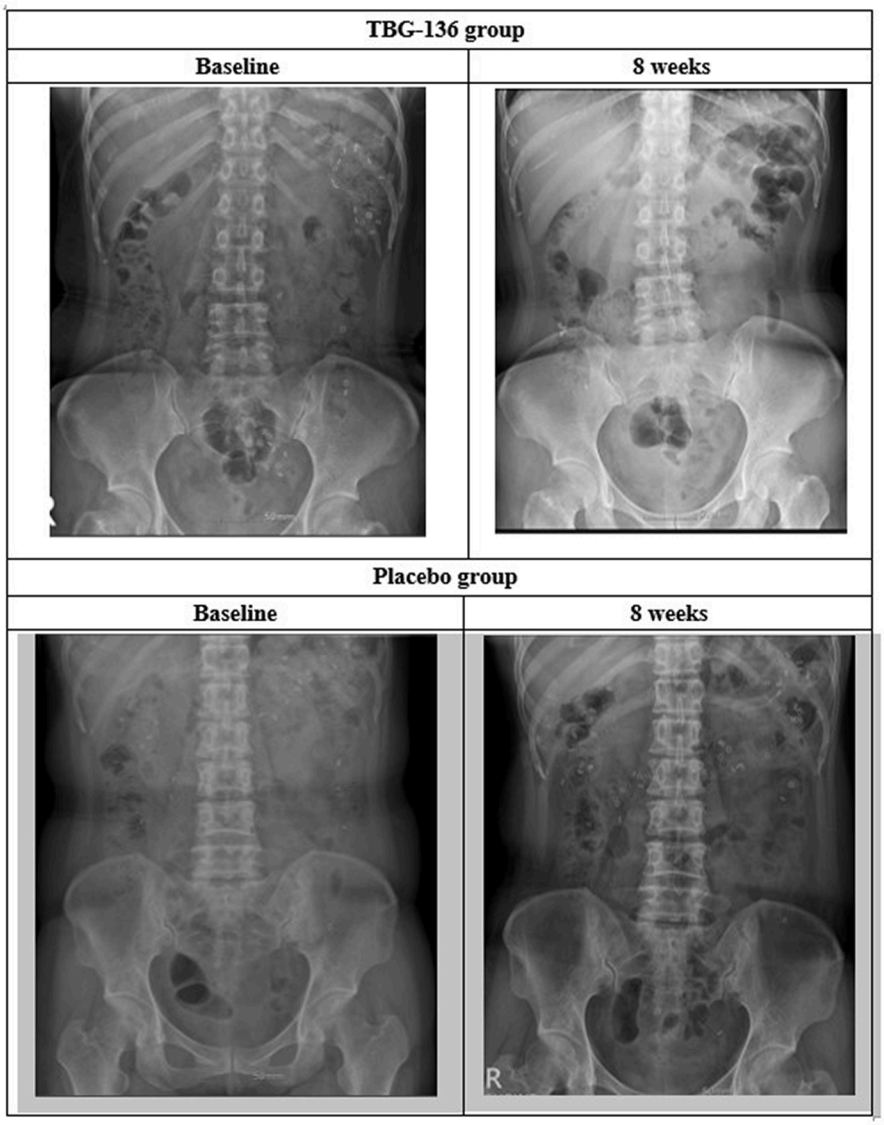

Fig. 3. Representative X-ray images of colon transit time (CTT) obtained 48 hours after the consumption of capsules by different groups.

5. Conclusion

In summary, the supplementation of β-glucan derived from Schizophyllum commune (TBG-136) has shown positive effects on intestinal health, such as an increase in beneficial bacteria and improvements in bowel movement frequency and colonic transit time. These findings indicate that TBG-136 may be beneficial for individuals suffering from constipation, ultimately enhancing their overall quality of life.

CRediT authorship contribution statement

Hui-Yeon Jang: Formal analysis, Investigation, Methodology, Project administration, Visualization, Writing – original draft. **Su-Jin Jung:** Conceptualization, Investigation, Methodology. **Eun-Ock Park:** Formal analysis, Investigation, Methodology. **Soo-Dong Kim:** Conceptualization, Funding acquisition. **Je-Kyoung Kim:** Conceptualization, Funding acquisition. **Soo-Wan Chae:** Conceptualization, Investigation, Methodology. **Youn–Soo Cha:** Writing – review & editing, Conceptualization, Methodology. **Seung-Ok Lee:** Conceptualization, Methodology, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors do not have permission to share data.

Acknowledgement

We would like to thank all participating researchers and our study staff members. We thank Quegen Biotech Co., Ltd, Siheung, Republic of Korea, for providing the test product, TBG-136.

Ethics statement

This study was approved by the Institutional Review Board (IRB) of Jeonbuk National University Hospital (IRB No. 2019-08-010, September/04/2019), and registered at Clinical Research Information Service (CRIS; KCT0006657).

Funding source

This research was a part of the project titled "Cooperative Research Program for Agriculture Science and Technology Development (Project No. PJ01331801)", funded by the Rural Development Administration, Republic of Korea.

Availability of data and materials

The datasets generated and/or analyzed during the current study are not publicly available to protect patient confidentiality but are available from the corresponding author on reasonable request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jff.2023.105668.

References

Ahn, J.-B., Park, J.-A., Jo, H.-J., Woo, I.-H., Lee, S.-H., & Jang, K.-I. (2012). Quality characteristics and antioxidant activity of commercial Doenjang and traditional Doenjang in Korea. *The Korean Journal of Food And Nutrition, 25*(1), 142–148.

Akramienė, D., Kondrotas, A., Didžiapetrienė, J., & Kėvelaitis, E. (2007). Effects of ß-glucans on the immune system. *Medicina, 43*(8), 597.

Armstrong, T., & Bull, F. (2006). Development of the world health organization global physical activity questionnaire (GPAQ). *Journal of Public Health, 14*(2), 66–70.

Arpaia, N., Campbell, C., Fan, X., Dikiy, S., Van Der Veeken, J., Deroos, P., & Coffer, P. J. (2013). Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature, 504*(7480), 451–455.

Bai, J., Zhao, J., Waleed, A.-A., Wang, J., Xue, L., Liu, J., & Li, Y. (2021). Oat β-glucan alleviates DSS-induced colitis via regulating gut microbiota metabolism in mice. *Food & function, 12*(19), 8976–8993.

Ballou, S., & Lembo, A. (2020). *symptom improvement does not equal satisfaction with treatment for constipation* (Vol. 51,, 909–910.

Cani, P. D., Amar, J., Iglesias, M. A., Poggi, M., Knauf, C., Bastelica, D., & Chabo, C. (2007). Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes, 56*(7), 1761–1772.

Carabotti, M., Scirocco, A., Maselli, M. A., & Severi, C. (2015). The gut-brain axis: Interactions between enteric microbiota, central and enteric nervous systems. *Annals of gastroenterology: Quarterly publication of the Hellenic Society of Gastroenterology, 28*(2), 203.

Cheung, P. C. (2008). *Mushrooms as functional foods*. John Wiley & Sons.

De Gregoris, T. B., Aldred, N., Clare, A. S., & Burgess, J. G. (2011). Improvement of phylum-and class-specific primers for real-time PCR quantification of bacterial taxa. *Journal of microbiological methods, 86*(3), 351–356.

Den Besten, G., Van Eunen, K., Groen, A. K., Venema, K., Reijngoud, D.-J., & Bakker, B. M. (2013). The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *Journal of lipid research, 54*(9), 2325–2340.

Deng, C., Hu, Z., Fu, H., Hu, M., Xu, X., & Chen, J. (2012). Chemical analysis and antioxidant activity in vitro of a β-D-glucan isolated from Dictyophora indusiata. *International journal of biological macromolecules, 51*(1–2), 70–75.

Drossman, D. A. (2006). The functional gastrointestinal disorders and the Rome III process. *gastroenterology, 130*(5), 1377–1390.

Du, B., Bian, Z., & Xu, B. (2014). Skin health promotion effects of natural beta-glucan derived from cereals and microorganisms: A review. *Phytotherapy Research, 28*(2), 159–166.

Du, B., Lin, C., Bian, Z., & Xu, B. (2015). An insight into anti-inflammatory effects of fungal beta-glucans. *Trends in Food Science & Technology, 41*(1), 49–59.

Du, B., Meenu, M., Liu, H., & Xu, B. (2019). A concise review on the molecular structure and function relationship of β-glucan. *International journal of molecular sciences, 20*(16), 4032.

Dukas, L., Willett, W. C., & Giovannucci, E. L. (2003). Association between physical activity, fiber intake, and other lifestyle variables and constipation in a study of women. *The American journal of gastroenterology, 98*(8), 1790–1796.

Endo, A., Futagawa-Endo, Y., & Dicks, L. (2009). Lactobacillus and Bifidobacterium diversity in horse feces, revealed by PCR-DGGE. *Current microbiology, 59*, 651–655.

Frank, L., Kleinman, L., Farup, C., Taylor, L., & Miner, P. (1999). Psychometric validation of a constipation symptom assessment questionnaire. *Scandinavian journal of gastroenterology, 34*(9), 870–877.

Goldin, B. R. (1990). Intestinal microflora: Metabolism of drugs and carcinogens. *Annals of medicine, 22*(1), 43–48.

Heijtz, R. D., Wang, S., Anuar, F., Qian, Y., Björkholm, B., Samuelsson, A., … Pettersson, S. (2011). Normal gut microbiota modulates brain development and behavior. *Proceedings of the National Academy of Sciences, 108*(7), 3047–3052.

Jayachandran, M., Chen, J., Chung, S. S. M., & Xu, B. (2018). A critical review on the impacts of β-glucans on gut microbiota and human health. *The Journal of nutritional biochemistry, 61*, 101–110.

Jung, S.-J., Oh, M.-R., Park, S.-H., & Chae, S.-W. (2020). Effects of rice-based and wheat-based diets on bowel movements in young Korean women with functional constipation. *European journal of clinical nutrition, 74*(11), 1565–1575.

Korczak, R., & Slavin, J. (2013). Effects of Oats and *β*-Glucan on Gut Health. *Oats nutrition and technology*, 299–309.

Kumar, V., Sinha, A. K., Makkar, H. P., De Boeck, G., & Becker, K. (2012). Dietary roles of non-starch polysachharides in human nutrition: A review. *Critical reviews in food science and nutrition, 52*(10), 899–935.

Lee, J.-S., Lee, S.-H., Jang, Y.-M., Lee, J.-D., Lee, B.-H., & Jung, J.-Y. (2011). Macrophage and anticancer activities of feed additives on β-glucan from Schizophyllum commune in breast cancer cells. *Journal of the Korean Society of Food Science and Nutrition, 40*(7), 949–955.

Lee, J.-W. (2014). Antioxidant and Immunological Activities of Polysaccharide Extracted from Cultured Mycelia of Schizophyllum commune. *Journal of the Korean Society of Food Science and Nutrition, 43*(9), 1334–1341.

Marquis, P., De La Loge, C., Dubois, D., McDermott, A., & Chassany, O. (2005). Development and validation of the Patient Assessment of Constipation Quality of Life questionnaire. *Scandinavian journal of gastroenterology, 40*(5), 540–551.

Mearin, F., Ciriza, C., Mínguez, M., Rey, E., Mascort, J. J., Peña, E., … Júdez, J. (2016). Clinical practice guideline: Irritable bowel syndrome with constipation and functional constipation in the adult. *Rev Esp Enferm Dig, 108*(6), 332–363.

Metcalf, A. M., Phillips, S. F., Zinsmeister, A. R., MacCarty, R. L., Beart, R. W., & Wolff, B. G. (1987). Simplified assessment of segmental colonic transit. *Gastroenterology, 92*(1), 40–47.

Mio, K., Otake, N., Nakashima, S., Matsuoka, T., & Aoe, S. (2021). Ingestion of High β-glucan barley flour enhances the intestinal immune system of diet-induced obese mice by prebiotic effects. *Nutrients, 13*(3), 907.

Mitsou, E. K., Panopoulou, N., Turunen, K., Spiliotis, V., & Kyriacou, A. (2010). Prebiotic potential of barley derived β-glucan at low intake levels: A randomised, double-blinded, placebo-controlled clinical study. *Food Research International, 43*(4), 1086–1092.

Moayyedi, P., Mearin, F., Azpiroz, F., Andresen, V., Barbara, G., Corsetti, M., … Stanghellini, V. (2017). Irritable bowel syndrome diagnosis and management: A simplified algorithm for clinical practice. *United European gastroenterology journal, 5*(6), 773–788.

Muthuramalingam, K., Singh, V., Choi, C., Choi, S. I., Kim, Y. M., Unno, T., & Cho, M. (2020). Dietary intervention using (1, 3)/(1, 6)-β-glucan, a fungus-derived soluble prebiotic ameliorates high-fat diet-induced metabolic distress and alters beneficially the gut microbiota in mice model. *European Journal of Nutrition, 59*(6), 2617–2629.

Ng, M.-L., & Yap, A.-T. (2002). Inhibition of human colon carcinoma development by lentinan from shiitake mushrooms (Lentinus edodes). *The Journal of Alternative & Complementary Medicine, 8*(5), 581–589.

Quigley, E., Vandeplassche, L., Kerstens, R., & Ausma, J. (2009). Clinical trial: The efficacy, impact on quality of life, and safety and tolerability of prucalopride in severe chronic constipation–a 12-week, randomized, double-blind, placebo-controlled study. *Alimentary pharmacology & therapeutics, 29*(3), 315–328.

Raa, J. (2015). Immune modulation by non-digestible and non-absorbable beta-1, 3/1, 6-glucan. *Microbial ecology in health and disease, 26*(1), 27824.

Riegler, G., & Esposito, I. (2001). Bristol scale stool form. A still valid help in medical practice and clinical research. *Techniques in coloproctology, 5*(3), 163–164.

Russo, P., López, P., Capozzi, V., De Palencia, P. F., Dueñas, M. T., Spano, G., & Fiocco, D. (2012). Beta-glucans improve growth, viability and colonization of probiotic microorganisms. *International journal of molecular sciences, 13*(5), 6026–6039.

Singh, V., Muthuramalingam, K., Kim, Y. M., Park, S., Kim, S. H., Lee, J., … Cho, M. (2021). Synbiotic supplementation with prebiotic Schizophyllum commune derived β-(1, 3/1, 6)-glucan and probiotic concoction benefits gut microbiota and its associated metabolic activities. *Applied Biological Chemistry, 64*(1), 1–10.

Sun, S., Yang, Y., Lin, X., Chen, P., Ye, L., Zeng, L., . . . Shan, J. (2021). Qiweibaizhu Decoction Treats Diarrheal Juvenile Rats by Modulating the Gut Microbiota, Short-Chain Fatty Acids, and the Mucus Barrier. *Evidence-Based Complementary and Alternative Medicine, 2021*.

Tao, Y., Zhang, L., & Cheung, P. C. (2006). Physicochemical properties and antitumor activities of water-soluble native and sulfated hyperbranched mushroom polysaccharides. *Carbohydrate Research, 341*(13), 2261–2269.

Tsukada, C., Yokoyama, H., Miyaji, C., Ishimoto, Y., Kawamura, H., & Abo, T. (2003). Immunopotentiation of intraepithelial lymphocytes in the intestine by oral administrations of β-glucan. *Cellular immunology, 221*(1), 1–5.

Turnbaugh, P. J., Ley, R. E., Mahowald, M. A., Magrini, V., Mardis, E. R., & Gordon, J. I. (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. *nature, 444*(7122), 1027–1031.

Vetvicka, V., Vannucci, L., Sima, P., & Richter, J. (2019). Beta Glucan: Supplement or Drug? From Laboratory to Clinical Trials. *Molecules, 24*(7). https://doi.org/10.3390/molecules24071251

Vu, V., Muthuramalingam, K., Singh, V., Choi, C., Kim, Y. M., Unno, T., & Cho, M. (2022). Schizophyllum commune-derived β-glucan improves intestinal health demonstrating protective effects against constipation and common metabolic disorders. *Applied Biological Chemistry, 65*(1), 1–11.

Vu, V., Muthuramalingam, K., Singh, V., Hyun, C., Kim, Y. M., Unno, T., & Cho, M. (2022). Effects of β-glucan, probiotics, and synbiotics on obesity-associated colitis and hepatic manifestations in C57BL/6J mice. *European journal of nutrition, 61*(2), 793–807.

Wong, J. M., De Souza, R., Kendall, C. W., Emam, A., & Jenkins, D. J. (2006). Colonic health: Fermentation and short chain fatty acids. *Journal of clinical gastroenterology, 40*(3), 235–243.