A THESIS
FOR THE DEGREE OF MASTER OF SCIENCE

# *Schizophyllum commune* derived β-glucan improves gut healthiness

**Vu Van Vuong**

**(Supervised by Professor Moonjae Cho)**

Department of Interdisciplinary Graduated Program in Advanced Convergence Technology and Science

GRADUATE SCHOOL
JEJU NATIONAL UNIVERSITY

February 2022

# *Schizophyllum commune* derived β-glucan improves gut healthiness

**Vu Van Vuong**
(Supervised by Professor Moonjae Cho)

A dissertation submitted in partial fulfillment of the requirement for the degree of Master of Science

2021.11.30

This dissertation has been examined and approved by

.. .....

Dr. Moonjae Cho, Full Professor, Department of Interdisciplinary Graduated Program in Advanced Convergence Technology and Science, Jeju National University

.. .....

Dr. Tatsuya Unno, Associate Professor, Faculty of Biotechnology, School of Life Sciences, SARI, Jeju National University

.. .....

Dr. Somi Kim, Full Professor, Department of Interdisciplinary Graduated Program in Advanced Convergence Technology and Science, Jeju National University

2021. 11. 30

Date

Department of Interdisciplinary Graduate Program in Advanced Convergence Technology and Science

GRADUATE SCHOOL
JEJU NATIONAL UNIVERSITY

## CONTENTS

## LIST OF TABLES

## LIST OF FIGURES

## LIST OF ABBREVIATIONS

**ALP -** Alkaline phosphatase

**ALT -** Alanine transaminase

**AMOVA** - Analysis of molecular variance

**AST -** Aspartate transaminase

**BG –** β-glucan

**CD** – Crohn's disease

**COX-2** - cyclooxygenase-2

**DSS** - Dextran sulfate sodium

**E-cad** - Epithelial cadherin

**FC –** Functional constipation

**GAPDH** - Glyceraldehyde 3-phosphate dehydrogenase

**GLU** – Glucose

**H&E stain** - Hematoxylin and eosin stain

**HDL** - High-density lipoprotein

**HFD** – High-fat diet

**IBD** – Inflammatory bowel disease

**IL-1β** - interleukin-1β

**IL-6** - interleukin-6

**LDH** - Lactate dehydrogenase

**LEfSe** - Linear discriminant analysis effect size

**LPS** - Lipopolysaccharide

**NAFLD** - Non-alcoholic fatty liver disease

**ND** -Normal diet

**NMDS** - Non-metric multidimensional scaling

**NOX4** - NADPH oxidase 4

**PAS** - Periodic acid–Schiff

**PRO** – Probiotics

**OTUs** - Operational taxonomic units

**SCFAs** – Short chain fatty acids

**SYN** – Synbiotics

**TG** - Triglycerides

**TNF- α** - Tumor necrosis factor- α

**Total-Cho** - Total cholesterol

**UC** – Ulcerative colitis

**Vuong Vu.** *Schizophyllum commune* derived β-glucan improves gut overall healthiness

## ABSTRACT

The thesis demonstrates the novel investigation about the effect of *Schizophyllum commune*-derived β-glucan on gut-related conditions such as obesity-related inflammatory bowel disease (IBD) and constipation. Effects of the compound on two conditions were evaluated via evaluating its impact on the gastrointestinal tract, gut microbiota, and immune system.

Firstly, C57BL/6J mice fed on three diets consist of normal diet (ND), a normal diet supplemented with 3 g/kg (BG_low), and 5 g/kg (BG_high) of β-glucan (BG). After 12 weeks of treatment, β-glucan show a significant increase in goblet cells population and mucin production. Besides that, the compound bulked feces with lowered moisture content, decreased glucose serum level, and obesity-related biomarkers, however, intestinal transit rate was not increased which was observed in obese mice. The compound increased the abundance of beneficial bacteria while suppressing harmful strains in a dose-dependent manner. Specific short-chain fatty acid (SCFA)-producing strains, such as *Roseburia*, *Ruminococcus*, and *Bifidobacteria*, were selectively increased by β-glucan.

The effect of β-glucan on obesity-associated colitis was assessed by using C57BL/6J mice fed on five diets comprised of normal diet (ND), high-fat diet (HFD), and HFD implemented with β-glucan (BG), probiotics (PRO), and a mixture of BG and PRO (SYN) for 12 weeks. Dietary treatment was followed by 5 days of colitis induction using DSS. Colon and liver samples were sectioned for histological analysis while fecal microbiota was analyzed. DSS significantly disrupted the intestinal barrier, induced transmural inflammation and colitis-associated hepatic manifestation. Although BG and PRO secured intestinal tight junctions, these two treatments did not protect the colon from inflammatory cell infiltration concomitant with high inflammatory markers. In contrast, SYN demonstrated stronger and broader effects in reducing colonic inflammation. While BG treatment increased the abundance of

indigenous *Lactobacillus*, PRO treatment decreased bacterial diversity by suppressing the growth of several species of bacteria. SYN treatment groups, however, supported the growth of both indigenous and supplemented bacteria while maintaining bacterial diversity.

The two experiments consolidated the beneficial effect of *Schizophyllum commune*-derived β-glucan on gut-related diseases such as IBD, constipation, and metabolic syndromes. Even on basal state, consumption of β-glucan successfully improved intestinal health and shift gut microbiota toward a healthy population, resulting in downregulating the risk of IBD, constipation, and metabolic diseases. The second trial showed that administration of both β-glucan and probiotics results in synergistic effect with better outcomes. On the other hand, this thesis also pointed out the insignificant effect in immunomodulation of *Schizophyllum commune* β-glucan and other beneficial effects could be limited by the competition over nutrients between gut beneficial strains.

**부반부옹.** 치마버섯 유래 베타-글루칸이 장의 전반적인 건강상태를 개선한다.

## ABSTRACT - HANGUL

이 논문은 치마버섯 유래 베타-글루칸이 장 질환, 예를 들어 궤양성 대장염이나 변비와 같은 비만 관련 염증성 장 질환들(Inflammatory Bowel Disease, 이하 IBD)에 대해 갖는 영향을 새롭게 연구한 결과들을 보여준다. 위장관, 장내미생물총 그리고 면역계에 베타-글루칸이 미치는 영향을 연구함으로써 베타-글루칸이 앞의 두 가지 질환(궤양성 대장염과 변비)에 대해 어떤 치료 효과를 가질 수 있는지 검증해보았다.

우선, C57BL/6J 쥐들을 다음 3 가지의 식단으로 먹였다; 일반 식단 (Normal Diet, 이하 ND), 일반 식단에 베타-글루칸(BG)을 3 g/kg 으로 추가한 식단 (이하 BG-low), 그리고 일반 식단에 BG 를 5 g/kg 으로 추가한 식단 (이하 BG-high). 12 주 간 먹였을 때, 베타-글루칸은 술잔세포의 수와 뮤신의 생성을 크게 증가시켰다. 뿐만 아니라, 베타-글루칸은 수분이 적은 변의 크기를 증가시켰고, 혈당 수치와 비만 관련 생체표지들은 감소시켰다. 하지만 이전 연구에서 베타-글루칸이 비만 쥐에 대해서 장내 이동률도 증가시켰던 반면, 본 실험의 일반 쥐에 대해서는 그러한 효과를 보이지 않았다. 베타-글루칸은 그 양에 비례하게 유익한 세균의 수는 증가시킨 반면 해로운 종들의 수는 감소시켰다. 그리고 특정 단사슬 지방산을 생성하는 종, 예를 들어 *Roseburia, Ruminococcus, Bifidobacteria* 의 수가 베타-글루칸에 의해 증가되었다.

비만 관련 대장염에 미치는 베타-글루칸의 영향에 대해서도 C57BL/6J 쥐들을 이용하여 연구하였다. 이 쥐들은 다음 5 가지의 식단을 12 주 동안 먹었다; 일반 식단 (ND), 고지방 식단 (High-Fat Diet, 이하 HFD), 그리고 HFD 에 베타-글루칸을 추가한 식단 (BG), 프로바이오틱스를 추가한 식단 (Probiotics, 이하 PRO), 마지막으로 베타-글루칸과 프로바이오틱스를 혼합하여 추가한 식단 (SYN). 이러한 식단은, DSS 로 5 일 동안 대장염을 유도한 이후에 제공했다. 결장과 간은 조직분석을 위해 박편으로 만들었고, 변의 미생물총도 분석했다. DSS 는 장 벽을 심각하게 손상시켰으며 경벽성 염증과 대장염에 관련된 징후들을 간에서 유발하였다. BG 와 PRO 가 장의 밀착연접은 보호했지만, 염증성 세포 그리고 염증성 표지들의 결장 침투를 막지는 못 했다.

대조적으로, SYN 은 결장염증을 줄이는 데 있어서 더 효과가 있었다. BG 가 고유의 유산균을 증가시킨 반면, PRO 는 몇 가지 특정 세균의 성장을 막음으로써 세균의 다양성을 감소시켰다. SYN 은 세균의 다양성은 유지하면서 고유종과 새로운 세균의 성장을 모두 도왔다.

두 가지의 동물실험은 치마버섯 유래 베타-글루칸이 IBD, 변비 그리고 대사증후군과 같은 장 관련 질환들에 대해 갖는 유익한 영향들을 더욱 공고하게 만들었다. 기초 상태에서도, 베타-글루칸은 장 건강을 성공적으로 증진시켰고 장 미생물총을 더욱 건강한 구성으로 만듦으로써 IBD, 변비 그리고 대사증후군의 위험을 줄였다. 특히 두 번째 실험은 베타-글루칸과 프로바이오틱스를 모두 섭취하는 것이 시너지효과를 내어 더 나은 결과를 만든다는 것을 보여주었다. 반면, 본 연구는 치마버섯 유래 베타-글루칸이 면역조정에 있어서는 큰 효과가 없다는 것, 그리고 장에 유익한 세균들 사이의 영양분경쟁을 유발함으로써 다른 유익한 영향들이 제한될 수 있음을 지적한다.

# CHAPTER 1. INTRODUCTION

## 1.1 Characteristics of β-glucan derived *Schizophyllum commune*

B-glucan is a polysaccharide with the monomer residue is D-glucose which links together by a glycosidic bond. The compound is a natural bioactive carbohydrate that has a comprehensive investigation history in clinical application. Since β-glucan is an impactful modifier, its effect on various medical conditions such as cancer, obesity, diabetes, cardiovascular diseases, hyper-cholesterol, immunomodulation, etc is always a worth-focused research interest [1, 2]. It is proven beneficial effects could be observed in β-glucan from various sources such as bacteria, algae, yeast, protozoan, fungi, cereal like oats, wheat, barley, etc (**Fig. 1.1**).

| Component | Grain | Yeast | Mushroom | Bacteria |
|---|---|---|---|---|
| Structure | (1→3)(1→4) β-1,4 glucan Linear | (1→3)(1→6) β-1,6 branch Long branched | (1→3)(1→6) β-1,6 branch Short branched | (1→3) linear β-1,3 glucan |
| Source of origin | Oat, Barley, Rye | S.cerevisiae | Phellinus linteus, Coriolus versicolor, Ganoderma lucidum, Agaricus blazei murill | Agrobacterium sp. |
| Isolated part | Surface of grain | Cell wall of yeast | Mycelium fruit body | Exopolysaccharide |

**Figure 1. 1 Characteristic of four kinds of β-glucan.**

Come along with an extraordinary number of studies investigating the effect of this compound, β-glucan has been proven as showing significant benefit toward many conditions [2]. As the result of ample *in vivo*, *in vitro*, and clinical studies, β-glucan was proven to possess important therapeutic effects including antitumor [3-5], immunomodulation [6-8], bone regeneration and healing [9-11], anti-diabetic and anti-obesity [12-16], lowering cholesterol and blood pressure [17, 18], antigenotoxic, antimutagenic, and antioxidative [2].

It is widely known that β-glucan from different sources showed distinct degrees of impact on host physiology. Importantly, although there are various sources of β-glucan, different structures and different molecular weights β-glucan from one source could also show different effects [19, 20] (Fig. 1.2). Besides that, the spectrum of processing methods involved in extraction, purification, and functionalization could further exert influence on their physiochemical properties as branching, molecular weight, concentration, viscosity, solubility, glycosidic linkage, etc., results in different degrees of their physiological effects [21].

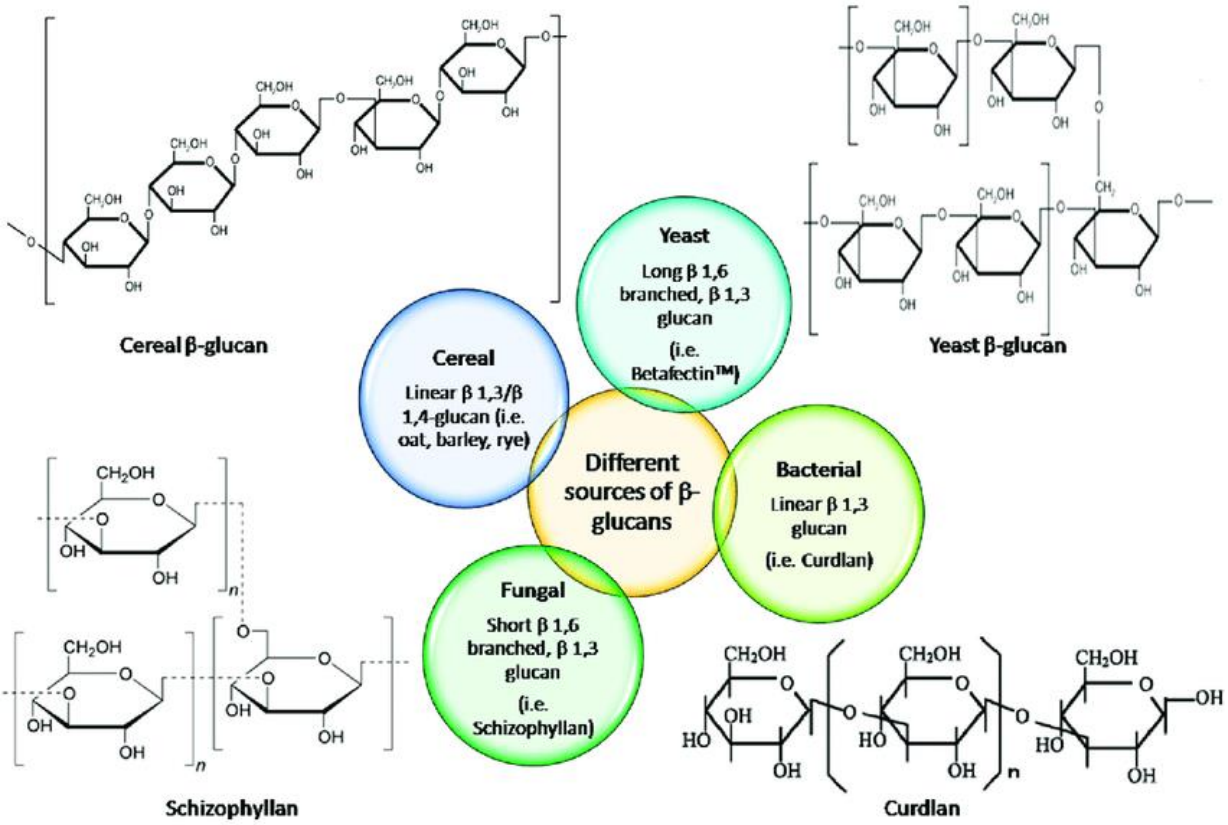



**Figure 1. 2 Structures of β-glucan from four different sources [22].**

This thesis aims to investigate the beneficial impact of a specific fiber, which is β-glucan derived from *Schizophyllum commune.* Since *Schizophyllum commune* is a species of fungus in the genus *Schizophyllum*, the β-glucan extracted from this source inherits most fungal characteristics as having short β 1,6 branched, β 1,3 glucan which is known as having immunomodulatory effect [23]. Fermented *Schizophyllum commune* derived

exopolysaccharide β-1,3/1,6 glucan has a molecular weight greater than 1.7 x $10^6$ Da and has a degree of branching of 0.33, the compound is high purity and water-soluble.

### 1.2 Gut-related disorders

#### 1.2.1 Constipation

Chronic constipation comprises functional constipation (FC) and constipation-type irritable bowel syndrome [24]. Infrequent and slow bowel movements are the two major conditions in functional constipation [25]. The multifactorial pathogenesis of constipation consists of dietary habits, genetic factors, colonic absorption, motility, and medication history [25]. Furthermore, unhealthy lifestyle habits, such as lack of fiber intake, high consumption of dairy products, dehydration, and a sedentary lifestyle could also lead to unsatisfactory defecation [25, 26]. Following constipation, patients usually meet two or more of the following symptoms: excessive straining needed during defecation; lumpy and hard stool scale 1–2 on the Bristol Stool Scale; sensation of incomplete evacuation and anorectal obstruction/blockage; manual maneuvers, such as digital evacuation and support of pelvic floor needed; and fewer than three bowel movements per week [27]. There is a wide range of treatments for constipation, and a medical approach using stimulant laxatives and osmotic could be used as the first strategy for patients with chronic constipation. If laxatives are ineffective, the use of lubiprostone, linaclotide, and prucalopride has also been suggested [25]. In addition, physiotherapy training of pelvic floor muscles and biofeedback therapy are especially useful for improving bowel symptoms, and in 70% of patients with gastrointestinal disorders, related symptoms have been eliminated using biofeedback therapy [25]. When other approaches are insufficient, surgical interventions can be applied [25]. Since therapies could be administered based on the mentioned etiologies, avoiding dehydration and increasing ingestion of fiber are the most reasonable and accessible treatments [26]. The clinical guideline/position papers published by the North American Society for Pediatric

Gastroenterology, Hepatology, and Nutrition (NASPGHAN) and the European Society of Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) did not support the ingestion of fiber as a sufficient therapy for FC [28]. However, 86% of gastroenterologists from Europe and 81% from the US commonly recommend increasing the amount of fiber ingestion as a daily habit to amend FC [26]. In addition, previous meta-analyses showed that fiber ingestion resulted in an increased frequency of defecation, stool softness, and faster transit rate [28]. Together, this suggests that the use of fibers is still controversial, and further research is required.

### 1.2.2 Inflammatory bowel disease

Inflammatory bowel disease (IBD) comprises Crohn's disease (CD) and ulcerative colitis (UC). CD and UC share symptoms such as weight loss, diarrhea, hematochezia, and abdominal pain; however, the severity, sites, and inflammatory complications differ [29, 30]. Both animal and human studies have demonstrated a significant increase in the production of pro-inflammatory cytokines in both acute and chronic UC and CD, concomitant with the significant infiltration of inflammatory cells into the mucosal layer [31, 32]. Interactions among intestinal epithelial cells, intraluminal microorganisms, and the immune system are key regulators of intestinal homeostasis [30]. It has been suggested that intestinal homeostasis breakdown - the disruption in dynamic crosstalk among intestinal epithelial cells, intestinal bacteria, and immune cells is a fundamental cause of IBD in humans and animals [30]. Various IBD cohorts have demonstrated that obesity affects the development of colitis and other autoimmune conditions, as well as the response of patients to therapies [33]. Gut dysbiosis, which is characterized by decreased bacterial diversity, suppressed populations of beneficial strains, and increased abundance of harmful bacteria can be caused by a Western-style diet and is known to worsen the symptoms of UC in humans [34]. In addition, large amounts of fat produce a variety of adipokines and transcriptional factors that affect the

severity of IBD in humans and animals [34, 35]. Previously, mice genetically susceptible to IBD showed exacerbated symptoms when fed a high-fat diet, suggesting that obesity is a risk factor for IBD [36]. IBD leads to extra-intestinal manifestations comprising various disorders of the liver and biliary tract [37]. IBD-induced disruption of overall nutritional equilibrium leads to the development of fatty liver, while portal vein thrombosis is caused by an increased platelet count and elevated levels of fibrinogen and factors V and VIII [37]. Fatty liver, liver thrombosis, and other conditions eventually lead to IBD-associated hepatitis. Dextran sulfate sodium (DSS), the most common chemical used to induce IBD in animal models, induces colitis by disrupting colonic epithelial cells, resulting in compromised epithelial barrier function and integrity [38].

### 1.3 Dietary treatment

#### 1.3.1 Dietary treatment for Constipation

Previous studies have identified the effects of many types of fiber on FC and gastrointestinal function, including inulin [39], partially hydrolyzed guar gum [40], lactulose [41], glucomannan [42, 43], galacto-oligosaccharides [44], psyllium, and ispaghula [45]. In addition to these fibers, β-glucan has also been proposed as a beneficial prebiotic, an animal study showed that bread yeast β-glucan enhanced intestinal motility and recovered intestinal microecology, resulting in an increase in neurotransmitter and tight junction protein expression on loperamide-induced constipation [46]. Importantly, different sources and different molecular weights of β-glucan have distinct effects on the gastrointestinal tract, which suggests that the benefits of this compound have not been fully discovered [20, 47, 48]. Soluble and non-digestible (1,3)/(1,6)-β-glucan produced from *Schizophyllum commune* was recently shown to have an effect on high-fat diet-induced gut dysbiosis and ameliorated obesity-related constipation [49, 50]. Since the gut dysbiosis plays an important role in the progression of metabolic disorders, such as obesity, diabetes, and cardiovascular disease, the

reformation of gut microbiota by this compound suggests protective effects against these diseases [51, 52]. Furthermore, administration of *Schizophyllum commune* β-glucan fostered the fiber fermentation process and increased short chain fatty acids (SCFAs), which are metabolites known to fuel intestinal epithelial cells [53, 54].

It has been demonstrated that colonic mucus is decreased in rats with loperamide-induced constipation, while the consumption of both water-soluble and -insoluble fibers could relieve this condition [55, 56]. Importantly, different types of fiber lead to different results; for example, a 1.5 mg/day administration of carrageenan and chondroitin sulfate or 5 mg/day of sodium alginate increased fecal exertion, mucin production, and mucous layer thickness after 2 days, while administration of cellulose at 5 mg/day was ineffective in mucin production [56]. Different effects of various fiber sources suggest that there are various beneficial fibers and effects that are yet to be thoroughly discovered. The composition of fecal and mucosal microbiota in patients with constipation is different from that in healthy individuals, suggesting a link between the gut microbiota and constipation [57]. It has been demonstrated that the population of Bacteroidetes is increased in the colonic mucosa of patients with constipation [57]. In addition, genera of Firmicutes, such as *Faecalibacterium*, *Lactococcus*, and *Roseburia,* correlated with fast colonic transit [57]. Previous studies have demonstrated that low levels of *Lactobacillus* spp. and high levels of *Bifidobacterium*, *Clostridia*, *Bacteroides* spp., *Parabacteroides* spp., and *Proteus mirabilis* were observed in children with functional constipation [58]. A meta-analysis demonstrated that the relationship between the gut microbiota and functional constipation is inconsistent, and no consensus exists [24]. However, low levels of *Bifidobacterium* in fecal samples and high levels of *Bacteroidetes* in the mucosa were observed in patients with irritable bowel syndrome-constipation [24]. The review also suggests that treatment with probiotics, such as *Lactobacillus* spp. and *Bifidobacterium* spp., prebiotics, synbiotics, and fecal microbiota transplantation are effective

for treating chronic constipation with insignificant side effects [24, 58]. In addition, previous meta-analyses showed that fiber ingestion resulted in an increased frequency of defecation, stool softness, and faster transit rate [28].

In this study, the effects of *Schizophyllum commune*-derived β-glucan on the gastrointestinal tract on basal state were evaluated, focusing on physical changes, gut microbiota modification, modifying intestinal mucin production, gastrointestinal transit rate, and regulating associated serum biomarkers. Based on the acquired results, the benefits of this compound on conditions such as constipation and common metabolic diseases could be observed even in the basal condition.

### 1.3.2 Dietary treatment for IBD

Currently, several compounds, such as 5-aminosalicylic acid (ASA)-based compounds, corticosteroids, azathioprine/6-mercaptopurine, methotrexate, and cyclosporine, have been approved for the management of IBD [59]. Over the past decade, dietary interventions, such as prebiotics, probiotics, and synbiotics, have been reported as promising therapies for IBD; moreover, they are easily accessible and have few side effects [50, 60]. Since disrupted homeostasis between the gut microbiome and host immune system is the key factor leading to IBD, dietary interventions with the potential to revert the microbiota to a healthy state are promising for IBD treatment [59]. Furthermore, prebiotics, probiotics, and synbiotics not only affect the microbiota but also strongly modulate the immune system and intestinal epithelium, implying that adequate administration of these compounds could ameliorate IBD [20, 59, 61-64].

Short-chain fatty acids (SCFAs) are a product of fiber fermentation by gut microbiota, which play an important role in maintaining intestinal homeostasis by fueling intestinal epithelial cells and strengthening the gut barrier [53]. Moreover, butyrate, one of the SCFAs which is

known to have immunomodulatory functions [53]. Importantly, SCFA yield is reduced in the mucosa and feces of IBD patients, which is explained by the reduction of SCFA-producing bacteria, including members of the phylum Firmicutes and the genus *Faecalibacterium prausnitzii* [53]. Due to the beneficial effects of SCFAs, some universal prebiotic dietary fibers, including fructooligosaccharides, galactooligosaccharides, and inulin, have recently been investigated as treatments for IBD [65]. Another notable source of fiber is β-glucan, which is extracted from various sources, such as yeast, fungi, and oat, which could be a promising therapeutic agent for IBD and was previously demonstrated to exert beneficial effects in patients with colitis and in animal models [20, 66]. Compared to commercial medicines, β-glucan is easy to access and exerts no significant side effects [49, 67]. Moreover, the effects of β-glucan vary based on different sources, suggesting the great undiscovered potential of β-glucan [20, 49, 66, 67]. Importantly, β-glucan possesses most of the abilities of conventional prebiotics, such as restoring healthy gut microbiota, creating a gastrointestinal barrier against intraluminal pathogens, and regulating the immune system [20].

Previous meta-analyses have indicated that probiotics are an effective dietary therapy for IBD [53]. Remission in UC patients could be achieved by administration of VSL#3, a mixture of strains of *Lactobacillus*, *Bifidobacterium*, and *Streptococcus salivarius* without significant side effects [53]. The daily administration of $3\times10^9$ live bacteria of mixture VSL#3 for 60 days ameliorated UC in mice [68]. In addition, *Lactobacillus rhamnosus* GG (LGG) is the most widely used probiotic with various beneficial effects, such as preventing gastrointestinal infections, diarrhea, and stimulating immune responses [69]. The administration of synbiotics composed of *Bifidobacterium* and oligofructose-enriched inulin reduced both the sigmoidoscopy score and histological damage of rectal mucosa, consistent with the decrease in the tumor necrosis factor- α (TNF- α) and interleukin-1β (IL-1β) in UC patients [70]. Some

synbiotics have been used in clinical practice, such as the co-administration of oat fiber with *Lactobacillus plantarum* and fructo-oligosaccharides with *L. sporogens,* which prevented intestinal infection and decreased the incidence of diarrhea [71]. Improvement of IBD by synbiotic treatment has only been demonstrated by a few studies that are insufficient to draw relevant conclusions, suggesting that further studies on the effect of synbiotics on IBD are required [71].

Previously, it is reported that *Schizophyllum commune*-derived β-glucan effectively modulated gut dysbiosis caused by a high-fat diet [49] and supported the beneficial activities of probiotics when supplemented together [50]. In this thesis, I investigated whether the modulation of the gut microbiota may ease the symptoms of obesity-associated colitis. I modulated the gut microbiota of mice with obesity-associated colitis using three different additives (β-glucan, probiotics, and synbiotics) to evaluate an effective approach for preventing and treating colitis in mice.

### 1.4 Objectives and scope of the thesis

In this thesis, I investigate the effect of a β-glucan from a specific source - *Schizophyllum commune* on constipation, inflammatory bowel disease, and common metabolic disorders. The strategy in which the compound exerts its impact was assessed via various well-planned experiments with reliable results.

### 1.5 Structure of the thesis

This thesis consists of 3 chapters as follows:

Chapter -1 presents the background knowledge of the characteristic of material and the strategy in which the material protects host from various diseases and conditions. This chapter also describes the characteristic of conditions, while demonstrates common treatments and promising dietary method.

Chapter -2 demonstrates the strategy in which *Schizophyllum commune* derived B-glucan affects intestinal epithelium, gut microbiota, and fecal morphology in normal state suggesting protective effect of the compound against constipation and metabolic dysfunction.

Chapter -3 presents the beneficial effect of *Schizophyllum commune* derived B-glucan against obesity-associated colitis and colitis-induced NAFLD via modifying gut microbiota, securing intestinal barrier, and reducing intestinal inflammation.

## CHAPTER 2: *Schizophyllum commune* derived β-glucan proposed protective effects against constipation and common metabolic disorders.

### 2.1 Abstract

It has been proven that β-glucan produced by *Schizophyllum commune* has beneficial effects on obesity, obesity-associated constipation, and colitis conditions; however, the protective effect of the compound on a basal state is yet to be investigated. C57BL/6J mice were fed with a normal diet (ND), normal diet supplemented with 3 g/kg (BG_low), and 5 g/kg (BG_high) of β-glucan (BG) for 12 weeks. Body weight, food and water intake and fecal status were monitored weekly. Periodic acid–Schiff (PAS) and Alcian Blue stained intestine was used to evaluate the mucin layer thickness and goblet cell population. Internal organs changes and intestinal motility were also assessed. Serum biomarkers for liver injury and glucose level were analyzed. Fecal microbiota and associated metabolic activities were also investigated. β-glucan bulked feces, and decreased fecal moisture; however, the intestinal transit rate did not increase. β-glucan proliferate goblet cells results in a thickened mucin layer. The compound increased the abundance of beneficial bacteria while suppressing harmful strains in a dose-dependent manner. Specific short chain fatty acid (SCFA)-producing strains, such as *Roseburia*, *Ruminococcus*, and *Bifidobacteria*, were selectively increased by β-glucan. In addition, consumption of β-glucan lowered level of obesity-associated biomarkers. *Schizophyllum commune* β-glucan showed an insignificant change in transit rate in healthy conditions when compared with obesity, despite similar effects on increasing mucus production and bulked feces. Nonetheless, the outcomes proposed protective effects against obesity, diabetes, inflammatory bowel diseases (IBD), and constipation, in which the modification of the gut microbiota by β-glucan is the largest contributor.

## 2.2 Methods

### 2.2.1 Animal and animal care

In the present study, 5-week-old male C57BL/6J mice were used as a target for the treatment of β-glucan. Animal nourishment and handling in this experiment were compliant with the guidelines framed by the Animal Care and Use Committee (ACUC No.:2018-0018) at Jeju National University. Immediately after arriving at the laboratory, the mice were acclimatized to the experimental environment in the animal room with a 12 h light/dark cycle. The room temperature was maintained constant during the experiment at 23 ± 2 $^{o}$C with a humidity of 55 ± 15%. After one week of acclimatization, mice were randomly divided into three groups (*n*=11 per group): normal diet (ND), ND supplemented with β-glucan at a concentration of 3 g/kg (BG_low), and 5 g/kg (BG_high). BG_low and BG_high were prepared by directly adding β-glucan to the normal diet ingredients. Pharmacologically active and purified β-glucan produced from *Schizophyllum commune* were provided by Quegen Biotech Co. Ltd. (Seoul, Republic of Korea). During the animal experiment, mice were housed with *ad libitum* access to food and water, and animal food was replaced every day, while the total consumption was monitored weekly.

### 2.2.2 Physiological measurement

Animal body weight and food and water consumption were monitored weekly throughout the study. Wooden chips were used as animal bedding, the bedding was replaced every week. At 24 h after bedding replacement, 25 pieces of feces from each cage were collected for weighing. Photographs of the feces were also taken for color, size, and morphology evaluation. After 12 weeks of animal study, mice were sacrificed and tissues such as the small intestine, colon, liver, and lung were collected and stored at -70$^{o}$C. The distance from the duodenum to the terminal ileum (small intestine) and the distance from the cecum to the rectum (colon) were measured. The liver and lung weights were also evaluated, the liver and

lung indices were calculated using the following formula: liver index (%) = liver weight (g) / body weight (g) × 100 (%). The increased percentage of body weight was calculated using the following formula: increased body weight (%) = $12^{th}$ body weight (g) / initial body weight (g) × 100.

### 2.2.3 Fecal moisture content analysis

Equal amounts of feces were collected from each group and kept in a hot air oven at 60 $^{o}$C for 2 days to measure dry weight. The moisture content was calculated by subtracting the dry weight from the wet weight. Moisture content = (wet weight − dry weight)/wet weight × 100.

### 2.2.4 Feed efficiency calculation

Body weight and food consumption were monitored weekly. Feed efficiency was calculated as follows: feed efficiency (%) = increased body weight (g) / total food intake (g) × 100.

### 2.2.5 Fecal transit rate

The intestinal mobility rate was calculated using activated charcoal (100uL). The mice were starved overnight and were administered with activated charcoal 20 min before sacrifice. The length of the small intestine and the distance travelled by charcoal were measured. The transit rate was calculated as follows:

Intestinal transit rate (%) = charcoal travelled distance (cm) / small intestinal length (cm) × 100

### 2.2.6 Blood profiling

Immediately before sacrifice, blood was drawn from each mouse. Serum was collected by centrifuging blood at 500 ×*g* for 10 min, and analyzed by ChemOn Inc. (Republic of Korea) to test biomarkers associated with liver damage and overall energy homeostasis, such as aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), glucose (GLU), lactate dehydrogenase (LDH), and high-density lipoprotein (HDL). A clinical

biochemical analyzer (AU680, Beckman Coulter, Japan) was used to analyze these parameters.

### 2.2.7 Histopathological analysis

The small intestinal samples were sectioned and subjected to periodic acid–Schiff (PAS) and Alcian blue staining. Images of the histological samples were taken using an Olympus BX51 microscope. ImageJ software was used for histological analysis, and the mucus thickness layer was evaluated using stained intestine images (ten measurements per section per animal, four animals per group; mucus layers include mucosal and submucosal layers).

### 2.2.8 Gut microbiota analysis

Deoxyribonucleic acid (DNA) was extracted from the mouse fecal samples using the QIAamp PowerFecal Pro DNA Kit (QIAamp, USA). The V3-4 regions of the 16S rRNA gene were amplified using region-specific primers 341F and 806R. The MiSeq library was prepared according to the protocol provided by Illumina Inc. (USA). MiSeq sequencing was performed at Macrogen (Seoul, South Korea) according to the manufacturer's instructions. Mothur was used for MiSeq data analysis [72]. Briefly, the MiSeq output was first paired-end assembled and then aligned to Silva_v138 [73] using the align.seqs. Low-quality reads, such as ambiguous base calls and lengths not between 400 bp and 600 bp, were removed. The pre.cluster Mothur subroutine was applied to correct the machinery errors. Chimeric sequences were detected using VSEARCH [74] and removed using the remove.seqs Mothur subroutine. Taxonomy classification was performed using the Ribosomal Database Project (RDP) trainset18 [75] and classify.seqs Mothur subroutine. The mitochondrial or chloroplast sequences were removed. Operational taxonomic units (OTUs) were determined using the OptiClust [76], and were used to calculate the Bray-Curtis distances. Non-metric multidimensional scaling (NMDS) analysis was performed to estimate the microbial shifts among the groups. The differential abundance test was performed using the linear

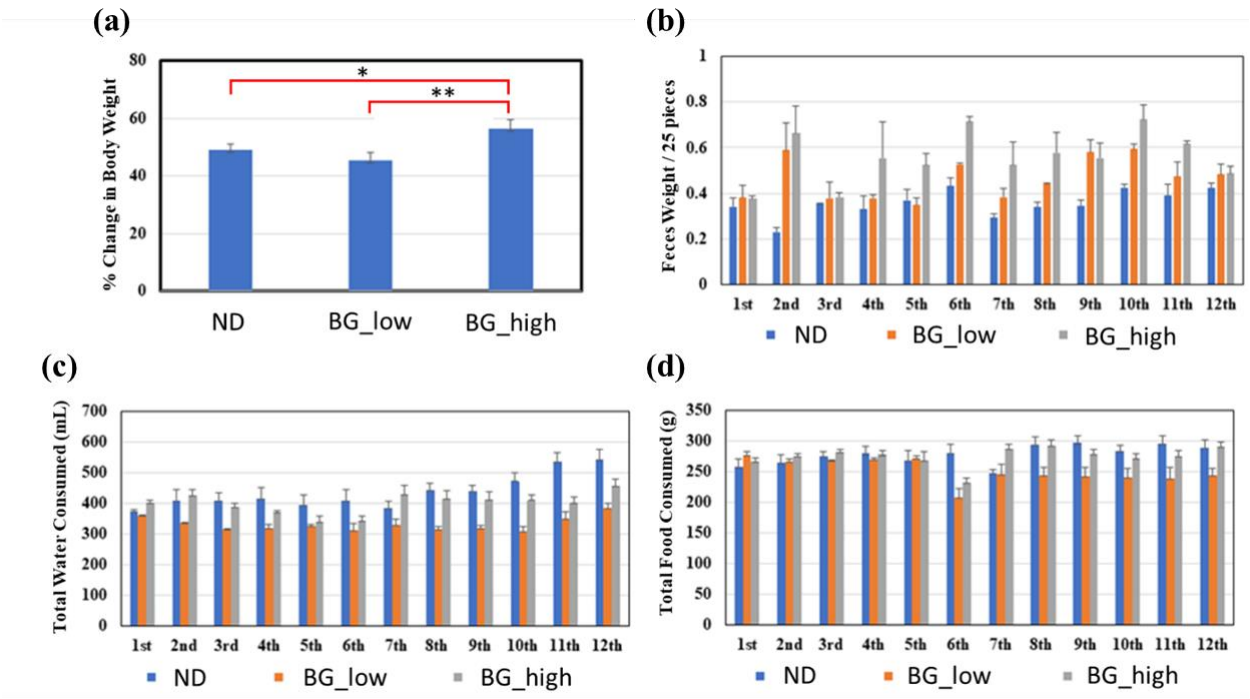
discriminant analysis effect size (LEfSe) [77] implemented in Mothur. PICRUSt2 was used to predict the intestinal metabolic activity [78]. STAMP [79] was used to identify differentially abundant metabolic activities with significance at P <0.05, adjusted with Benjamini-Hochber multiple test correction. Heatmaps were drawn using the heatmap.2 R package. Analysis of molecular variance (AMOVA) was used to estimate the significant differences in the NMDS plots.

### 2.2.9 Statistical analysis

Student's t-test was used to analyze significant differences in most experiments, and the difference with a p-value of less than 0.05, was considered significant. The data in this paper are presented as the mean ± SD.

## 2.3 Result

### 2.3.1 *Schizophyllum commune* β-glucan increased feed efficiency, fecal moist and changed fecal morphology



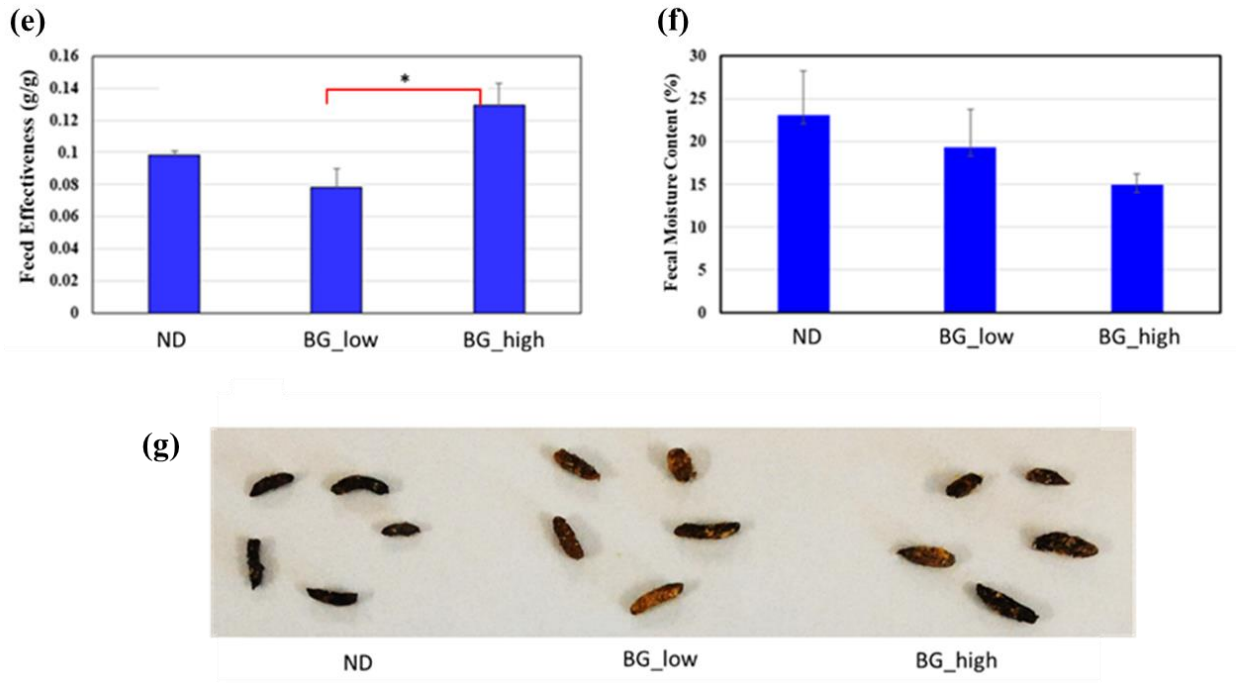



**Figure 2. 1 Effect of β-glucan on food and water consumption and fecal status.** After 12 weeks of feeding, several parameters are evaluated and compared among groups (n= 11 per group). (a) Body weight; (b) Fecal weight; (c) Total water consumed; (d) Total food consumed; (e) Feed effectiveness; (f) Fecal moisture content; (g) Fecal morphology. Statistically significant results are labeled as $P<0.05$ - *; $P<0.01$ - **.

After 12 weeks of treatment, the BG_high diet significantly increased body weight compared to the normal diet and BG_low treated groups (Fig. 2.1a). Both β-glucan treated groups showed high fecal weight throughout the 12-week feeding period, although not significant (Fig. 2.1b). Similar food intake is observed among the three groups, while mice in β-glucan treated groups tend to consume less water (Fig. 2.1c; 2.1d). Treatment of 5 g/kg of β-glucan markedly increased feed efficiency compared to 3 g/kg group (Fig. 2.1e). Concomitant with a decreased fecal moisture content in the β-glucan treated group, stools from β-glucan treated mice had larger size and lighter color compared to the normal diet and BG_low treated groups (Fig. 2.1f, 2.1g).

### 2.3.2 β-glucan proliferated goblet cells and increased mucin production

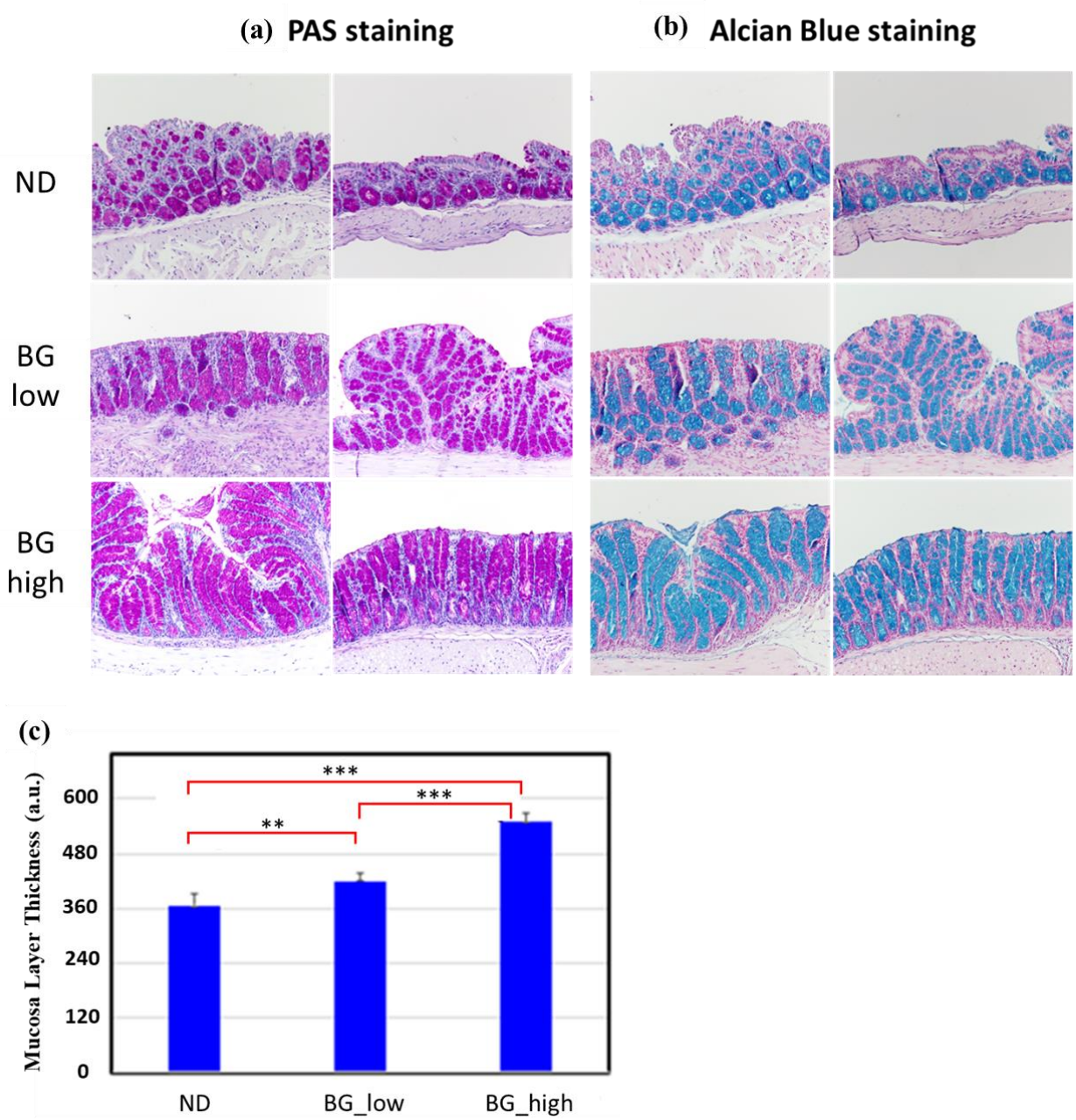



**Figure 2. 2 Effect of β-glucan on mucus thickness and goblet cell proliferation.** (a) The intracellular mucus of the goblet cells is stained using periodic acid–Schiff (PAS); (b) However, PAS could not stain the acidic mucus, which is stained by Alcian blue instead; (c) Mucosa layer thickness was measured by applying ImageJ software to analyze sectioned intestine images. Statistically significant results are labeled as P<0.05 - *; P<0.01 - **; P<0.001 - ***.

Both BG_low and BG_high diets increased mucus secretion, which is displayed by the pink color area in PAS staining images and blue color area in Alcian blue staining images (Fig. 2.2a; 2.2b). The thickness of the mucosa layer was measured using PAS and Alcian blue staining images (Fig. 2.2c). Administration of high concentrations of β-glucan resulted in a significantly thicker mucosal layer (Fig. 2.2c). PAS staining of intracellular mucus clearly showed that the increase in mucin production is due to the induced proliferation of the goblet cells by β-glucan.

### 2.3.3 β-glucan increased intestinal length and liver and lung weight

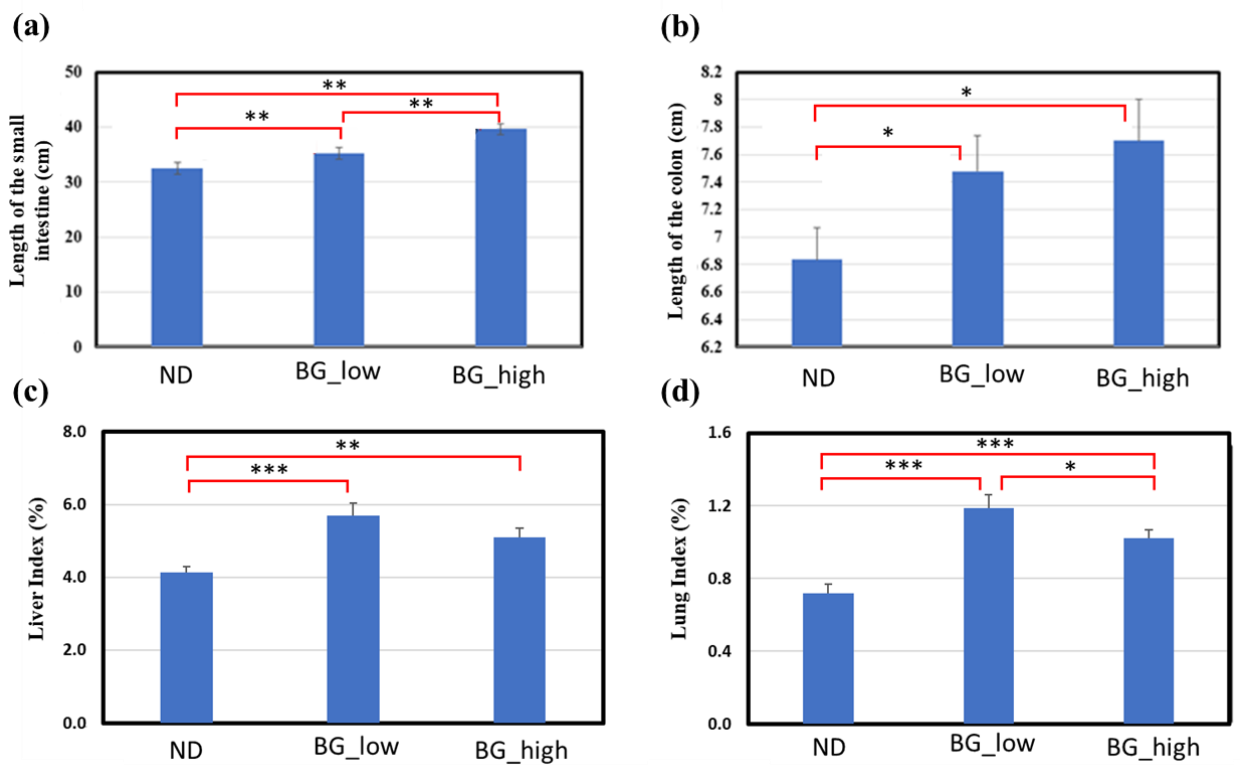



**Figure 2. 3 Effect of β-glucan on anatomic changes in C57BL/6J mice.** The small intestine and colon of the mice are measured by a ruler, while the animal liver and lung are weighed. (a) small intestine length; (b) colon length; (c) Liver index; (d) Lung index. Statistically significant results are labeled as $P<0.05$ - *; $P<0.01$ - **; $P<0.001$ - ***.

β-glucan lengthened the small intestine and colon in the animals (Fig. 2.3a, 2.3b). In addition, consumption of β-glucan increased the weight of the liver and lungs (Fig. 2.3c, 2.3d).

Measurement of intestinal motility using activated carbon showed that β-glucan did not improve the intestinal transit rate (data not shown); however, our previous study using a high-fat diet as a control, HFD supplemented with β-glucan, showed a significant increase in the gastrointestinal transit rate [49]. Blood profiling assessment demonstrated that β-glucan significantly decreased the level of liver injury biomarkers, such as AST, ALT, and ALP, in a dose-dependent manner (Table 2.1). In addition, glucose levels were significantly low in the BG-treated groups, especially in the BG_high group. LDH and HDL serum levels were upregulated in the β-glucan-treated group, with high levels in BG_high-treated mice (Table 2.1).

**Table 2. 1 Blood profiling for serum markers of liver injury and cardiovascular disease.**

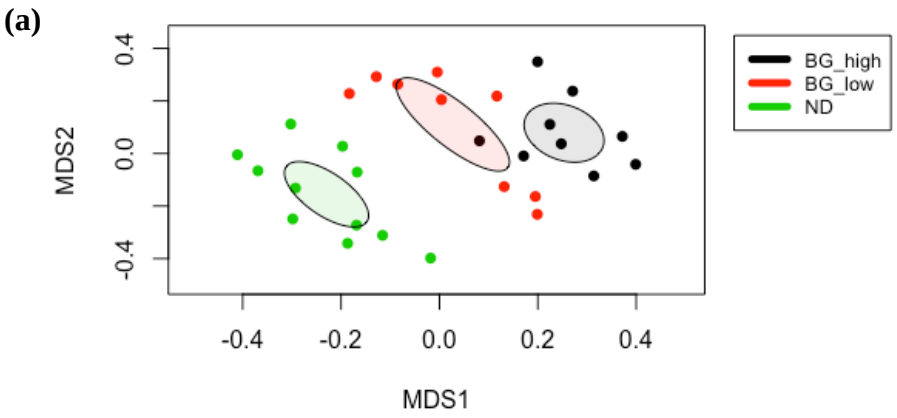
| Blood profiling | ND | BG_low | BG_high |
|---|---|---|---|
| AST (U/L) | 166.2 ± 63.0 | 164.6 ± 58.3 | 149.7 ± 48.0 |
| ALT (U/L) | 39.7 ± 14.0 | 36.6 ± 4.3 | 34.6 ± 2.1 |
| ALP (U/L) | 82.8 ± 13.3 | 81.1 ± 6.2 | 80.4 ± 13.0 |
| GLU (mg/dL) | 173.2 ± 27.6 | 121.9 ± 26.6* | 107.5 ± 45.4* |
| HDL (mg/dL) | 66.7 ± 3.3 | 83.5 ± 6.9* | 83.0 ± 5.3* |
| LDL (mg/dL) | 24.3 ± 6 | 24.2 ± 1.1 | 22 ± 3 |

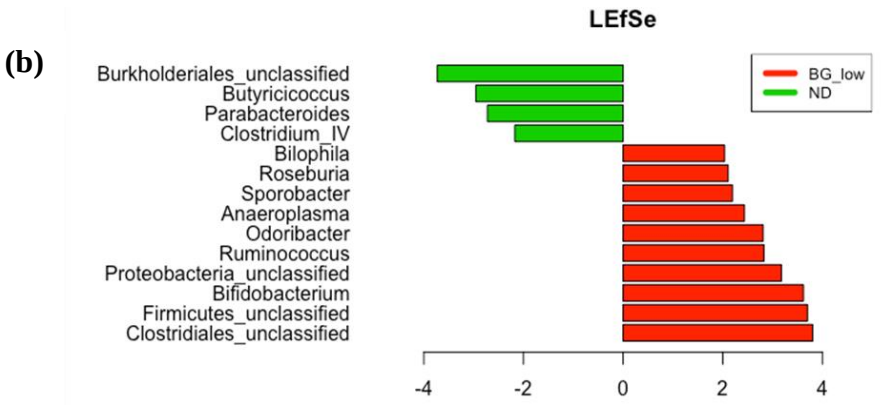
* compared to normal diet: $P<0.05$ - *.

### 2.3.4 β-glucan decreased harmful strains while selectively fostered SCFAs-producing strains

In this study, 2,043,883 clean reads were obtained and 10,000 reads per sample were used for analyses. Alpha-diversity analysis showed no difference between dietary groups, neither for species richness nor evenness (Fig. S2.1). Taxonomic composition analysis showed a clear difference between ND and BG groups at the phylum level, where a high abundance of *Verrucomicrobia* is observed (Fig. S2.2A). Distinct taxonomic compositions were not observed at the family and genus levels (Fig. S2.2B, and C). However, beta-diversity analysis

showed significant microbiota differences among the groups (P<0.01) (Fig. 2.4a). Differential abundance tests at the genus level showed that Butyricicoccus, Parabacteroides, and Clostridium_IV were decreased by β-glucan treatment (Fig. 2.4b, 2.4c). The high concentration of β-glucan further decreased the abundance of the genus *Akkermansia*. In contrast, β-glucan treatment increased the abundance of *Bifidobacterium*, *Ruminococcus*, and *Roseburia*.



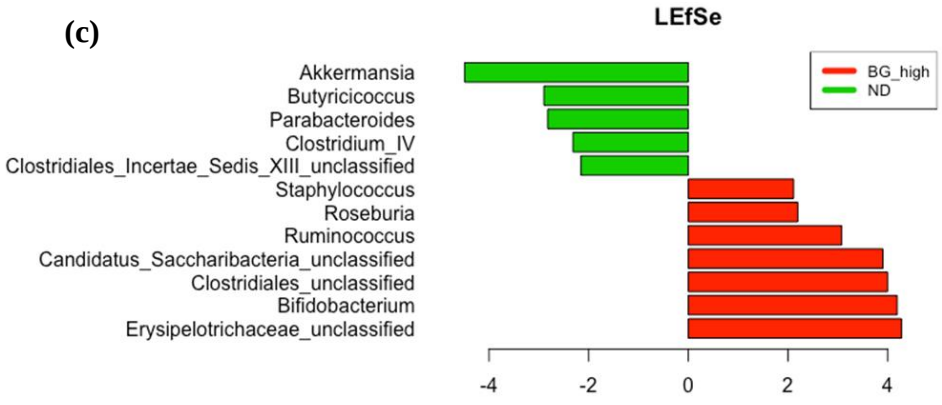



**Figure 2. 4 Effect of β-glucan on microbiota modification in C57BL/6J mice.** (a) Beta-diversity comparison among the dietary groups through non-multidimensional scaling analysis; (b) Differentially abundant genera between ND and BG_low; (c) ND and BG_high.

Differentially abundant genera between ND and Metabolic activities predicted by the PICRUSt2 showed that high concentrations of β-glucan significantly enriched and depleted 11 and 8 metabolisms, respectively. However, only four metabolic pathways were increased, and two pathways were decreased by low concentrations of β-glucan. However, hexitol degradation is the only metabolic activity with significant differential abundance between low and high concentrations of BG (Fig. S2.3). Enriched metabolism due to high concentrations of β-glucan is mostly related to nucleotide degradation and amino acid biosynthesis, while depleted metabolism is related to menaquinol biosynthesis (Fig. S2.3; Table 2.2).

**Table 2. 2 Metabolic activity differences between ND and BG_high (Effect size >0.1)**

| Enriched group | Pathways | Descriptions | Effect size difference |
|---|---|---|---|
| BG_high | SALVADEHYPOX-PWY | adenosine nucleotides degradation II | 0.24 |
| BG_high | PWY-6608 | guanosine nucleotides degradation III | 0.22 |
| BG_high | P161-PWY | acetylene degradation | 0.15 |
| BG_high | PWY-1861 | formaldehyde assimilation II (RuMP Cycle) | 0.15 |
| BG_high | PWY-5347 | superpathway of L-methionine biosynthesis (transsulfuration) | 0.13 |
| BG_high | PWY-6353 | purine nucleotides degradation II (aerobic) | 0.13 |
| BG_high | P4-PWY | superpathway of L-lysine, L-threonine and L-methionine biosynthesis I | 0.12 |
| BG_high | PWY-6901 | superpathway of glucose and xylose degradation | 0.12 |
| BG_high | PWY0-781 | aspartate superpathway | 0.11 |
| BG_high | MET-SAM-PWY | superpathway of S-adenosyl-L-methionine biosynthesis | 0.11 |
| BG_high | RUMP-PWY | formaldehyde oxidation I | 0.10 |
| ND | PWY-5838 | superpathway of menaquinol-8 biosynthesis I | 0.17 |

| | | | |
|---|---|---|---|
| ND | PWY-5840 | superpathway of menaquinol-7 biosynthesis | 0.16 |
| ND | PWY-5899 | superpathway of menaquinol-13 biosynthesis | 0.16 |
| ND | PWY-5897 | superpathway of menaquinol-11 biosynthesis | 0.16 |
| ND | PWY-7315 | dTDP-N-acetylthomosamine biosynthesis | 0.13 |
| ND | PWY-5861 | superpathway of demethylmenaquinol-8 biosynthesis | 0.13 |
| ND | FASYN-ELONG-PWY | fatty acid elongation -- saturated | 0.11 |
| ND | P108-PWY | pyruvate fermentation to propanoate I | 0.10 |

## 2.4 Discussion

### 2.4.1 β-glucan showed protective effect against constipation and metabolic disorders

After 12 weeks of treatment, 3 g/kg of *Schizophyllum commune-derived* β-glucan did not increase the animals' body weight; however, consumption of 5 g/kg β-glucan resulted in a significant increase in body weight and feed efficiency. Although it is widely known that consumption of fiber is related to lowered body weight [80], in the present study, consumption of *Schizophyllum commune* β-glucan increased not only the body weight, but also the lung and liver index significantly. An increase in body weight usually increases the risk of obesity and dysfunction of overall nutrition metabolism, followed by the upregulation of specific serum markers, such as AST, ALT, ALP, and cholesterol. Despite the increases in body weight and liver weight, biomarkers for liver injury, such as AST, ALT, and ALP were downregulated in both β-glucan-treated groups, suggesting that β-glucan has the potential to reduce the risk of liver damage. These results suggest a healthy weight gain by β-glucan and the protective effect of β-glucan against liver injury, which was also reported in a high-fat diet-induced obesity study [49]. Better nutrition metabolism and water absorption could explain the weight gain since our concurrent study showed that β-glucan successfully decreased HFD-induced weight gain and metabolic distress [49]. In addition, a significant decrease in the glucose level was observed, suggesting a protective effect against diabetes. HDL cholesterol is known as good cholesterol that has effect on cholesterol clearance, thus preventing atherosclerosis [81, 82], which was increased by β-glucan treatment. In contrast,

low-density lipoprotein (LDL) cholesterol, also called bad cholesterol, was slightly decreased at high doses of β-glucan.

Together with an increase in BW, mice treated with β-glucan had high fecal weight, large size, and light-colored feces; however, the fecal transit rate was not increased by β-glucan treatment. In comparison with our previous study using an HFD-fed model, mice in both normal and obese conditions showed bulked feces during β-glucan treatment, and the significantly increased transit rate was only observed in the obese mice [49]. In addition, mice that consumed β-glucan tended to drink less water, which resulted in a low moisture content. Controversially, it has been reported that statements such as bulking feces by fiber treatment can result in better bowel movement are illogical [83], however, a meta-analysis strongly stated that dietary fiber is a potential therapeutic compound that could be used to ameliorate constipation [84]. The interesting controversy about the effect of fiber on constipation symptoms raised the need to investigate the effect of a certain fiber on the host in both healthy and diseased conditions. Our results suggested that the effect of β-glucan could be varied due to many aspects, such as types of hosts (human or mouse), host conditions (healthy or obesity), dietary habits (normal diet and high-fat diet with low or high fiber), and most importantly the amount of water consumption. The results showed that β-glucan treatment under normal conditions did not lead to a significant decrease in body weight or intestinal transit rate, which was observed in obese conditions [49]. However, treatment with β-glucan successfully decreased obesity followed serum factors, induced better water absorption, bulked feces, proliferated goblet cell populations, and increased mucin production even under normal conditions, which implies a protective effect against obesity, diabetes, and constipation.

### 2.4.2 β-glucan improved intestinal overall healthiness

Administration of β-glucan increased mucin production, suggesting an important role of β-glucan in regulating the gut permeability and providing a lubricant that improves stool transition, implying the ability of β-glucan to relieve constipation. SCFAs, a product of fiber fermentation produced by gut microbiota, are important nutrients for intestinal epithelial cells that improve intestinal integrity and enhance mucin production [49, 53]. Increased production of SCFAs in mice was observed following the administration of β-glucan [54]. The increased mucus secretion mainly results from an increased number of mucus-secreting goblet cells. Our previous study clearly demonstrated that treatment with *Schizophyllum commune*-derived β-glucan increased proliferation and differentiation markers of intestinal cells such as cyclin D1, cyclin E, K14, p21, and p27 [49]. In this study, treatment with β-glucan significantly increased the length of the small intestine and colon. A longer gastrointestinal tract allows more absorption of water, which results in a lower moisture content in stools. In addition, treatment of high-fat diet incorporated with β-glucan on DSS-induced colitis showed increased expression level of tight-junction proteins (claudin-1) and adhesion proteins (E-cad) compared to consumption of a high-fat diet alone [49, 54]. The population of healthy epithelial cells and increased expression of tight-junction proteins are essential for gut permeability and intestinal barrier regulation, which prevents transmural infiltration of bacteria and microbial lipopolysaccharide (LPS), causing inflammation of the intestine, liver, and adipose tissue [52, 85]. These results suggest that β-glucan could protect the gut from IBD and IBD-induced non-alcoholic fatty liver disease (NAFLD), which was proven in our previous study on the effect of β-glucan on ulcerative colitis [54].

### 2.4.3 *Schizophyllum commune* β-glucan shifted gut microbiota to the healthy population

It has been proven that SCFAs are important metabolites produced via fiber fermentation by the gut microbiota, which fuels the intestinal epithelial cells, are increased under treatment with *Schizophyllum commune* β-glucan [54]. In the present study, consumption of β-glucan

dose-dependently supports beneficial gut microbiota, such as *Anaerostipes*, *Roseburia*, and *Coprobacillus*, which play roles in fermenting glucose to lactate and catabolizing lactate to produce butyrate [86-88]. In addition, the population of obesity-associated bacteria, such as *Parabacteroides* and *Lactococcus* is decreased by treatment with β-glucan, suggesting an anti-obesity effect of this compound [49]. β glucan decreases many SCFA-producing bacteria, such as *Butyricicoccus* and *Parabacteroides*, and fiber degraders, such as *Clostridium*_IV and *Akkermansia*, while significantly increases other SCFA-producing bacteria and probiotics, such as *Roseburia*, *Ruminococcus*, and *Bifidobacteria*. Therefore, it is likely that the abundance of these species changed due to the competition over nutrient availability in the gut; that is, β-glucan supplementation selectively increasess some species of SCFA-producing bacteria**.** β-glucan consumption suppresses *the Akkermansia* population, in which the strain is known to degrade intestinal mucin and lead to stool drying and hard defecation [89]. Comparison of metabolic activities indicated that higher concentrations of β-glucan affected intestinal/bacterial metabolic activities in the gut stronger than lower concentrations of β-glucan, although the two groups showed relatively similar metabolic activities. β-glucan increases degradation of purine nucleotides, such as adenosine and guanosine, the metabolism of which has been reported to increase the immune system in humans [90]. In contrast, β-glucan decreased menaquinol (reduced vitamin K2) biosynthesis, in which menaquinol-8 (PWY-5838) was reported to be positively associated with type 2 diabetes [91]. In conclusion, *Schizophyllum commune* β-glucan improved intestinal health by enhancing water absorption, proliferating epithelial cells, and increasing lubricating mucin production, which is essential for the treatment of constipation. Moreover, administration of β-glucan prevents the risk of conditions, such as obesity, diabetes, atherosclerosis, and intestinal inflammation diseases that are observed with reduction in serum glucose levels and biomarkers associated with liver injury, while upregulating HDL. Modification of the gut

microbiota toward a healthy community due to the compound plays a major role in obtaining these benefits. The study consolidated the protective effect of *Schizophyllum commune* against various diseases in basal state; however, further studies using specific models are needed to comprehensibly conclude the effect of *Schizophyllum commune* β-glucan on these conditions.

### 2.5 Supplemental Materials

**Fig. S2.1.** Alpha-diversity comparison among dietary groups for species richness (a) and evenness (b) estimated by Chao I and Shannon, respectively.

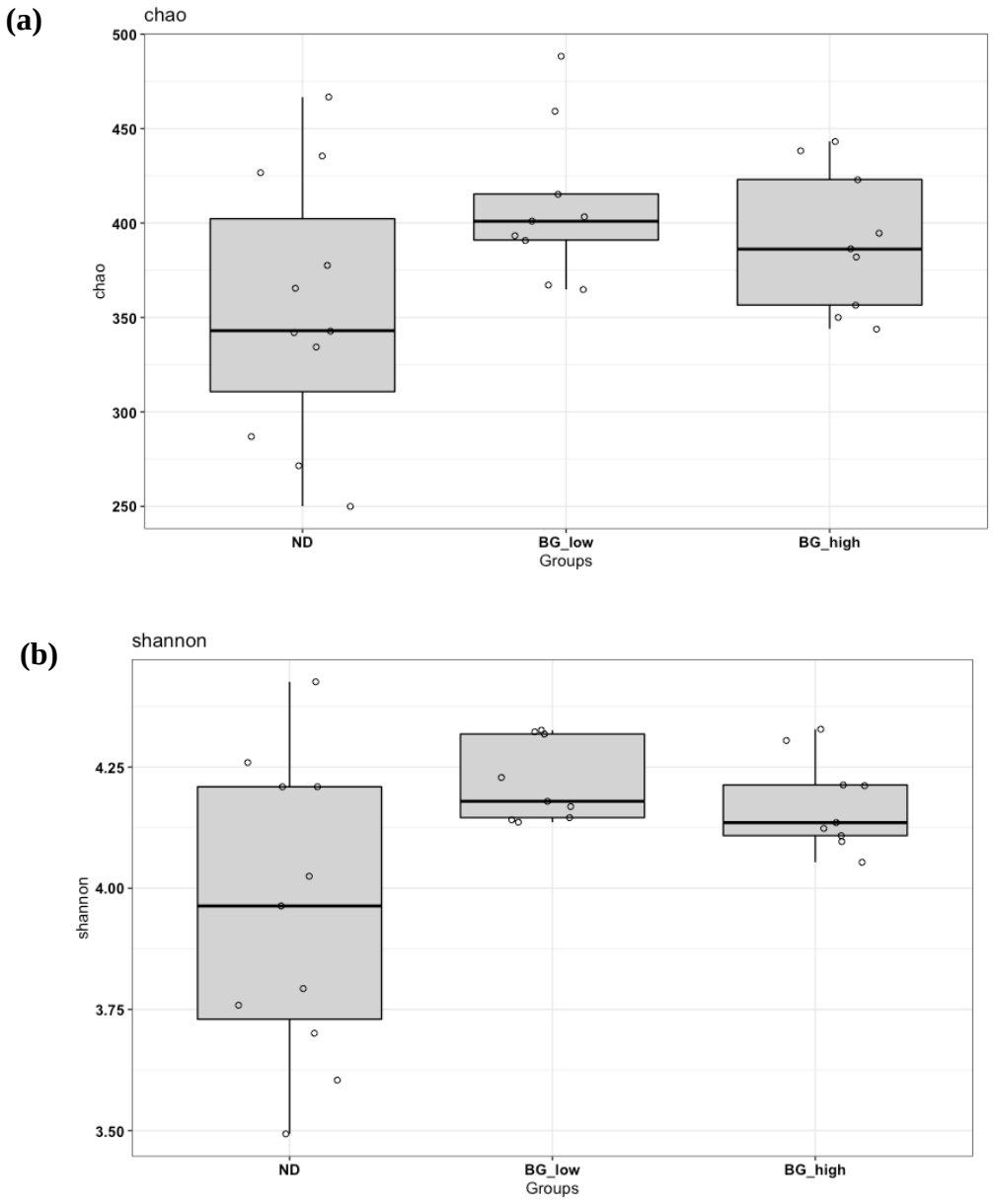
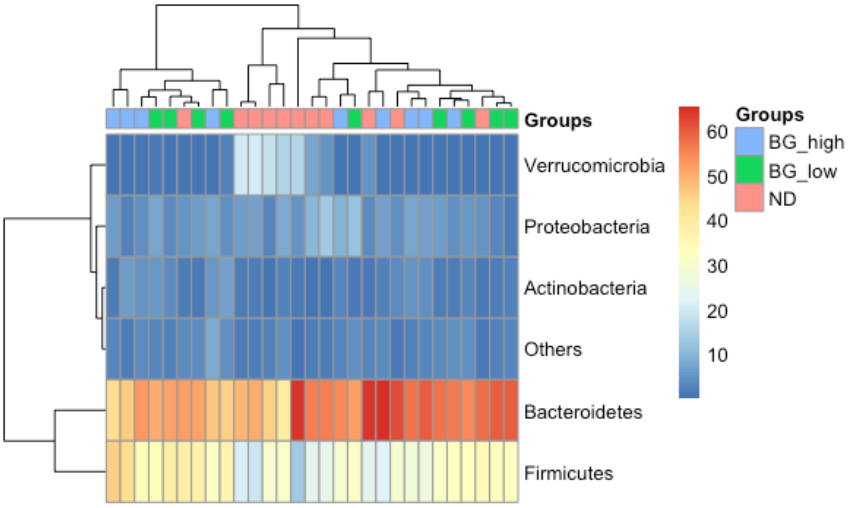
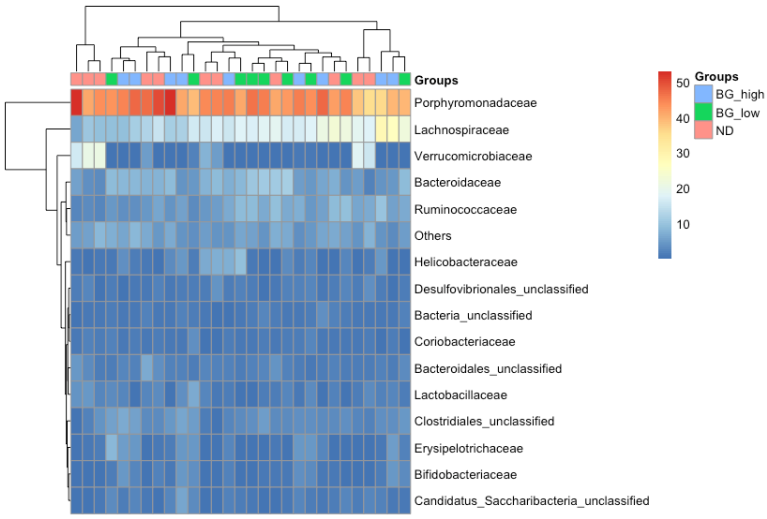


**Fig. S2.2.** Taxonomic composition analysis among dietary groups at the phylum (a), family (b), and genus levels (c).

**(a)**




**(b)**



**(c)**

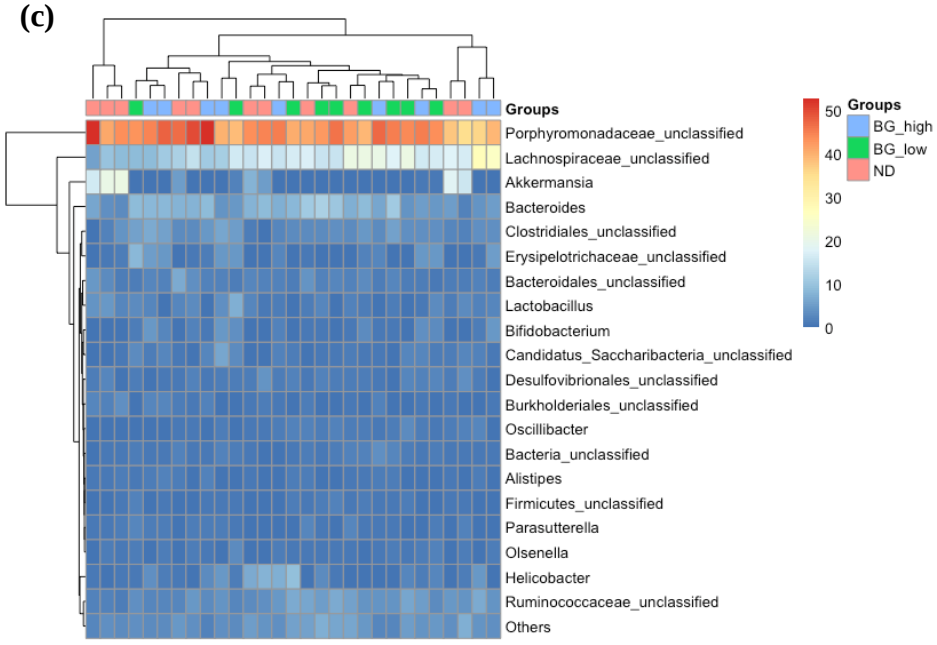
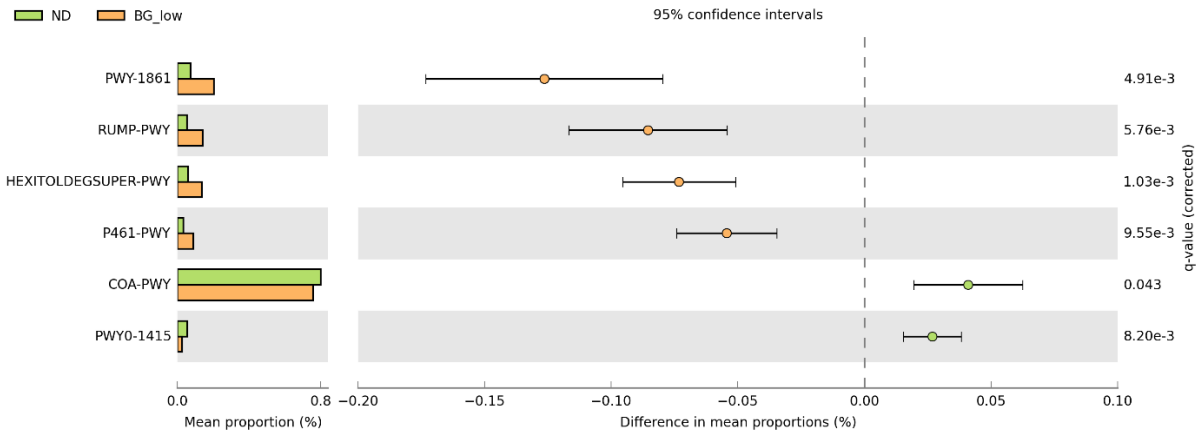



**Fig. S2.3.** Metabolic activity differences between normal diet and BG_low groups (a), BG_low and BG_high groups (b), and ND and BG_high groups (c).

**(a)**




PWY-1861: Formaldehyde assimilation II (RuMP Cycle)

RUMP-PWY: Formaldehyde oxidation I

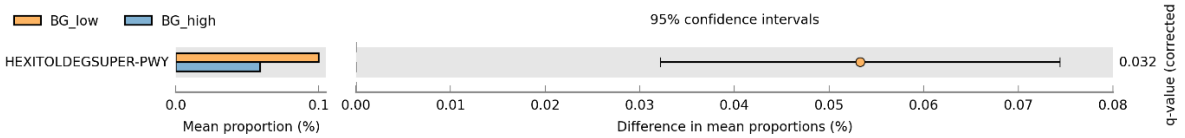
HEXITOLDEGSUPER-PWY: Superpathway of hexitol degradation (bacteria)

P461-PWY: Hexitol fermentation to lactate, formate, ethanol and acetate

COA-PWY: Coenzyme A biosynthesis I

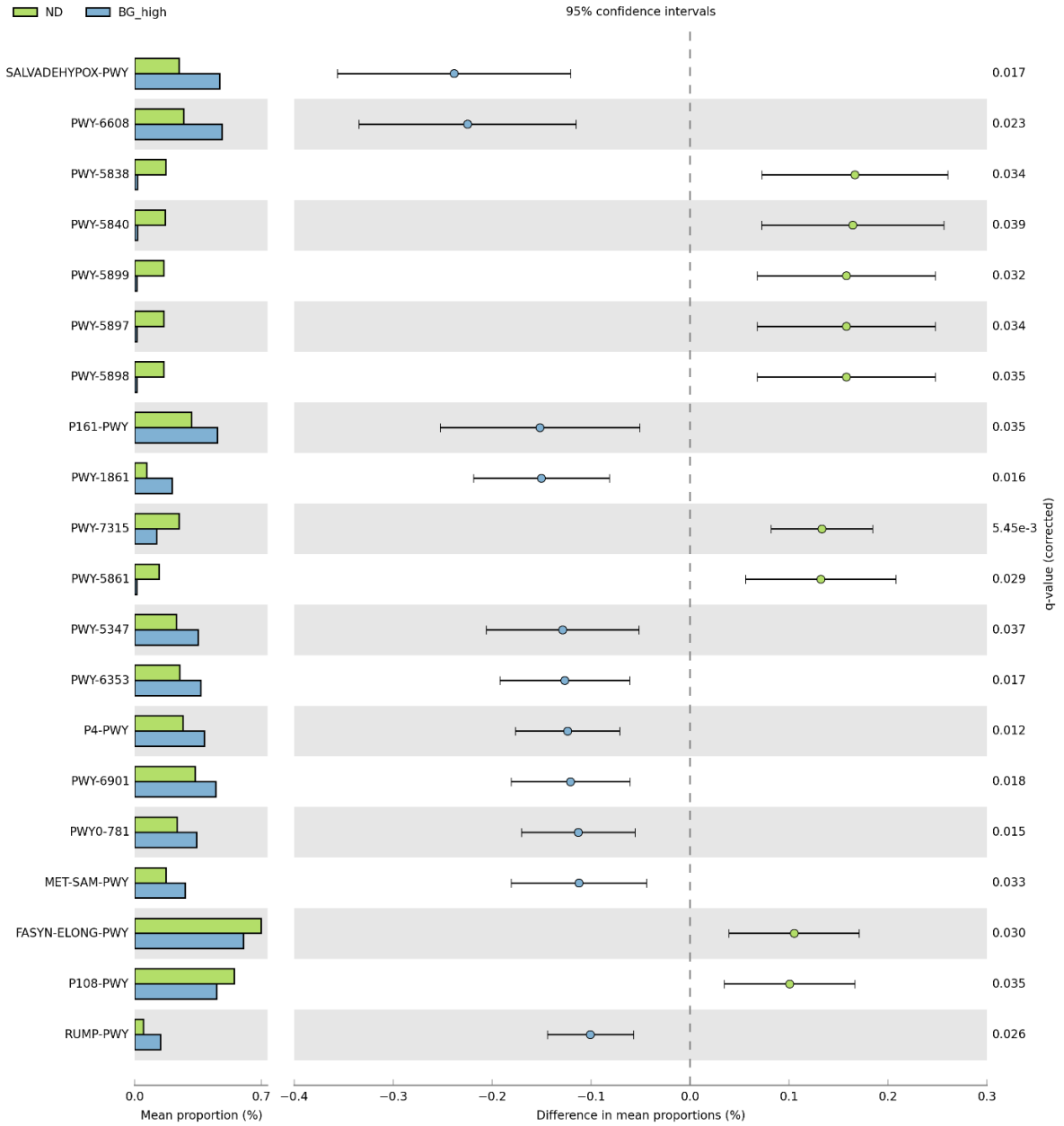
PWY0-1415: Superpathway of heme biosynthesis from uroporphyrinogen-III

**(b)**




HEXITOLDEGSUPER-PWY: Superpathway of hexitol degradation (bacteria)

**(c)**




* Descriptions of these pathways are shown in Table 1.

## CHAPTER 3. Effects of β-glucan with and without co-treated probiotics on obesity-associated colitis and hepatic manifestations

### 3.1 Abstract

Probiotics and prebiotics are commonly used to improve the gut microbiota. Since prebiotics can support the growth of probiotics, co-administration of these is called synbiotics. It has been demonstrated that obesity-induced gut dysbiosis can worsen inflammatory bowel disease symptoms. This study evaluated how modulation of gut microbiota with *Schizophyllum commune*-derived β-glucan (BG), probiotics (PRO), and synbiotics containing both BG and PRO (SYN) could improve the symptoms of obesity-associated colitis and hepatic manifestation. Mice were fed a normal diet (ND), high-fat diet (HFD), and HFD with different additives (BG, PRO, and SYN) for 12 weeks, followed by 5 days of colitis induction. Mice were sacrificed before and after colitis induction. During the experiment, body weight, food and water consumption, and rectal bleeding were monitored. Proteins from the colon were subjected to western blotting, and serum biomarkers such as alanine transaminase, alkaline phosphatase, triglycerides, and total cholesterol were analyzed. Colon and liver samples were sectioned for histological analysis. The fecal microbiota was analyzed based on partial 16S rRNA gene sequences. Although BG and PRO secured intestinal tight junctions, these two treatments did not modulate inflammatory cell infiltration and inflammatory markers (i.e., IL-6 and TNF-α). In contrast, SYN demonstrated stronger and broader effects in reducing colonic inflammation. While BG treatment increased the abundance of indigenous *Lactobacillus*, PRO treatment decreased bacterial diversity by suppressing the growth of several species of bacteria. SYN treatment groups, however, supported the growth of both indigenous and supplemented bacteria while maintaining bacterial diversity. Obesity-associated colitis can be improved by modulating gut bacteria

with β-glucan and probiotics. The co-administration of both outperformed β-glucan and probiotic treatment alone by fostering both indigenous and supplemented probiotic strains.

### 3.2 Methods

#### 3.2.1 Animals and Animal Care

Seven-week-old male C57BL/6J mice were used in this study. Animal experiments were performed in accordance with the guidelines of the Animal Care and Use Committee approved by the Jeju National University (ACUC No. 2018-0018). Immediately after arrival, the mice were acclimatized to the experimental conditions with a 12/12 h light/dark cycle and 55 ± 15% humidity at 23 ± 2 °C. After seven days of acclimatization, the mice (average body weight: 22.4 g) were divided into five groups (seven mice per cage) according to the following diets: normal diet (ND), high-fat diet (HFD), HFD with β-glucan (BG), HFD with probiotics (PRO), and HFD with synbiotics (SYN); n = 7 for the ND group and n=14 for the other groups. The synbiotics is a co-administration of β-glucan and probiotics used in this study. The HFD was prepared by adding 45% kcal of lard to the ND. β-glucan-, probiotic-, and synbiotic-complemented HFD were processed by directly adding β-glucan, probiotics, and synbiotics to the sterilized HFD ingredients and stored at -20 oC until used. Purified and pharmacologically active (1,3)/(1,6)-β-glucan extracted from Schizophyllum commune was provided by Quegen Biotech Co. Ltd., Republic of Korea, and its purity was approximately 95%. The soluble β-1,6 branching β-1,3-glucan has a molecular weight of approximately 1.78-1.79 × 106 Da. The BG diet was prepared by adding 3 g of β-glucan to 1 kg of HFD, the amount of which was proven to show anti-obesity effects in mice fed with HFD in our previous study [22]. The probiotic composition comprised a mixture (VSL#3) of S. thermophilus, B. lactis, B. breve, L. helveticus, L. bulgaricus, L. plantarum Q180, L. plantarum K50, and Lactobacillus rhamnosus, LGG® (CHr. Hansen) provided by CKD BiO, Seoul, Republic of Korea, was added to the HFD as shown in Table 3.1.

**Table 3. 1 Probiotic concentrations used in the study.**

| Bacterial strains | Bacterial loads (CFU/g) |
|---|---|
| *S. thermophilus* CKDB027 | 6.4 E+11 |
| *B. lactis* CKDB005 | 1.3 E+12 |
| *B. breve* CKDB002 | 7.1 E+11 |
| *L. helveticus* CKDB001 | 1.5 E+11 |
| *L. bulgaricus* CKDB001 | 1.0 E+11 |
| *L. phantarum* Q180 | 1.0 E+11 |
| *L. phantarum* K50 | 1.0 E+11 |
| LGG (CHr. Hansen) | 3.0 E+11 |

The probiotic mixture was added to the high-fat diet at a concentration of 15 g/kg according to the manufacturer's instruction. The synbiotic-supplemented diet was prepared by adding probiotics to the BG diet according to Table 3.2.

**Table 3. 2 Composition and calories of diets used in this study.**

| Treatment type | AIN 93M purified control diet (ND) | | AIN 93M modified high-fat diet (45% Kcal) (HFD) | | AIN 93M modified high-fat diet (45% Kcal) with 3g/kg of β-glucan (BG) | | AIN 93M modified high-fat diet (45% Kcal) with probiotics (PRO) | | AIN 93M modified high-fat diet (45% Kcal) with 3g/kg of β-glucan and probiotics (SYN) | |
|---|---|---|---|---|---|---|---|---|---|---|
| Ingredient | Gm* | Kcal | Gm | Kcal | Gm | Kcal | Gm | Kcal | Gm | Kcal |
| Casein, lactic | 140 | 560 | 140 | 560 | 140 | 560 | 140 | 560 | 140 | 560 |
| L-Cystine | 1.8 | 7.2 | 1.8 | 7.2 | 1.8 | 7.2 | 1.8 | 7.2 | 1.8 | 7.2 |
| Corn starch | 465.7 | 1863 | 263 | 1052 | 263 | 1052 | 263 | 1052 | 263 | 1052 |
| Sucrose | 100 | 400 | 100 | 400 | 100 | 400 | 100 | 400 | 100 | 400 |
| Maltodextrin | 155 | 620 | 155 | 620 | 155 | 620 | 155 | 620 | 155 | 620 |
| Cellulose | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 | 50 | 0 |
| Soy bean oil | 40 | 360 | 43 | 387 | 43 | 387 | 43 | 387 | 43 | 387 |
| Lard | - | - | 200 | 1800 | 200 | 1800 | 200 | 1800 | 200 | 1800 |
| β-glucan | - | - | - | - | 3 | 0 | - | - | 3 | 0 |
| Probiotic mixture | - | - | - | - | - | - | 15 | 0 | 15 | 0 |
| *t*-Butylhydroquinone | 0.008 | 0 | 0.008 | 0 | 0.008 | 0 | 0.008 | 0 | 0.008 | 0 |
| AIN-93M mineral Mix | 35 | 0 | 35 | 0 | 35 | 0 | 35 | 0 | 35 | 0 |
| AIN-93M vitamin Mix | 10 | 40 | 10 | 40 | 10 | 40 | 10 | 40 | 10 | 40 |
| Choline bitartrate | 2.5 | 0 | 2.5 | 0 | 2.5 | 0 | 2.5 | 0 | 2.5 | 0 |
| Total | 1000 | 3850.2 | 1000 | 4866.2 | 1003 | 4866.2 | 1015 | 4866.2 | 1018 | 4866.2 |

*weight in gram

During the experiment, the mice were housed with free access to food and water with wood chips as animal bedding. Bedding and food were replaced daily, while food consumption was measured once a week. Colitis was induced by oral administration of 3% DSS (MP Biomedical) for five days and animals were sacrificed at the end of the experiment (Fig. 3.1, Fig. S3.1). Animal euthanasia was conducted using diethyl ether as an inhalant agent purchased from DaeJung Chemicals & Metals Co. Ltd., Republic of Korea. The feces, colon, liver, and cecum were obtained from all animals and immediately stored at -70 °C to preserve their integrity.

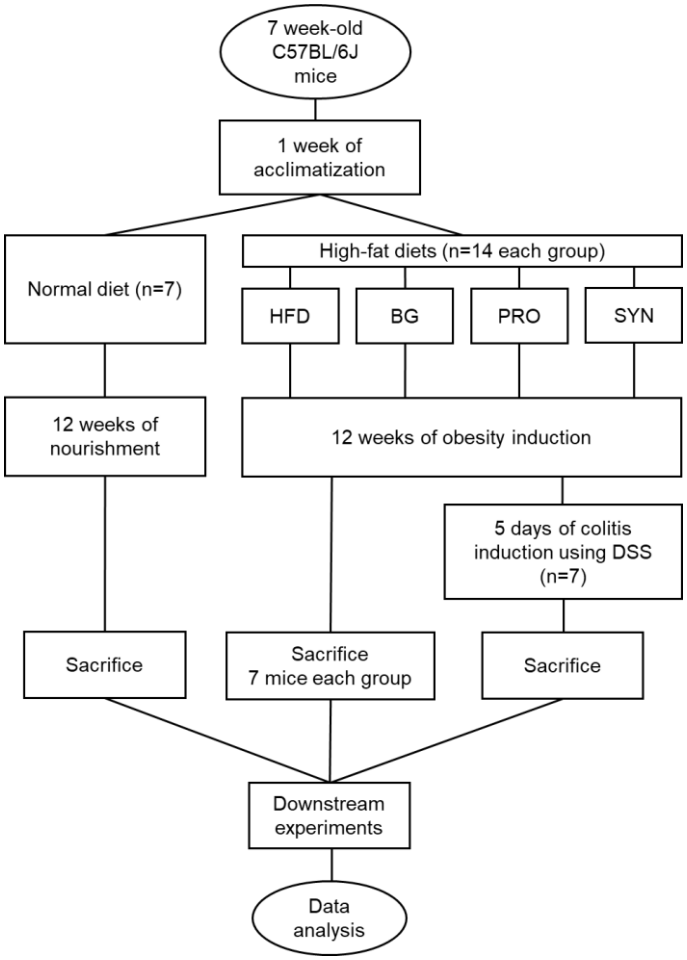



**Figure 3. 1 Schematic showing experimental procedure.** The mice underwent 12 weeks of obesity induction with different diets and five days of colitis induction. Seven mice in each

group were sacrificed at week 12 and the rest were sacrificed after 5 days following colitis induction. Fresh tissues were collected for downstream experiments.

### 3.2.2 Feed Conversion Ratio

The feed conversion ratio was calculated based on the change in mice body weight and total consumption of food after 12 weeks using the following equation:

Feed conversion ratio = total food intake (g) / Δ biomass (g)

### 3.2.3 Anatomical Analysis

Seven mice in each group were sacrificed at the end of the 12 week obesity-induction period. The remaining mice underwent 5 days of colitis induction and were then sacrificed. Before sacrifice, the mice were weighed, and their fecal samples were collected for microbiota analysis. Bleeding severity was assessed based on the amount of blood observed on the surface of individual stools and scored as (3) for high severity, (2) for medium severity, (1) for mild severity, and (0) for no bleeding. After sacrifice, fresh liver tissues were quickly washed twice with phosphate-buffered saline (PBS) and distilled water to remove blood, fat residue, and dust before being weighed. Fresh colon tissues were collected by cutting the gastrointestinal tract from the end of the cecum to the rectum. Subsequently, the colon tissues were washed twice and their lengths were measured.

### 3.2.4 Immunoblotting

Equivalent amounts of colon samples from the HFD, HFD+DSS, BG+DSS, PRO+DSS, and SYN+DSS groups (n = 7 in each group) were lysed and homogenized in T-PER buffer (Thermo Fisher Scientific, Rockford, USA). Protein samples were collected and stored at -20°C. The same amount of protein in each sample (20 μg) was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and detected using western blotting. ImageJ software was used to evaluate the intensity of the protein bands. The following primary antibodies were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA,

USA): anti-E-cadherin (CS 3195S), anti-COX-2 (H-3) (SC-376861), anti-IL-6 (SC-32296), anti-TNF-α (SC-52746), anti-Claudin-1 (sc-166338), and anti-GAPDH (SC-25778)] antibodies. The anti-NOX4 antibody was purchased from Proteintech (14347-1-AP). Secondary antibodies (rabbit and mouse) were procured from Koma Biotech (Seoul, Republic of Korea).

### 3.2.5 Blood Profiling

Blood samples were drawn after right ventricular cardiac puncture and centrifuged at 500 ×$g$ for 10 min, and serum samples were analyzed by ChemOn Inc. (Republic of Korea) to evaluate circulating biomarkers related to liver/kidney injuries, including alanine transaminase (ALT), alkaline phosphatase (ALP), triglycerides (TG), and total cholesterol (Total-Cho). A clinical biochemical analyzer (AU680, Beckman Coulter, Japan) was used to evaluate these parameters.

### 3.2.6 Histopathological Analysis

Hematoxylin and eosin (H&E) staining was performed on colon and liver tissues. H&E-stained colon images were acquired at different sites at three magnifications using an Olympus BX51 microscope. Microscopic damage to the colon caused by DSS was determined by the infiltration of inflammatory cells, which were evaluated by a pathological expert. H&E-stained liver images were subjected to semi-quantitative examinations for steatosis, and lobular and portal/periportal activity. Lobular and portal/periportal activity was scored using the Batts and Ludwig scoring system, while Brunt's scale was applied for liver steatosis (Table S3.1) [92, 93]. ImageJ software was used to measure droplets and cell surface area. The degree of thrombosis was determined by the number of veins and sinusoids congested with red blood cells (two images per individual; seven animals per group). All histological samples were blinded prior to the evaluation.

### 3.2.7 16S rRNA Gene Amplicon Sequencing

DNA was extracted from mouse fecal samples using the QIAamp PowerFecal Pro DNA Kit (QIAamp, USA). V3-4 regions of 16S rRNA gene were amplified using region-specific primers (341F-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG and 806R-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCT AATCC). The MiSeq library was prepared according to the protocol provided by Illumina Inc. (USA). MiSeq sequencing was performed at Macrogen (Seoul, South Korea) according to the manufacturer's instructions.

**3.2.8 Quantification of Short-Chain Fatty Acids**

Frozen fecal samples were thawed on ice and homogenized with absolute methanol in a defined ratio (1:4), by vortexing them for 2 mins. The pH of the homogenized mixture was maintained to 2 by adding 4N HCL, and solution was incubated for 10 min, with frequent vortexing in between, then centrifuged at 10,000 rpm for 5 min at 4 °C. The supernatant was collected using 1 ml syringe, and then filtered through 0.45 µm pore sized filter into a sterile vial. The three major SCFAs were quantified by gas chromatography (QP2010, Shimadzu, Japan) on a splitless mode, using DB-FRAP column (30m*0.25um*0.25um, Agilent, USA), while ethyl acetate (Sigma-Aldrich, St. Louis, USA) was used as an internal standard. For each GC run, the samples were injected onto the column maintained at 60 °C. After 3 min, the oven temperature was gradually increased to 200 °C at a rate of 20 °C per minute, held for 0.5 min, and then increased to 230 °C at a rate of 20 °C per minute and maintained for 1.5 min.

**3.2.9 Bioinformatic Analysis**

Mothur was used for MiSeq data analysis [72]. Briefly, MiSeq output was first paired-end assembled and then aligned to Silva_v138 [73] using the align.seqs Mothur subroutine. Reads with ambiguous base calls, lengths longer than 600 bp, or shorter than 400bp were removed.

Machinery errors were corrected using the pre.cluster Mothur subroutine. Chimeric sequences were removed using VSEARCH [74]. Taxonomy classification was performed based on the RDP trainset16 [75]. Reads classified as mitochondria or chloroplasts were removed. Operational taxonomic units (OTUs) were determined based on clustering results using OptiClust with 3% dissimilarity [76]. The number of reads was normalized by randomly sampling 10,000 reads from each sample using the subsample Mothur subroutine. Bray-Curtis distances were calculated and used for non-metric multidimensional scaling (NMDS) analysis to estimate the microbial shifts among the different treatment groups. The differential abundance test was performed using linear discriminant analysis effect size (LEfSe) [77] implemented in Mothur and run with a strict multiple testing correction by default. The PICRUSt2 algorithm with the ALDEx2 Bioconductor package was used to identify significantly affected metabolic activities [78]. These differentially abundant genera and predicted metabolic activities were considered significant at $P < 0.05$. The results were visualized in a heatmap using the heatmap.2 R package.

### 3.2.10 Statistical Analysis

One-way ANOVA was used to analyze the statistical significance between the control group (ND) and each treatment group. Adjusted P-values were obtained through Tukey HSD test using the multcomp R package, and values less than 0.05 were considered significant. Data are presented as means +/- SD, and normal distribution was confirmed using the Shapiro–Wilk test in R prior to the statistical analyses. ANOVA was performed using R version 3.6.1. Spearman correlation analysis was performed using the otu.association Mothur subroutine.

## 3.3 RESULTS

### 3.3.1 High-fat diet induced obesity

Body weight, food, water, and calorie intake were monitored weekly (Fig. 3.2a, Fig. S3.2). In this study, β-glucan, probiotics, and synbiotics had no significant effect on HFD-induced

weight gain (Fig. 3.2a, 3.2b). Weekly body weight and food consumption data showed that the high-fat diet had a lower feed conversion ratio compared with the normal diet (Fig. 3.2c). Obesity induction was confirmed by histopathological analysis, and liver sections stained with H&E clearly showed a higher number of lipid droplets in the livers of HFD-fed mice (Fig. 3.5a). Blood profiling analysis showed that HFD-fed mice had higher serum levels of triglyceride (TG) and total cholesterol (Total-Cho) than ND-fed mice (Fig. 3.2d). However, ALT and ALP levels were not increased by HFD, suggesting that the effects of HFD may not have caused hepatotoxicity. Interestingly, mice in the BG group showed a higher level of ALT compared to the HFD group.

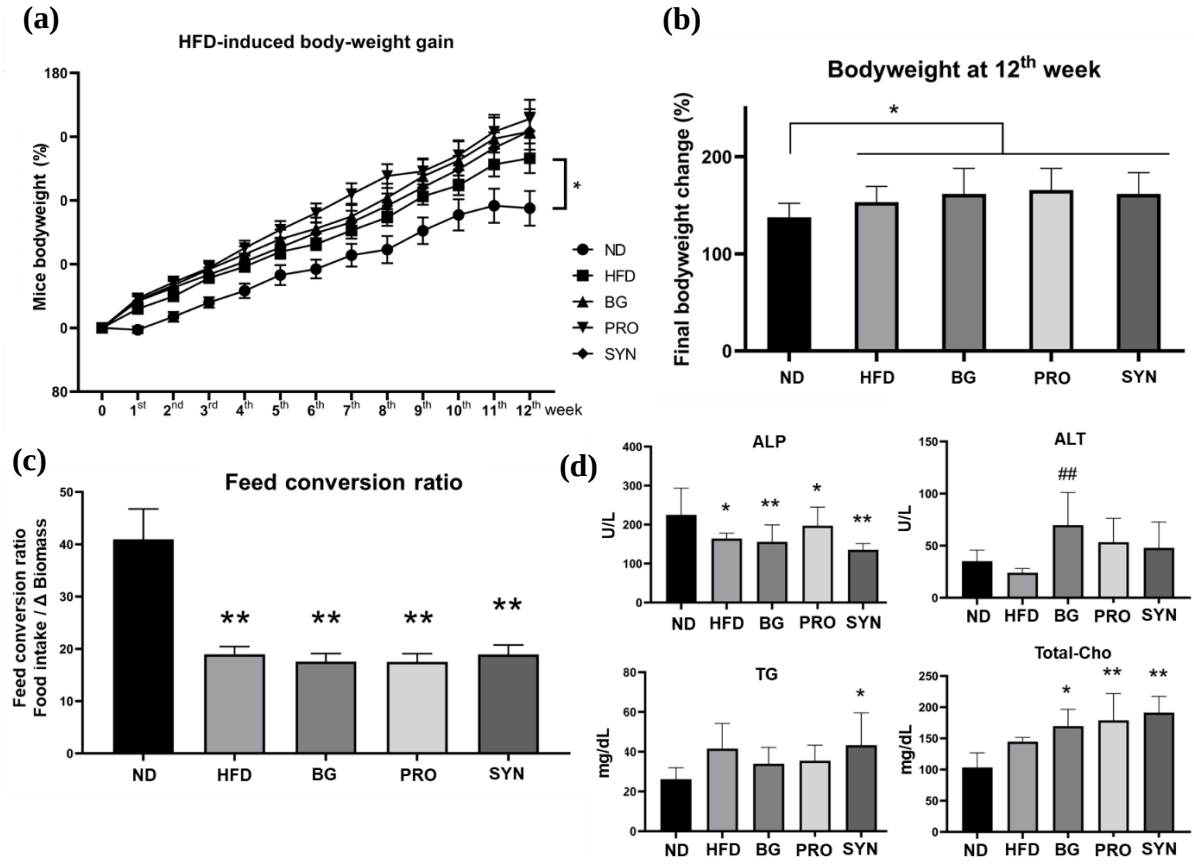



**Figure 3. 2 Effect of supplementary BG, PRO, and SYN on obesity management. (a)** Trend of body-weight gain over 12-weeks; **(b)** Final body-weight change at the end of the obesity-inducing period; **(c)** Feed conversion ratio; **(d)** Blood profiling analysis. Statistically significant results are labeled as * compared to ND, # compared to HFD; P<0.05 - *, #;

P<0.01 - **, ##; P<0.001 - ***, ###. ND, Normal diet; HFD, High-fat diet; BG, High-fat diet + β-glucan (BG); PRO, High-fat diet + probiotics (PRO); SYN, High-fat diet + synbiotic (SYN)

### 3.3.2 Intervention with BG, PRO, and SYN ameliorated DSS-induced colitis in obese mice

Five days of DSS administration led to weight loss (approximately 4-7%), while the PRO and BG groups showed significantly lower weight reduction (Fig. 3.3a). Additionally, all mice subjected to DSS administration showed severe rectal bleeding (Fig. 3.3b). However, DSS and treatments did not affect colon length or liver weight in this study (Fig. 3.3c, 3.3d).

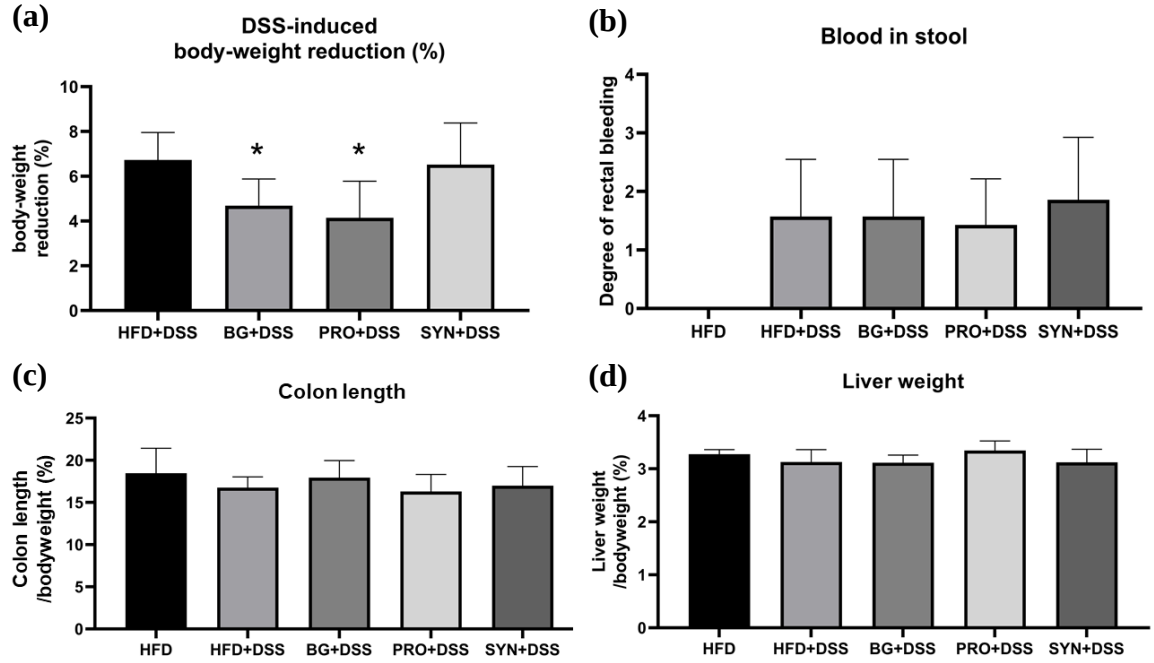
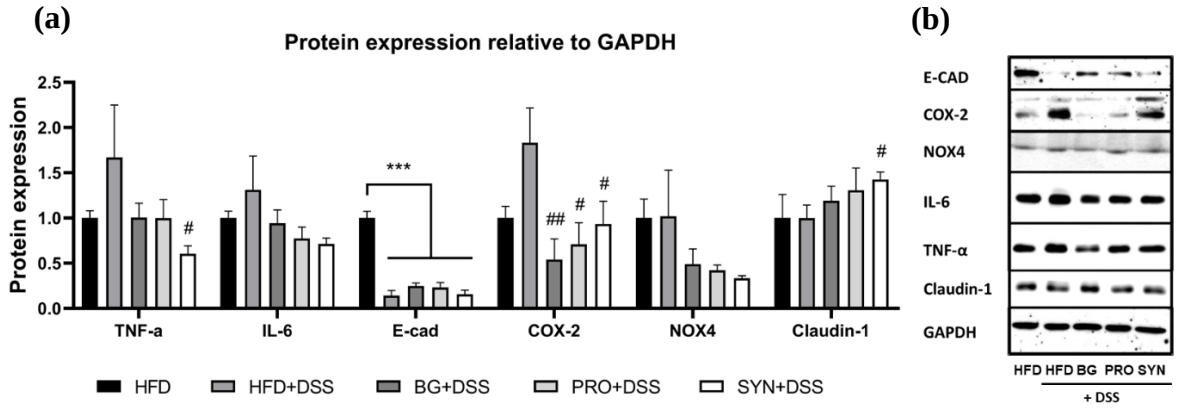



**Figure 3. 3 Effects of DSS on anatomic changes. (a)** DSS treatment for five days induced weight loss in all mice; **(b)** Rectal bleeding caused by DSS was evaluated by the amount of blood in stools; **(c)** colon length was measured in mice treated with and without DSS; **(d)** Liver weight was measured in mice treated with and without DSS. Statistically significant results are labeled as * compared to HFD+DSS; P<0.05 - *; P<0.01 - **; P<0.001 - ***.

HFD, High-fat diet; BG, High-fat diet + β-glucan (BG); PRO, High-fat diet + probiotics (PRO); SYN, High-fat diet + synbiotic (SYN)

DSS increased the expression of COX-2 by 1.8-fold, while treatment with BG, PRO, and SYN increased COX-2 expression in the HFD+DSS group by 1.5-, 1.7-, and 1.9-fold respectively (Fig. 3.4a, 3.4b). DSS decreased E-cad expression by 7-fold, while BG and PRO increased E-cad expression in the HFD+DSS group by 1.25- and 1.23-fold, respectively (Fig. 3.4a, 3.4b). Treatment with DSS showed no effect on the expression of claudin-1; however, the administration of dietary compounds, particularly synbiotics, increased claudin-1 expression (Fig. 3.4a, 3.4b). Mice subjected to DSS administration experienced intestinal transmural inflammation, which was assessed by inflammatory cell infiltration and accompanying ulcers (Fig. 3.4c). The inflammatory condition was milder in the SYN-treated cases (Fig. 3.4c).



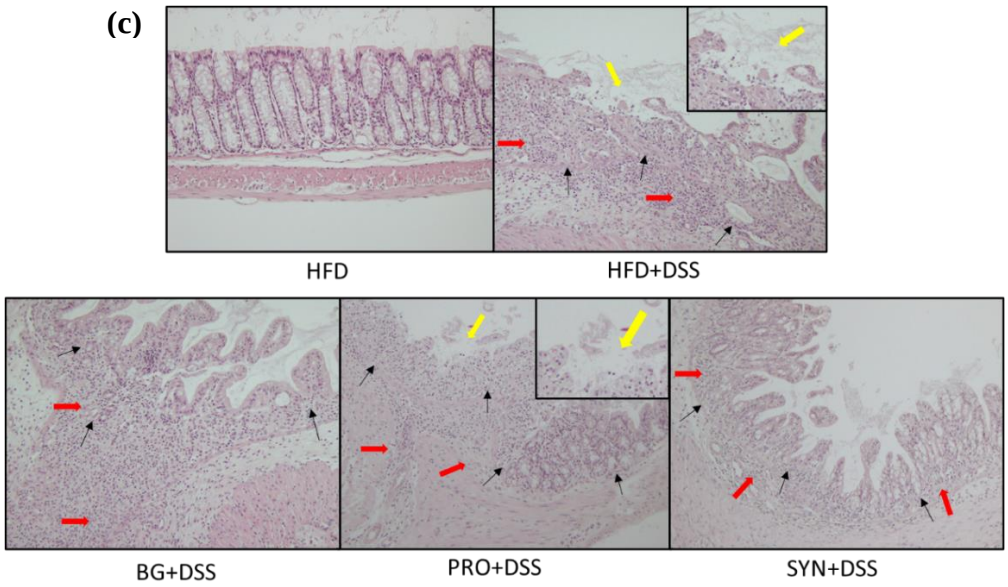



**Figure 3. 4 Effects of BG, PRO, and SYN on DSS-induced colonic inflammation. (a)** Immunoblotting analysis of proteins involved in intestinal inflammation and permeability, 7 colon samples from each group were used to evaluate the protein expression level; **(b)** Western blotting (WB) using the same total protein amount of each sample (20 μg), WB result demonstrated the relative expression level of each target protein, the samples closest to the average were used to represent the graph; **(c)** Histological analysis of colon tissues showed transmural inflammation, crypt distortion, and inflammatory cell infiltration (red arrow: cells infiltration; yellow arrow: ulceration; black arrow: crypt distortion). Statistically significant results are labeled as * compared to HFD, # compared to HFD+DSS; $P<0.05$ - *, #; $P<0.01$ - **, ##; $P<0.001$ - ***, ###. HFD, High-fat diet; BG, High-fat diet + β-glucan (BG); PRO, High-fat diet + probiotics (PRO); SYN, High-fat diet + synbiotic (SYN)

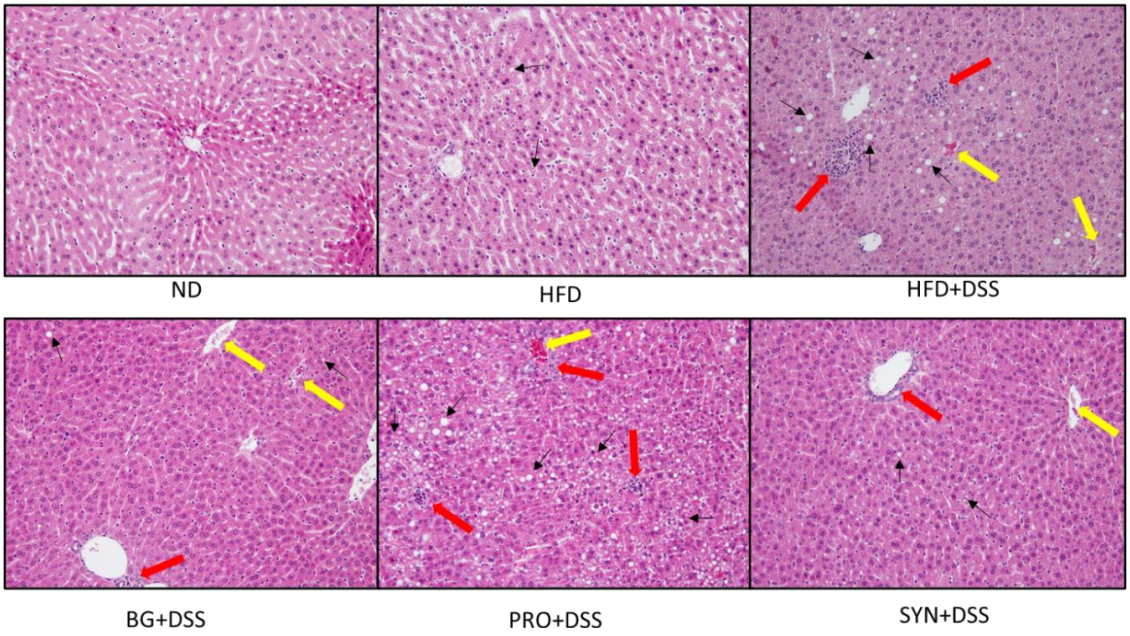
Concomitant with the recruitment of inflammatory cells into the mucosal layer, the secretion of proinflammatory cytokines, such as TNF-α and IL-6, was increased in the DSS-treated group (Fig. 3.4a, 3.4b). While treatment with BG and PRO slightly affected pro-inflammatory marker expression, SYN significantly decreased the expression of TNF-α and IL-6 compared with the HFD+DSS group (Fig. 3.4a, 3.4b). The expression of NOX4 was not

affected by DSS administration or dietary intervention (Fig. 3.4a, 3.4b). Consistent with the downregulation of TNF-α and IL-6, SYN treatment also decreased the expression of NOX4.

### 3.3.3 Protective effects of BG, PRO, and SYN in improving colitis-related nonalcoholic fatty liver disease (NAFLD)

In this study, DSS administration significantly induced NAFLD manifestations, including steatosis, thrombosis, and inflammation (Fig. 3.5a). BG, PRO, and SYN reduced liver thrombosis as revealed by the ratios of congested veins to total veins (Fig. 3.5c). While SYN significantly ameliorated liver steatosis and hepatitis, PRO had little effect (Fig. 3.5b, 3.5d, 3.5e). DSS increased the ratio of lipid droplet area to cellular area from 8.9% to 17.7%, while treatment with BG, PRO, and SYN lowered the ratio by 3.9%, 1.7%, and 4.5%, respectively (Fig. 3.5f). In general, SYN treatment showed the best therapeutic effect on DSS-induced colitis and demonstrated the greatest improvement in all liver conditions. However, the significant correlation between cecal SCFA concentrations and steatosis grades using Pearson's correlation analysis was not observed.

**(a)**



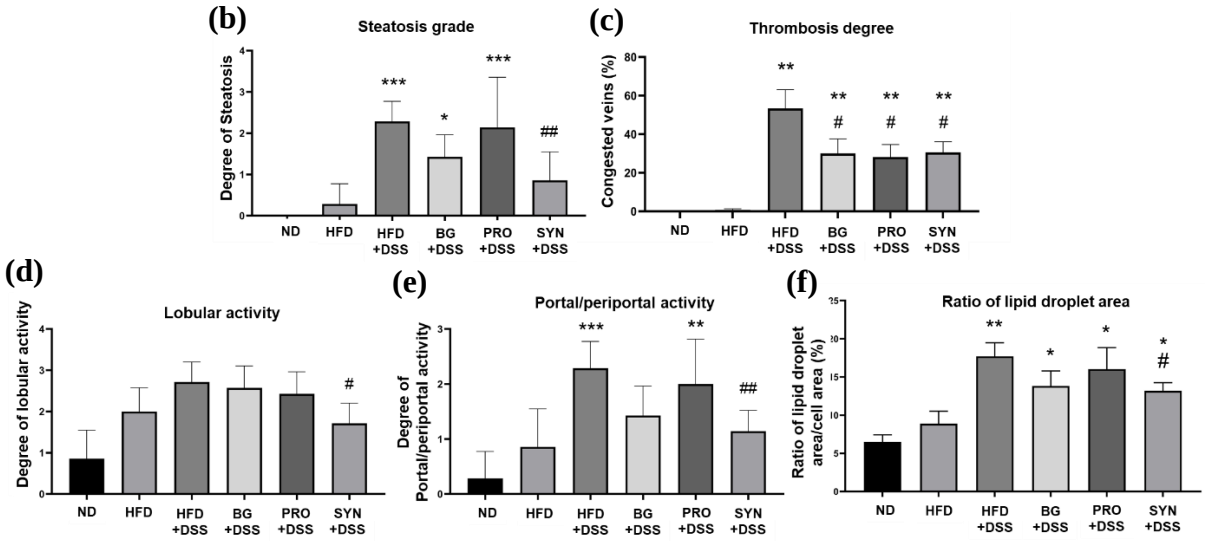



**Figure 3. 5 Effects of BG, PRO, and SYN on IBD-induced liver disorders. (a)** Histological analysis of liver tissues at 20x magnification showed the presence of lipid droplets, portal vein thrombosis, and granulomatous hepatitis (red arrow: lobular and portal/periportal hepatitis; yellow arrow: portal vein and sinusoid thrombosis; black arrow: lipid droplet); **(b)** Grading of steatosis; **(c)** Grading of liver thrombosis; **(d)** Grading of lobular activity; **(e)** Grading of portal/periportal activity; **(f)** Ratio of lipid droplet area to cell area. Statistically significant results are labeled as * compared to HFD, # compared to HFD+DSS; P<0.05 - *, #; P<0.01 - **, ##; P<0.001 - ***, ###. ND, Normal diet; HFD, High-fat diet; BG, High-fat diet + β-glucan (BG); PRO, High-fat diet + probiotics (PRO); SYN, High-fat diet + synbiotic (SYN)

### 3.3.4 BG, PRO, and SYN modulated the fecal microbial communities to different extents

In this study, more than 99% Good's coverage, an estimator for a portion of non-singleton reads, was obtained for all the samples, suggesting that sequence depth was sufficient, and significantly lower species richness and evenness were observed in the PRO group (Fig. S3.3). Bacterial composition analysis showed that PRO treatment increased the abundance of *Akkermansia*, while other genera did not show clear differences in their abundance (Fig.

S3.4). NMDS analysis showed that all additives, particularly PRO and SYN, significantly modulated the gut microbiota and increased the abundance of probiotic bacteria, including butyrate producers (Fig. 3.6a; Fig. 3.6b).

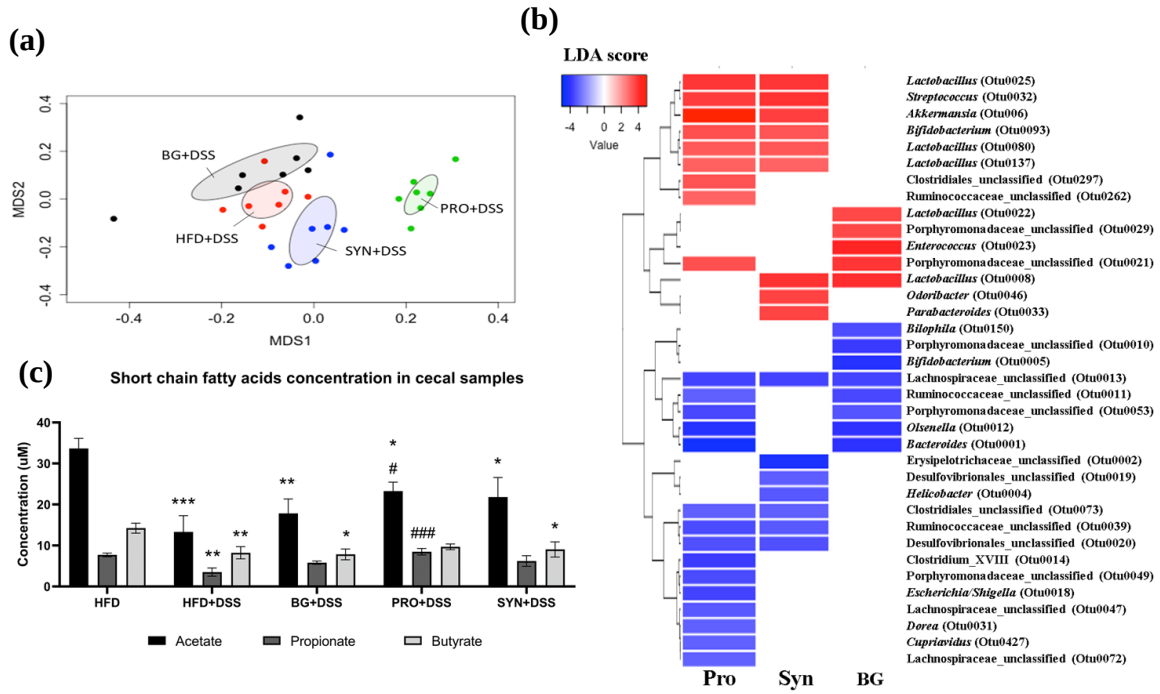


**Figure 3. 6 Effect of different additives on gut microbiota composition. (a)** Microbial community comparison based on non-metric multidimensional scaling (NMDS) analysis. Ellipses were drawn with 95% confidence calculated based on standard error using Vegan R package; **(b)** Differentially abundant OTUs by LEfSe (LDA>3, $p<0.05$); **(c)** Amount of short-chain fatty acids in cecum. Statistically significant results are labeled as * compared to HFD, # compared to HFD+DSS; $p<0.05$ - *, #; $p<0.01$ - **, ##; $p<0.001$ - ***, ###; **(d)** Predicted metabolic activities with differential abundance identified by ALDEx2 (effect size >1, mean difference >0.5). HFD, High-fat diet; BG, High-fat diet + β-glucan (BG); PRO, High-fat diet + probiotics (PRO); SYN, High-fat diet + synbiotic (SYN)

Compared to the HFD group, the BG, PRO, and SYN groups showed a significant increase in probiotic strains such as *Lactobacillus* and *Bifidobacterium,* whereas only PRO and SYN increased the abundance of *Akkermansia* (Fig. 3.6b). However, the BG and PRO treatments significantly reduced the populations of *Bifidobacterium* and *Bacteroides*. The analysis of differentially abundant predicted metabolic activities showed that the SYN treatment has the effects observed for both BG and PRO on metabolic activities compared to mice fed a high-fat diet followed by DSS administration (Fig. 3.6d). Among them, succinate fermentation to butanoate was only enhanced by the SYN treatment, while menaquinol biosynthesis and fucose degradation were enhanced only by the PRO treatment. DSS significantly reduced SCFA concentration in cecal samples, but all additives increased the amount of SCFAs, although only the changes in acetate and propionate in the PRO-treated group were significant ($p<0.05$) (Fig. 3.6c).

**3.4 DISCUSSION**

In this study, I investigated the effects of β-glucan, probiotics, and synbiotics on dietary-induced obese mice that were also administered DSS. Our results showed that these treatments generally ameliorated the symptoms caused by DSS (Table S3.2). However, gut

microbiota analysis showed that β-glucan and probiotics shifted gut microbiota differently, while gut microbiota shifts caused by synbiotics seemed to result from the combined effects of probiotics and β-glucan. This study highlighted the potential of synbiotics in mitigating obesity-associated colitis and hepatic manifestations by effectively nourishing both administered and indigenous beneficial strains.

### 3.4.1 β-glucan, probiotics, and synbiotics shifted the gut microbiota to the healthy population

Obesity and colitis can be caused partly by the breakdown of gut microbiota homeostasis, a state that is individually dependent and characterized in terms of composition, diversity, richness, dynamics, and resilience [94]. The breakdown of microbiota homeostasis is defined as an imbalance in composition and/or function in the microbial ecology that exceeds its resistance and resilience [95]. Dysbiosis has been known to play a role in the development of various disorders, including IBD and NAFLD [95]. Our study demonstrated that this could be amended by administering β-glucan, probiotics, and synbiotics. In this study, mice fed a HFD supplemented with additives had slightly higher hepatotoxicity and body weights compared with HFD-fed mice, which was explained by Kindt et al. as microbiota-derived SCFAs, especially acetate, could promote fatty acid metabolism in the liver [96]. In addition, higher ALT levels were observed in the BG treatment group than in the HFD group. It has been reported that metabolites from fiber fermentation such as glucose, fructose, and acetate could induce NAFLD by promoting de novo lipogenesis [35]. In this study, overconsumption of dietary carbohydrates may have resulted in undesirable hepatic injury in the BG treatment group. Administration of BG, PRO, and SYN markedly modulated the gut bacterial community in HFD-fed mice. The abundance of *Lactobacillus* increased with the administration of BG, PRO, and SYN. In addition, administration of both PRO and SYN increased the abundance of *Akkermansia*, which exerts anti-obesity and anti-inflammatory

effects [97]. Several OTUs were significantly decreased in response to probiotic treatment, which could be due to competition for nutrients or adhesion on intestinal epithelium between ingested probiotic strains and indigenous gut microbiota [98, 99].

### 3.4.2 Dietary treatment effect on anatomic change in C57BL/6J mice after 5 days DSS-induced colitis

DSS-induced colitis is usually accompanied by several anatomical changes, including weight loss, blood in feces, a shortened colon, and an enlarged liver [31, 38]. In the present study, the effect of DSS on inducing weight loss and rectal bleeding was significant, and all mice that underwent 5 days of DSS-induced colitis showed blood in stool and decreased body weight. However, 5 days of administration of DSS did not significantly enlarge the liver or shorten the colon, suggesting that a longer induction period is needed for these effects. In addition, common symptoms of IBD often include diarrhea, because inflammation may shorten fecal transit rate which could lead to diarrhea [100, 101]. However, diarrhea symptoms were not evaluated in this study, further study should include assessing fecal moist content as a subsequent colitis symptom.

### 3.4.3 Dietary treatment secured intestinal barrier and amended colitis-related inflammation

In this study, DSS-treated mice showed a significant increase in COX-2 expression, suggesting a highly inflammatory state in the colon. In addition, the expression of E-cad was strongly decreased, indicating the breakdown of the intestinal barrier. BG, PRO, and SYN treatments restored COX-2 and E-cad levels. E-cad, an important component of epithelial adherence junctions, is strongly expressed by epithelial cells and plays a key role in the development, differentiation, and maintenance of intestinal tissue integrity [102]. In contrast, the expression of COX-2, an inflammatory marker expressed by epithelial cells, is

upregulated in the large intestine during IBD [103]. Our results indicated that BG, PRO, and SYN secured the epithelial barrier by slightly upregulating E-cad and significantly decreasing COX-2 expression. In this study, DSS did not decrease claudin-1, a main tight-junction protein, while dietary treatments increased claudin-1 expression. This result suggests that these treatments, especially SYN, could be used to secure intestinal tight junctions. Although DSS administration induced colonic transmural inflammation, a large number of infiltrated cells did not markedly increase the expression of pro-inflammatory factors, such as TNF-α, IL-6, and NOX4. While TNF-α and IL-6 are mainly secreted by inflammatory cells, NOX4 is expressed in various cell types and generates reactive oxygen species (ROS). Notably, SYN treatment reduced cell infiltration and the expression of IL-6, TNF-α, and NOX4 among all treatments. This study suggested that β-glucan, probiotics, and synbiotics ameliorated obesity-associated colitis to varying degrees. β-glucan and probiotics may have protected the intestinal epithelium from the damaging effects of DSS mainly by securing epithelial tight junctions and reducing epithelial inflammation, while synbiotics may have decreased inflammatory cell infiltration and the expression of pro-inflammatory factors.

### 3.4.4 Dietary treatment significantly improved colitis-induced NAFLD

The H&E-stained liver sections displayed DSS-induced colitis related to NAFLD, observed as increased liver steatosis, thrombosis, and hepatitis due to colitis. Previous clinical studies have demonstrated a relationship between IBD and NAFLD, in which dietary habits have been suggested as the main causative factor [37]. In this study, mice in the HFD and HFD + DSS groups were fed the same diet, implying that colitis impaired nutrient and fat metabolism, resulting in NAFLD. In the present study, NAFLD symptoms such as steatosis, liver thrombosis, and hepatitis were induced, and the treatment with dietary compounds, especially synbiotics, significantly ameliorated colitis-associated hepatic conditions. Although all additives had similar effects on liver thrombosis, their impacts on steatosis and

inflammatory activity were different. The results showed that probiotic treatment had a lack of therapeutic effects on steatosis and lobular and portal/periportal activity, while those conditions were milder in the β-glucan-treated group; however, this effect was not significant compared to the HFD+DSS group. The administration of synbiotics significantly ameliorated colitis-induced NAFLD symptoms in comparison with the HFD+DSS group. The greater improvement of BG and SYN on hepatic manifestation compared to PRO suggests that their effect on fostering indigenous strains is the preferred approach for nutrient disruption-related conditions such as NAFLD.

### 3.4.5 Short-chain fatty acids could play an important role in the effects of dietary treatment

The metabolic activity prediction showed that PRO and SYN treatments could downregulate sulfur oxidation and taurine degradation pathways, demonstrating the anti-inflammatory and cytoprotective effects of these treatments [104]. In addition, only SYN treatment enhanced butyrate production through succinate fermentation in response to DSS administration when compared to the HFD-fed group (Fig. 3.6d). However, it should be noted that most SCFAs are immediately absorbed by the intestinal epithelium; thus, precise quantification of luminal SCFAs is usually difficult [105]. Additionally, the absorption of microbiome-produced SCFAs in the gut is believed to exert beneficial effects in colitis patients [53, 106]. This study demonstrated that BG, PRO, and SYN could increase the population of beneficial bacteria producing metabolites such as SCFAs, which secure epithelial tight junctions, reduce intestinal inflammation, and improve whole-body energy metabolism. Since SCFAs produced in the gut are mainly taken up by the liver (83.6% for propionate, 66.7% for butyrate, and 3% for acetate) [107], the change in the production of SCFAs due to dietary administration can significantly affect the liver. The beneficial effects of SCFAs on steatosis could be exerted by two strategies: the absorption of SCFAs by the intestinal epithelium, thereby improving the

body energy metabolism or the delivery of SCFAs from the gut to the liver via the portal vein [107, 108]. Notably, in this study, the SYN treatment ameliorated the liver damage caused by DSS administration. In contrast, the PRO treatment significantly increased propionate, but did not ameliorate colitis-associated steatosis and hepatitis. These results suggest that microbiome-produced SCFAs alone may not play a role in improving IBD-associated hepatic manifestations. It should be noted that the effectiveness of SCFAs can be regulated by gut microbial composition. Further studies are warranted to elucidate the mechanisms underlying this strategy.

### 3.4.6 Fostering both indigenous and given probiotics is essential for the synergistic effects and better outcomes

Orally administered probiotics increase the population of beneficial bacterial species. However, in our study, the administration of probiotics led to a significant reduction in the population of indigenous microbial diversity (Fig. S3.1, Fig. 3.5b). In contrast, the consumption of β-glucan increased the population of indigenous beneficial strains, while enhancing the mevalonate pathway, which was reported to ameliorate DSS-induced colitis [109]. Synbiotics, however, completely shifted the microbial community toward a healthy one in which both administered and indigenous beneficial strains may have been fostered by β-glucan. Previously, Ma et al. reported that supplementing prebiotics with probiotics (as in synbiotics) fosters both exogenous and indigenous probiotics [110]. Therefore, synbiotic treatment can generally avoid conflicts between administered and indigenous probiotics by supplementation with sufficient nutrients. The study suggests that nourishing the endogenous beneficial strains in the host by prebiotics is recommended not only for obesity-associated colitis but also for colitis-associated NAFLD susceptibility. In this study, each treatment group (n=7) was housed in the same cage. Recent studies have demonstrated that co-housed mice tend to have similar microbiomes. Laukens et al. suggested that the number of cages

should be maximized to increase the number of biological replicates [111]. Therefore, the results observed from the beta-diversity analysis in this study could be over-expressed due to co-housing effects. However, other studies have reported that co-housing does not normalize the mucosal microbiome [112] thus, host phenotypes derived from the mucosal microbiome may be independent among co-housed mice. Nevertheless, it is recommended to apply multiple cages to increase the accuracy of investigating the effects of additives on gut microbial community shifts.

This study demonstrated the beneficial effects of β-glucan and probiotics in a mouse model of obesity-associated colitis. Most importantly, our results indicated that synbiotic dietary intervention outperformed β-glucan and probiotics in alleviating obesity-associated colitis and hepatic manifestations. Further human-based studies are needed to confirm their therapeutic effects in clinical management.

## 3.5 SUPPLEMENTAL MATERIALS

**Fig. S3.1:** Infographic abstract

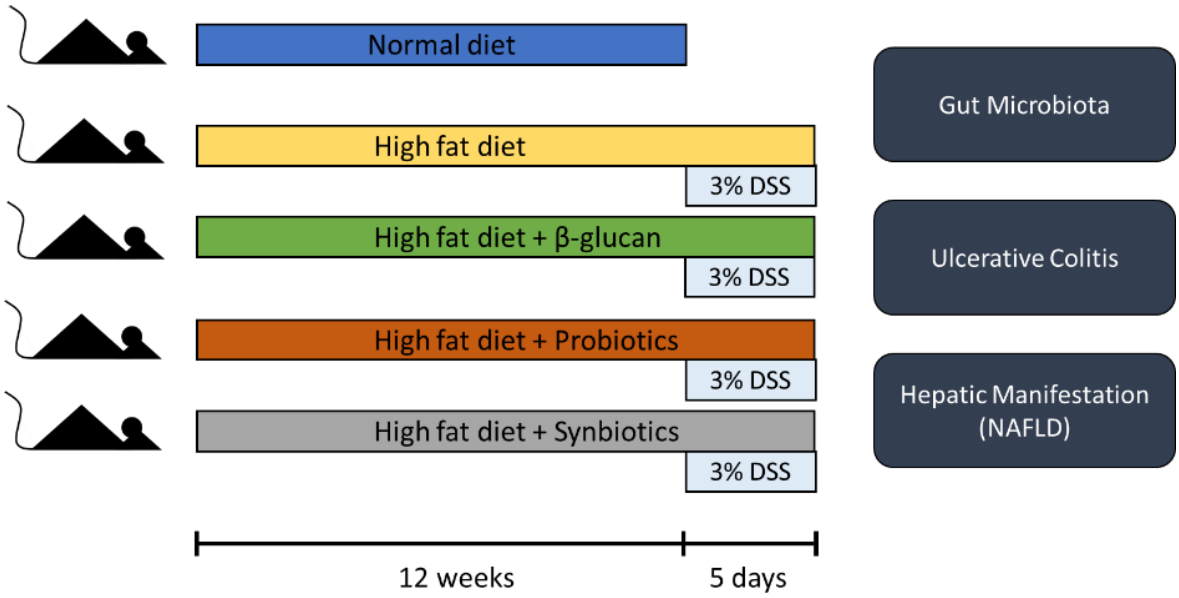



**Fig. S3.2:** Total and weekly intake of food, water, and calory

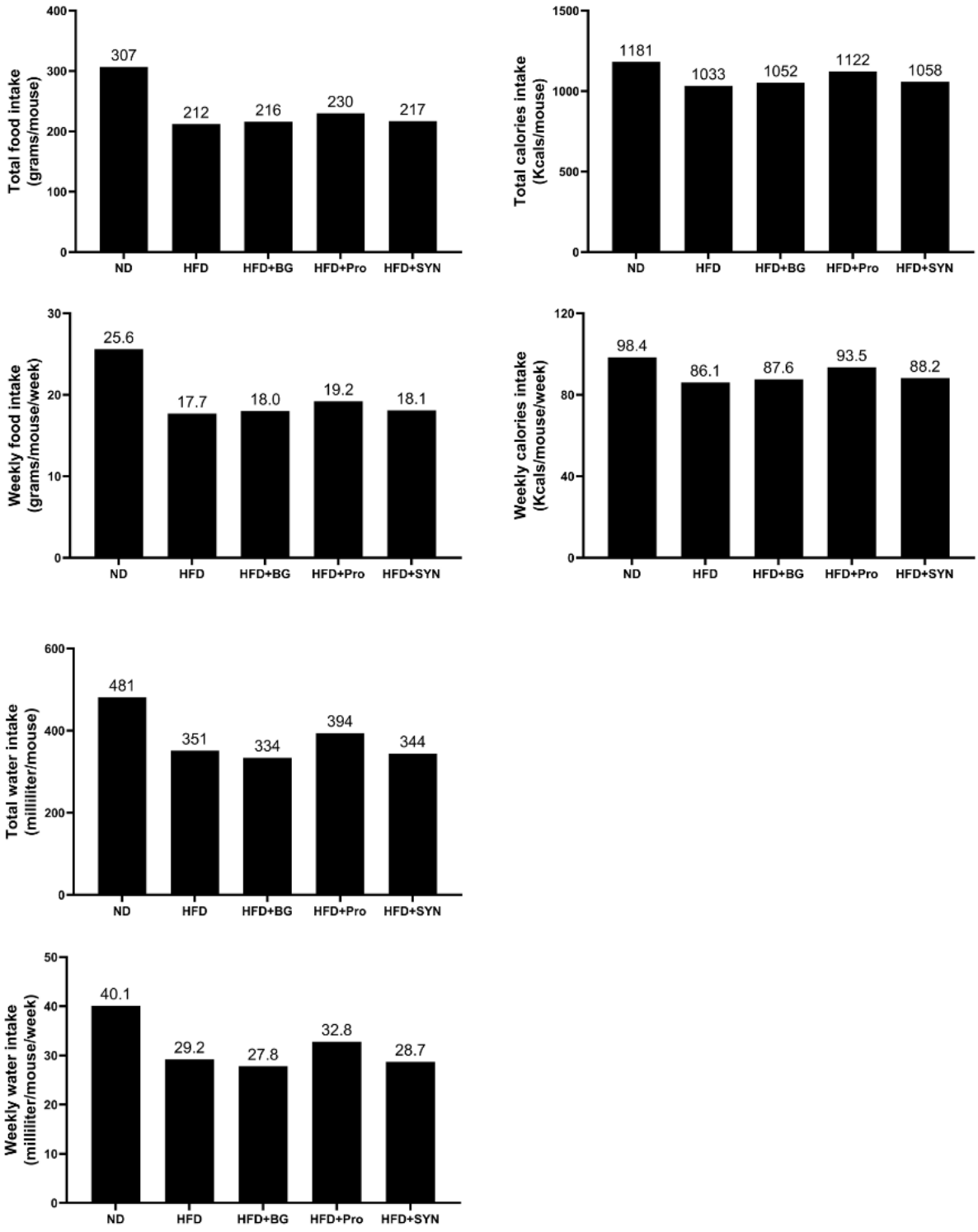



**Fig. S3.3:** Alpha diversity analysis for coverage (a), species richness (b), and species evenness

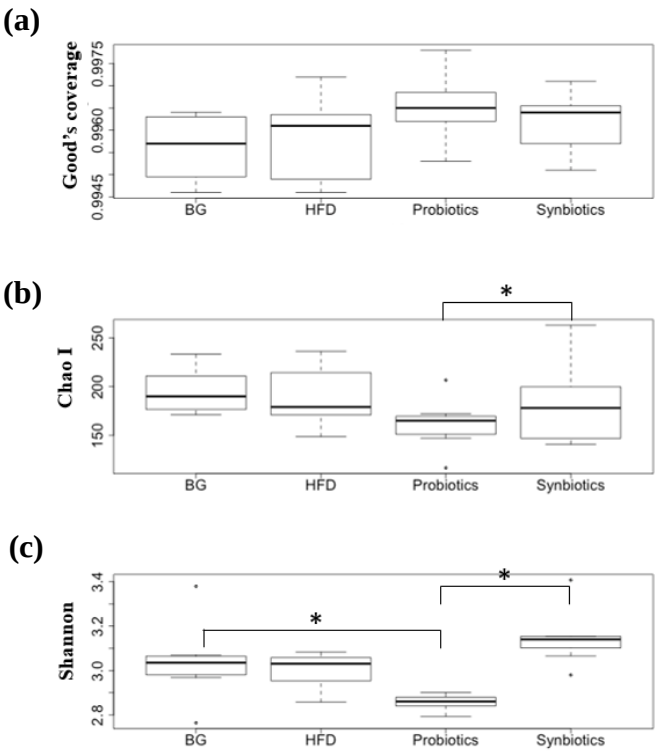
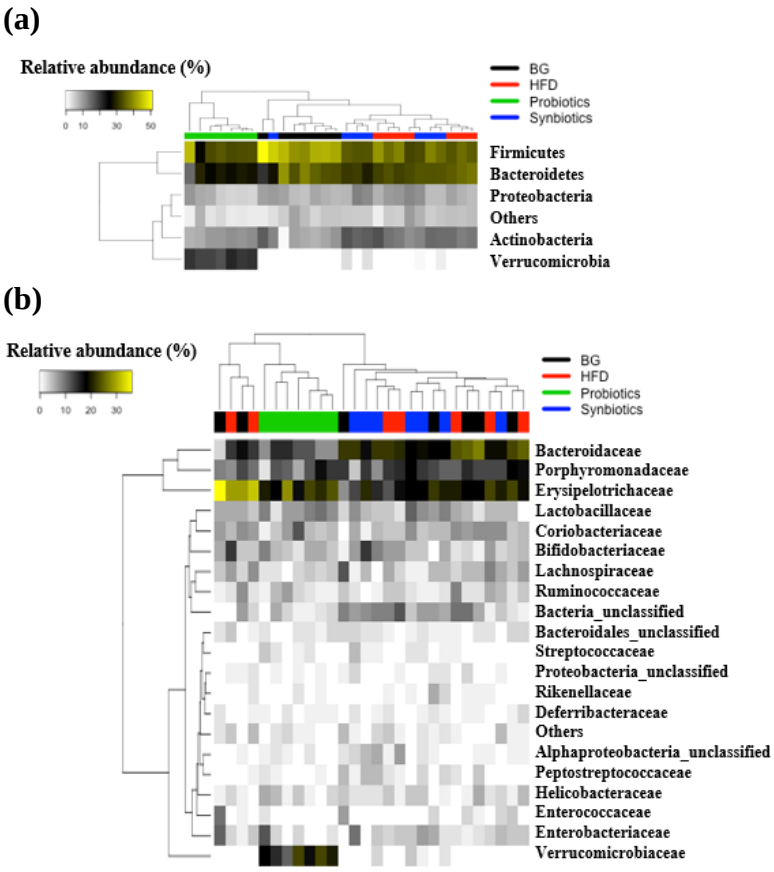



**Fig. S3.4:** Bacterial composition at the phylum level (a), the family level (b) and the genus level (c).



**(c)**

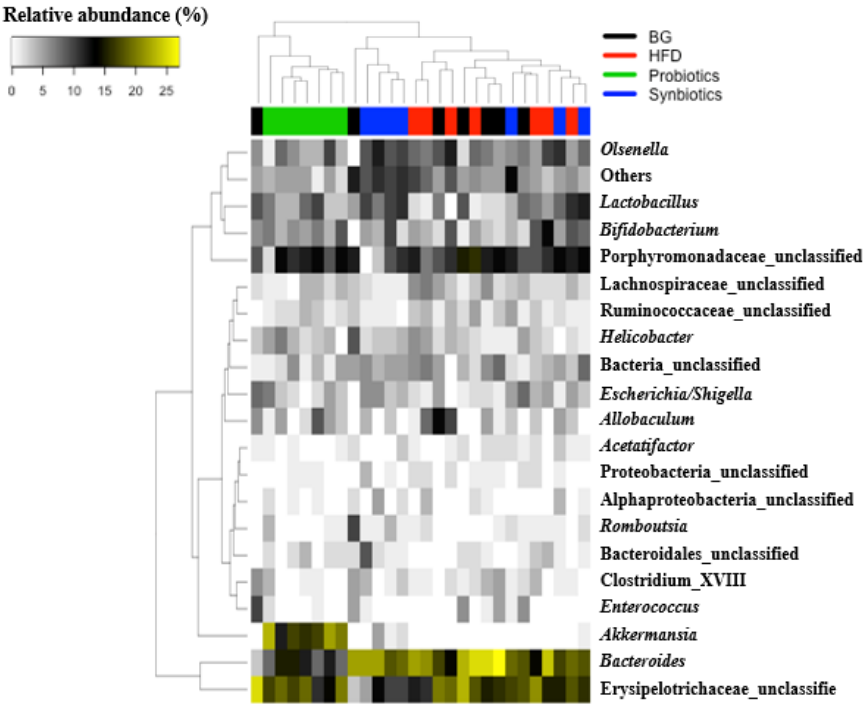



**Table S3.1:** Liver disorders scoring system

| **Portal/periportal activity** | **Score** | **Lobular activity** | **Score** |
|---|---|---|---|
| None or minimal | 0 | None | 0 |
| Portal inflammation only | 1 | Inflammatory cells but no hepatocellular death | 1 |
| Mild interface hepatitis | 2 | Focal cell death | 2 |
| Moderate interface hepatitis | 3 | Severe focal cell death, +/++ confluent necrosis without bridging | 3 |
| Severe interface hepatitis | 4 | Damage includes bridging necrosis | 4 |

| **Liver steatosis scoring systems – Brunt's scale** | **Score** |
|---|---|
| 0% hepatocytes affected | 0 |
| < 33% hepatocytes affected | 1 |
| 33% - 66% hepatocytes affected | 2 |
| > 66% hepatocytes affected | 3 |

**Table S3.2:** Improvement obesity-associated colitis symptoms by each treatment

| Measurement | β-Glucan | Probiotics | Synbiotics |
|---|---|---|---|
| Weight loss | ↓ DSS-induced weight loss by 2% | ↓ DSS-induced weight loss by 2.6% | ↓ DSS-induced weight loss by 0.2% |

| | | | |
|---|---|---|---|
| Gut microbiota | ↑ indigenous probiotic strains | ↑ probiotic strains<br>↓ gut microbiota diversity | ↑ probiotic strains while maintaining diversity |
| SCFAs production | ↑ acetate, propionate | ↑ acetate, propionate, and butyrate | ↑ acetate, propionate, and butyrate |
| Colitis:<br>Intestinal barrier | ↑ E-cad<br>↓ COX-2 | ↑ E-cad<br>↓ COX-2 | ↑ Claudin-1<br>↓ COX-2 |
| Inflammatory regulation | No effects | No effects | ↓ TNF-a, IL-6, and NOX-4<br>↓ cell infiltration and colonic ulceration |
| Hepatic manifestations | ↓ steatosis, liver thrombosis, portal/periportal activity | ↓ liver thrombosis | ↓ steatosis, liver thrombosis, lobular and portal/periportal activity |

## SUMMARY OF THE THESIS

### 1. Effect of *Schizophyllum commune* β-glucan on gastrointestinal tract

In summary, this thesis pointed out the beneficial effect of a specific fiber, *Schizophyllum commune* β-glucan, on gut overall health. The compound was proven to have effects on various aspects such as intestinal epithelium, immunomodulation, and gut microbiota modification. Toward the epithelium, β-glucan is a source of fiber which is fermented by gut microbiota to produce SCFAs which fuels intestinal cells, resulted in increased tight-junction and adherent. High expression of those markers prevents intestinal permeability which is essential to prevent transmural infiltration of bacteria and microbial lipopolysaccharide.

### 2. Effect of *Schizophyllum commune* β-glucan on gut microbiota

On the other hand, *Schizophyllum commune* β-glucan has the potential to modify gut microbiota toward a healthy state by reducing harmful strains such as obesity and constipation-related strains, while fostering SCFAs-producing bacteria such as *Lactobacillus*. The increased production of SCFAs was observed in the trial while treating with β-glucan and synbiotics. Gut modification is concomitant with a decrease in various obesity-related metabolic pathways while the butyrate-producing pathway is upregulated via β-glucan treatment. **Immunomodulatory effect of *Schizophyllum commune* β-glucan**

As an immune modifier, *Schizophyllum commune* β-glucan successfully downregulates ubiquitous inflammatory markers such as COX-2 and NOX-4 induced by DSS treatment. However, inflammatory markers specifically expressed by macrophages were not decreased by β-glucan. Those results suggest the preference of interaction and regulation of the compound toward intestinal epithelial cells in comparison with macrophages.

### 3. Protective effect against constipation

The study did not show increased intestinal transit rate at basal state, the increase was proven in obese mice. However, consumption of the compound results in goblet cell proliferation

and high lubricating mucin production, which is essential for comfortable bowel movement. The advantage prepared by β-glucan at basal condition would protect against constipation to some extent proven as increasing transit rate in obese model.

**4. Protective effect on inflammatory bowel diseases**

By combining the benefits on the intestinal epithelium, immunomodulation, and gut microbiota modification, the compound successfully ameliorates DSS-induced colitis. Consumption of the fiber decreased inflammatory markers and increased expression of tight-junction and adherent proteins results in securing the intestinal barrier and decreasing gut permeability. Since the gut barrier was secured by β-glucan and probiotic treatments, following hepatic manifestation was significantly improved.

**5. Benefits on metabolic syndromes (obesity, diabetes, atherosclerosis, etc.)**

Besides constipation and IBD, *Schizophyllum commune* β-glucan also has effects on common metabolic conditions such as obesity, diabetes, and atherosclerosis. Treatment of β-glucan decreased glucose level and obesity-related serum biomarkers such as ALP, ALT, AST, and cholesterol even at basal state. The modification of gut microbiota by the dietary treatment is suggested as playing a vital part in improving gut overall health.

## REFERENCES


1. El Khoury, D., C. Cuda, et al. "Beta Glucan: Health Benefits in Obesity and Metabolic Syndrome." *Journal of nutrition and metabolism* 2012 (2012): 851362-62. https://doi.org/10.1155/2012/851362.
2. Bashir, Khawaja Muhammad Imran, and Jae-Suk Choi. "Clinical and Physiological Perspectives of B-Glucans: The Past, Present, and Future." *International Journal of Molecular Sciences* 18, no. 9 (2017): 1906. https://doi.org/10.3390/ijms18091906.
3. Berovic, M., J. Habijanic, et al. "Submerged Cultivation of Ganoderma Lucidum Biomass and Immunostimulatory Effects of Fungal Polysaccharides." *J Biotechnol* 103, no. 1 (2003): 77-86. https://doi.org/10.1016/s0168-1656(03)00069-5.
4. Ohno, N., T. Miura, et al. "Physicochemical Characteristics and Antitumor Activities of a Highly Branched Fungal (1----3)-Beta-D-Glucan, Ol-2, Isolated from Omphalia Lapidescens." *Chem Pharm Bull (Tokyo)* 40, no. 8 (1992): 2215-8. https://doi.org/10.1248/cpb.40.2215.
5. Weitberg, A. B. "A Phase I/Ii Trial of Beta-(1,3)/(1,6) D-Glucan in the Treatment of Patients with Advanced Malignancies Receiving Chemotherapy." *J Exp Clin Cancer Res* 27, no. 1 (2008): 40. https://doi.org/10.1186/1756-9966-27-40.
6. Sandula, J., E. Machová, et al. "Mitogenic Activity of Particulate Yeast Beta-(1-->3)-D-Glucan and Its Water-Soluble Derivatives." *Int J Biol Macromol* 17, no. 6 (1995): 323-6. https://doi.org/10.1016/0141-8130(96)81839-3.
7. Hara, C., Y. Kumazawa, et al. "Mitogenic and Colony-Stimulating Factor-Inducing Activities of Polysaccharide Fractions from the Fruit Bodies of Dictyophora Indusiata Fisch." *Chem Pharm Bull (Tokyo)* 39, no. 6 (1991): 1615-6. https://doi.org/10.1248/cpb.39.1615.
8. Babineau, T. J., A. Hackford, et al. "A Phase Ii Multicenter, Double-Blind, Randomized, Placebo-Controlled Study of Three Dosages of an Immunomodulator (Pgg-Glucan) in High-Risk Surgical Patients." *Arch Surg* 129, no. 11 (1994): 1204-10. https://doi.org/10.1001/archsurg.1994.01420350102014.
9. Turnbull, J. L., M. L. Patchen, et al. "The Polysaccharide, Pgg-Glucan, Enhances Human Myelopoiesis by Direct Action Independent of and Additive to Early-Acting Cytokines." *Acta Haematol* 102, no. 2 (1999): 66-71. https://doi.org/10.1159/000040972.
10. Tohamy, A. A., A. A. El-Ghor, et al. "Beta-Glucan Inhibits the Genotoxicity of Cyclophosphamide, Adriamycin and Cisplatin." *Mutat Res* 541, no. 1-2 (2003): 45-53. https://doi.org/10.1016/s1383-5718(03)00184-0.
11. Choi, J. S., M. Y. Park, et al. "Safety and Efficacy of Polycalcium for Improving Biomarkers of Bone Metabolism: A 4-Week Open-Label Clinical Study." *J Med Food* 16, no. 3 (2013): 263-7. https://doi.org/10.1089/jmf.2012.2537.
12. Hashimoto, T., Y. Nonaka, et al. "Suppressive Effect of Polysaccharides from the Edible and Medicinal Mushrooms, Lentinus Edodes and Agaricus Blazei, on the Expression of Cytochrome P450s in Mice." *Biosci Biotechnol Biochem* 66, no. 7 (2002): 1610-4. https://doi.org/10.1271/bbb.66.1610.

13. Neyrinck, A. M., S. Possemiers, et al. "Dietary Modulation of Clostridial Cluster Xiva Gut Bacteria (Roseburia Spp.) by Chitin-Glucan Fiber Improves Host Metabolic Alterations Induced by High-Fat Diet in Mice." *J Nutr Biochem* 23, no. 1 (2012): 51-9. https://doi.org/10.1016/j.jnutbio.2010.10.008.
14. Lim, M. K., S. K. Ku, et al. "Effect of Polycan, a B-Glucan Originating from Aureobasidium, on a High-Fat Diet-Induced Hyperlipemic Hamster Model." *Exp Ther Med* 9, no. 4 (2015): 1369-78. https://doi.org/10.3892/etm.2015.2238.
15. Tappy, L., E. Gügolz, et al. "Effects of Breakfast Cereals Containing Various Amounts of Beta-Glucan Fibers on Plasma Glucose and Insulin Responses in Niddm Subjects." *Diabetes Care* 19, no. 8 (1996): 831-4. https://doi.org/10.2337/diacare.19.8.831.
16. Bourdon, I., W. Yokoyama, et al. "Postprandial Lipid, Glucose, Insulin, and Cholecystokinin Responses in Men Fed Barley Pasta Enriched with Beta-Glucan." *Am J Clin Nutr* 69, no. 1 (1999): 55-63. https://doi.org/10.1093/ajcn/69.1.55.
17. Vetvicka, V., and J. Vetvickova. "Effects of Yeast-Derived Beta-Glucans on Blood Cholesterol and Macrophage Functionality." *J Immunotoxicol* 6, no. 1 (2009): 30-5. https://doi.org/10.1080/15476910802604317.
18. Davidson, M. H., L. D. Dugan, et al. "The Hypocholesterolemic Effects of Beta-Glucan in Oatmeal and Oat Bran. A Dose-Controlled Study." *Jama* 265, no. 14 (1991): 1833-9.
19. Błaszczyk, Katarzyna, Jacek Wilczak, et al. "Impact of Low and High Molecular Weight Oat Beta-Glucan on Oxidative Stress and Antioxidant Defense in Spleen of Rats with Lps Induced Enteritis." *Food Hydrocolloids* 51 (2015): 272-80. https://doi.org/https://doi.org/10.1016/j.foodhyd.2015.05.025.
20. Żyła, E., K. Dziendzikowska, et al. "Beneficial Effects of Oat Beta-Glucan Dietary Supplementation in Colitis Depend on Its Molecular Weight." *Molecules* 24, no. 19 (2019). https://doi.org/10.3390/molecules24193591.
21. Ames, Nancy P., Joanne Storsley, et al. "Effects of Processing on Physicochemical Properties and Efficacy of ²-Glucan from Oat and Barley." *Cereal Foods World* 60 (2015): 4-8.
22. Du, Bin, Maninder Meenu, et al. "A Concise Review on the Molecular Structure and Function Relationship of B-Glucan." *International Journal of Molecular Sciences* 20, no. 16 (2019): 4032.
23. Stier, H., V. Ebbeskotte, et al. "Immune-Modulatory Effects of Dietary Yeast Beta-1,3/1,6-D-Glucan." *Nutr J* 13 (2014): 38. https://doi.org/10.1186/1475-2891-13-38.
24. Ohkusa, Toshifumi, Shigeo Koido, et al. "Gut Microbiota and Chronic Constipation: A Review and Update." *Frontiers in medicine* 6 (2019): 19-19. https://doi.org/10.3389/fmed.2019.00019.
25. Forootan, Mojgan, Nazila Bagheri, et al. "Chronic Constipation: A Review of Literature." *Medicine* 97, no. 20 (2018): e10631-e31. https://doi.org/10.1097/MD.0000000000010631.
26. Axelrod, Cara Hannah, and Miguel Saps. "The Role of Fiber in the Treatment of Functional Gastrointestinal Disorders in Children." *Nutrients* 10, no. 11 (2018): 1650. https://doi.org/10.3390/nu10111650.

27. Prichard, David O., and Adil E. Bharucha. "Recent Advances in Understanding and Managing Chronic Constipation." *F1000Research* 7 (2018): F1000 Faculty Rev-640. https://doi.org/10.12688/f1000research.15900.1.
28. Tabbers, M. M., C. DiLorenzo, et al. "Evaluation and Treatment of Functional Constipation in Infants and Children: Evidence-Based Recommendations from Espghan and Naspghan." *J Pediatr Gastroenterol Nutr* 58, no. 2 (2014): 258-74. https://doi.org/10.1097/mpg.0000000000000266.
29. Kim, D. H., and J. H. Cheon. "Pathogenesis of Inflammatory Bowel Disease and Recent Advances in Biologic Therapies." *Immune Netw* 17, no. 1 (2017): 25-40. https://doi.org/https://doi.org/10.4110/in.2017.17.1.25.
30. Maloy, K. J., and F. Powrie. "Intestinal Homeostasis and Its Breakdown in Inflammatory Bowel Disease." *Nature* 474, no. 7351 (2011): 298-306. https://doi.org/https://doi.org/10.1038/nature10208.
31. Xavier, R. J., and D. K. Podolsky. "Unravelling the Pathogenesis of Inflammatory Bowel Disease." *Nature* 448, no. 7152 (2007): 427-34. https://doi.org/https://doi.org/10.1038/nature06005.
32. Strober, W., and I. J. Fuss. "Proinflammatory Cytokines in the Pathogenesis of Inflammatory Bowel Diseases." *Gastroenterology* 140, no. 6 (2011): 1756-67. https://doi.org/https://doi.org/10.1053/j.gastro.2011.02.016.
33. Harper, J. W., and T. L. Zisman. "Interaction of Obesity and Inflammatory Bowel Disease." *World journal of gastroenterology* 22, no. 35 (2016): 7868-81. https://doi.org/https://doi.org/10.3748/wjg.v22.i35.7868.
34. Jarmakiewicz-Czaja, Sara, Aneta Sokal, et al. "What Was First, Obesity or Inflammatory Bowel Disease? What Does the Gut Microbiota Have to Do with It?" *Nutrients* 12, no. 10 (2020): 3073. https://doi.org/https://doi.org/10.3390/nu12103073.
35. Versini, Mathilde, Pierre-Yves Jeandel, et al. "Obesity in Autoimmune Diseases: Not a Passive Bystander." *Autoimmunity Reviews* 13, no. 9 (2014): 981-1000. https://doi.org/https://doi.org/10.1016/j.autrev.2014.07.001.
36. Paik, Jisun, Yvette Fierce, et al. "High-Fat Diet-Induced Obesity Exacerbates Inflammatory Bowel Disease in Genetically Susceptible Mdr1a−/− Male Mice." *The Journal of Nutrition* 143, no. 8 (2013): 1240-47. https://doi.org/https://doi.org/10.3945/jn.113.174615.
37. Uko, V., S. Thangada, et al. "Liver Disorders in Inflammatory Bowel Disease." *Gastroenterol Res Pract* 2012 (2012): 642923. https://doi.org/https://doi.org/10.1155/2012/642923.
38. Chassaing, B., J. D. Aitken, et al. "Dextran Sulfate Sodium (Dss)-Induced Colitis in Mice." *Curr Protoc Immunol* 104 (2014): 15.25.1-15.25.14. https://doi.org/https://doi.org/10.1002/0471142735.im1525s104.
39. Closa-Monasterolo, R., N. Ferré, et al. "The Use of Inulin-Type Fructans Improves Stool Consistency in Constipated Children. A Randomised Clinical Trial: Pilot Study." *Int J Food Sci Nutr* 68, no. 5 (2017): 587-94. https://doi.org/10.1080/09637486.2016.1263605.

40. Polymeros, D., I. Beintaris, et al. "Partially Hydrolyzed Guar Gum Accelerates Colonic Transit Time and Improves Symptoms in Adults with Chronic Constipation." *Dig Dis Sci* 59, no. 9 (2014): 2207-14. https://doi.org/10.1007/s10620-014-3135-1.
41. Üstündağ, G., Z. Kuloğlu, et al. "Can Partially Hydrolyzed Guar Gum Be an Alternative to Lactulose in Treatment of Childhood Constipation?" *Turk J Gastroenterol* 21, no. 4 (2010): 360-4. https://doi.org/10.4318/tjg.2010.0121.
42. Staiano, A., D. Simeone, et al. "Effect of the Dietary Fiber Glucomannan on Chronic Constipation in Neurologically Impaired Children." *J Pediatr* 136, no. 1 (2000): 41-5. https://doi.org/10.1016/s0022-3476(00)90047-7.
43. Loening-Baucke, V., E. Miele, et al. "Fiber (Glucomannan) Is Beneficial in the Treatment of Childhood Constipation." *Pediatrics* 113, no. 3 Pt 1 (2004): e259-64. https://doi.org/10.1542/peds.113.3.e259.
44. Beleli, C. A., M. A. Antonio, et al. "Effect of 4'galactooligosaccharide on Constipation Symptoms." *J Pediatr (Rio J)* 91, no. 6 (2015): 567-73. https://doi.org/10.1016/j.jped.2015.01.010.
45. Suares, N. C., and A. C. Ford. "Systematic Review: The Effects of Fibre in the Management of Chronic Idiopathic Constipation." *Aliment Pharmacol Ther* 33, no. 8 (2011): 895-901. https://doi.org/10.1111/j.1365-2036.2011.04602.x.
46. Chen, Z., S. Lin, et al. "Effects of Bread Yeast Cell Wall Beta-Glucans on Mice with Loperamide-Induced Constipation." *J Med Food* 22, no. 10 (2019): 1009-21. https://doi.org/10.1089/jmf.2019.4407.
47. Miyamoto, J., K. Watanabe, et al. "Barley B-Glucan Improves Metabolic Condition Via Short-Chain Fatty Acids Produced by Gut Microbial Fermentation in High Fat Diet Fed Mice." 13, no. 4 (2018): e0196579. https://doi.org/10.1371/journal.pone.0196579.
48. Schwartz, B., and Y. Hadar. "Possible Mechanisms of Action of Mushroom-Derived Glucans on Inflammatory Bowel Disease and Associated Cancer." *Ann Transl Med* 2, no. 2 (2014): 19. https://doi.org/10.3978/j.issn.2305-5839.2014.01.03.
49. Muthuramalingam, K., V. Singh, et al. "Dietary Intervention Using (1,3)/(1,6)-B-Glucan, a Fungus-Derived Soluble Prebiotic Ameliorates High-Fat Diet-Induced Metabolic Distress and Alters Beneficially the Gut Microbiota in Mice Model." *Eur J Nutr* 59, no. 6 (2020): 2617-29. https://doi.org/10.1007/s00394-019-02110-5.
50. Singh, Vineet, Karthika Muthuramalingam, et al. "Synbiotic Supplementation with Prebiotic Schizophyllum Commune Derived B-(1,3/1,6)-Glucan and Probiotic Concoction Benefits Gut Microbiota and Its Associated Metabolic Activities." *Applied Biological Chemistry* 64, no. 1 (2021): 7. https://doi.org/10.1186/s13765-020-00572-4.
51. Aron-Wisnewsky, Judith, Edi Prifti, et al. "Major Microbiota Dysbiosis in Severe Obesity: Fate after Bariatric Surgery." *Gut* 68, no. 1 (2019): 70-82. https://doi.org/10.1136/gutjnl-2018-316103.
52. Dabke, Kruttika, Gustaf Hendrick, et al. "The Gut Microbiome and Metabolic Syndrome." *J Clin Invest* 129, no. 10 (2019): 4050-57. https://doi.org/10.1172/JCI129194.

53. Parada Venegas, D., M. K. De la Fuente, et al. "Short Chain Fatty Acids (Scfas)-Mediated Gut Epithelial and Immune Regulation and Its Relevance for Inflammatory Bowel Diseases." *Front Immunol* 10 (2019): 277. https://doi.org/10.3389/fimmu.2019.00277.
54. Vu, Vuong, Karthika Muthuramalingam, et al. "Effects of B-Glucan, Probiotics, and Synbiotics on Obesity-Associated Colitis and Hepatic Manifestations in C57bl/6j Mice." *European Journal of Nutrition* (2021). https://doi.org/10.1007/s00394-021-02668-z.
55. Shimotoyodome, Akira, Shinichi Meguro, et al. "Decreased Colonic Mucus in Rats with Loperamide-Induced Constipation." *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 126, no. 2 (2000): 203-12. https://doi.org/10.1016/S1095-6433(00)00194-X.
56. Shimotoyodome, A., S. Meguro, et al. "Sulfated Polysaccharides, but Not Cellulose, Increase Colonic Mucus in Rats with Loperamide-Induced Constipation." *Dig Dis Sci* 46, no. 7 (2001): 1482-9. https://doi.org/10.1023/a:1010644021888.
57. Parthasarathy, Gopanandan, Jun Chen, et al. "Relationship between Microbiota of the Colonic Mucosa Vs Feces and Symptoms, Colonic Transit, and Methane Production in Female Patients with Chronic Constipation." *Gastroenterology* 150, no. 2 (2016): 367-79.e1. https://doi.org/10.1053/j.gastro.2015.10.005.
58. Avelar Rodriguez, David, Jelena Popov, et al. "Functional Constipation and the Gut Microbiome in Children: Preclinical and Clinical Evidence." *Frontiers in pediatrics* 8 (2021): 595531-31. https://doi.org/10.3389/fped.2020.595531.
59. Looijer-van Langen, M. A., and L. A. Dieleman. "Prebiotics in Chronic Intestinal Inflammation." *Inflamm Bowel Dis* 15, no. 3 (2009): 454-62. https://doi.org/https://doi.org/10.1002/ibd.20737.
60. Tsai, Y. L., T. L. Lin, et al. "Probiotics, Prebiotics and Amelioration of Diseases." *J Biomed Sci* 26, no. 1 (2019): 3. https://doi.org/https://doi.org/10.1186/s12929-018-0493-6.
61. Casellas, F., N. Borruel, et al. "Oral Oligofructose-Enriched Inulin Supplementation in Acute Ulcerative Colitis Is Well Tolerated and Associated with Lowered Faecal Calprotectin." *Aliment Pharmacol Ther* 25, no. 9 (2007): 1061-7. https://doi.org/https://doi.org/10.1111/j.1365-2036.2007.03288.x.
62. Lindsay, J. O., K. Whelan, et al. "Clinical, Microbiological, and Immunological Effects of Fructo-Oligosaccharide in Patients with Crohn's Disease." *Gut* 55, no. 3 (2006): 348-55. https://doi.org/https://doi.org/10.1136/gut.2005.074971.
63. Roy, U., E. J. C. Gálvez, et al. "Distinct Microbial Communities Trigger Colitis Development Upon Intestinal Barrier Damage Via Innate or Adaptive Immune Cells." *Cell Rep* 21, no. 4 (2017): 994-1008. https://doi.org/https://doi.org/10.1016/j.celrep.2017.09.097.
64. Eom, T., Y. S. Kim, et al. "Current Understanding of Microbiota- and Dietary-Therapies for Treating Inflammatory Bowel Disease." *J Microbiol* 56, no. 3 (2018): 189-98. https://doi.org/https://doi.org/10.1007/s12275-018-8049-8.

65. Davani-Davari, Dorna, Manica Negahdaripour, et al. "Prebiotics: Definition, Types, Sources, Mechanisms, and Clinical Applications." *Foods (Basel, Switzerland)* 8, no. 3 (2019): 92. https://doi.org/https://doi.org/10.3390/foods8030092.
66. Liu, B., Q. Lin, et al. "Oat B-Glucan Ameliorates Dextran Sulfate Sodium (Dss)-Induced Ulcerative Colitis in Mice." *Food Funct* 6, no. 11 (2015): 3454-63. https://doi.org/https://doi.org/10.1039/c5fo00563a.
67. Sun, Y., X. Shi, et al. "Inhibition of Dextran Sodium Sulfate-Induced Colitis in Mice by Baker's Yeast Polysaccharides." *Carbohydr Polym* 207 (2019): 371-81. https://doi.org/https://doi.org/10.1016/j.carbpol.2018.11.087.
68. Liu, Xiao-jing, Ran Yu, et al. "Probiotic Mixture Vsl#3 Alleviates Dextran Sulfate Sodium-Induced Colitis in Mice by Downregulating T Follicular Helper Cells." *Current Medical Science* 39, no. 3 (2019): 371-78. https://doi.org/https://doi.org/10.1007/s11596-019-2045-z.
69. Segers, Marijke E., and Sarah Lebeer. "Towards a Better Understanding of Lactobacillus Rhamnosus Gg--Host Interactions." *Microbial cell factories* 13 Suppl 1, no. Suppl 1 (2014): S7-S7. https://doi.org/https://doi.org/10.1186/1475-2859-13-S1-S7.
70. Furrie, E., S. Macfarlane, et al. "Synbiotic Therapy (Bifidobacterium Longum/Synergy 1) Initiates Resolution of Inflammation in Patients with Active Ulcerative Colitis: A Randomised Controlled Pilot Trial." *Gut* 54, no. 2 (2005): 242-9. https://doi.org/https://doi.org/10.1136/gut.2004.044834.
71. Pandey, K. R., S. R. Naik, et al. "Probiotics, Prebiotics and Synbiotics- a Review." *J Food Sci Technol* 52, no. 12 (2015): 7577-87. https://doi.org/https://doi.org/10.1007/s13197-015-1921-1.
72. Schloss, Patrick D., Sarah L. Westcott, et al. "Introducing Mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities." *Applied and Environmental Microbiology* 75, no. 23 (2009): 7537-41. https://doi.org/10.1128/AEM.01541-09.
73. Quast, C., E. Pruesse, et al. "The Silva Ribosomal Rna Gene Database Project: Improved Data Processing and Web-Based Tools." *Nucleic Acids Res* 41, no. Database issue (2013): D590-6. https://doi.org/10.1093/nar/gks1219.
74. Rognes, T., T. Flouri, et al. "Vsearch: A Versatile Open Source Tool for Metagenomics." *PeerJ* 4 (2016): e2584. https://doi.org/10.7717/peerj.2584.
75. Cole, J. R., Q. Wang, et al. "Ribosomal Database Project: Data and Tools for High Throughput Rrna Analysis." *Nucleic Acids Res* 42, no. Database issue (2014): D633-42. https://doi.org/10.1093/nar/gkt1244.
76. Westcott, S. L., and P. D. Schloss. "Opticlust, an Improved Method for Assigning Amplicon-Based Sequence Data to Operational Taxonomic Units." *mSphere* 2, no. 2 (2017): e00073-17. https://doi.org/10.1128/mSphereDirect.00073-17.
77. Segata, N., J. Izard, et al. "Metagenomic Biomarker Discovery and Explanation." *Genome Biol* 12, no. 6 (2011): R60. https://doi.org/10.1186/gb-2011-12-6-r60.
78. Douglas, G. M., and V. J. Maffei. "Picrust2 for Prediction of Metagenome Functions." *Nat Biotechnol* 38, no. 6 (2020): 685-88. https://doi.org/10.1038/s41587-020-0548-6.

79. Parks, D. H., and R. G. Beiko. "Identifying Biologically Relevant Differences between Metagenomic Communities." *Bioinformatics* 26, no. 6 (2010): 715-21. https://doi.org/10.1093/bioinformatics/btq041.
80. Slavin, Joanne. "Fiber and Prebiotics: Mechanisms and Health Benefits." *Nutrients* 5, no. 4 (2013): 1417-35. https://doi.org/10.3390/nu5041417.
81. Mahdy Ali, K., A. Wonnerth, et al. "Cardiovascular Disease Risk Reduction by Raising Hdl Cholesterol--Current Therapies and Future Opportunities." *British journal of pharmacology* 167, no. 6 (2012): 1177-94. https://doi.org/10.1111/j.1476-5381.2012.02081.x.
82. Kosmas, C. E., I. Martinez, et al. "High-Density Lipoprotein (Hdl) Functionality and Its Relevance to Atherosclerotic Cardiovascular Disease." *Drugs Context* 7 (2018): 212525. https://doi.org/10.7573/dic.212525.
83. Ho, Kok-Sun, Charmaine You Mei Tan, et al. "Stopping or Reducing Dietary Fiber Intake Reduces Constipation and Its Associated Symptoms." *World journal of gastroenterology* 18, no. 33 (2012): 4593-96. https://doi.org/10.3748/wjg.v18.i33.4593.
84. Yang, Jing, Hai-Peng Wang, et al. "Effect of Dietary Fiber on Constipation: A Meta Analysis." *World journal of gastroenterology* 18, no. 48 (2012): 7378-83. https://doi.org/10.3748/wjg.v18.i48.7378.
85. Fournier, B. M., and C. A. Parkos. "The Role of Neutrophils During Intestinal Inflammation." *Mucosal Immunology* 5, no. 4 (2012): 354-66. https://doi.org/10.1038/mi.2012.24.
86. Ye, J., L. Lv, et al. "Butyrate Protects Mice against Methionine-Choline-Deficient Diet-Induced Non-Alcoholic Steatohepatitis by Improving Gut Barrier Function, Attenuating Inflammation and Reducing Endotoxin Levels." *Front Microbiol* 9 (2018): 1967. https://doi.org/10.3389/fmicb.2018.01967.
87. Sato, T., K. Matsumoto, et al. "Isolation of Lactate-Utilizing Butyrate-Producing Bacteria from Human Feces and in Vivo Administration of Anaerostipes Caccae Strain L2 and Galacto-Oligosaccharides in a Rat Model." *FEMS Microbiol Ecol* 66, no. 3 (2008): 528-36. https://doi.org/10.1111/j.1574-6941.2008.00528.x.
88. Koh, A., F. De Vadder, et al. "From Dietary Fiber to Host Physiology: Short-Chain Fatty Acids as Key Bacterial Metabolites." *Cell* 165, no. 6 (2016): 1332-45. https://doi.org/10.1016/j.cell.2016.05.041.
89. Cao, Hailong, Xiang Liu, et al. "Dysbiosis Contributes to Chronic Constipation Development Via Regulation of Serotonin Transporter in the Intestine." *Scientific Reports* 7, no. 1 (2017): 10322. https://doi.org/10.1038/s41598-017-10835-8.
90. Hawkes, J. S., R. A. Gibson, et al. "Effect of Dietary Nucleotide Supplementation on Growth and Immune Function in Term Infants: A Randomized Controlled Trial." *Eur J Clin Nutr* 60, no. 2 (2006): 254-64. https://doi.org/10.1038/sj.ejcn.1602310.
91. Dash, N. R., and M. T. Al Bataineh. "Metagenomic Analysis of the Gut Microbiome Reveals Enrichment of Menaquinones (Vitamin K2) Pathway in Diabetes Mellitus." *Diabetes Metab J* 45, no. 1 (2021): 77-85. https://doi.org/10.4093/dmj.2019.0202.

92. Batts, K. P., and J. Ludwig. "Chronic Hepatitis. An Update on Terminology and Reporting." *Am J Surg Pathol* 19, no. 12 (1995): 1409-17. https://doi.org/https://doi.org/10.1097/00000478-199512000-00007.
93. Brunt, E. M., C. G. Janney, et al. "Nonalcoholic Steatohepatitis: A Proposal for Grading and Staging the Histological Lesions." *Am J Gastroenterol* 94, no. 9 (1999): 2467-74. https://doi.org/https://doi.org/10.1111/j.1572-0241.1999.01377.x.
94. Lozupone, Catherine A., Jesse I. Stombaugh, et al. "Diversity, Stability and Resilience of the Human Gut Microbiota." *Nature* 489, no. 7415 (2012): 220-30. https://doi.org/https://doi.org/10.1038/nature11550.
95. Das, B., and G. B. Nair. "Homeostasis and Dysbiosis of the Gut Microbiome in Health and Disease." *J Biosci* 44, no. 5 (2019). https://doi.org/https://doi.org/10.1007/s12038-019-9926-y.
96. Kindt, A., and G. Liebisch. "The Gut Microbiota Promotes Hepatic Fatty Acid Desaturation and Elongation in Mice." *Nature Communications* 9, no. 1 (2018): 3760. https://doi.org/https://doi.org/10.1038/s41467-018-05767-4.
97. Everard, A., C. Belzer, et al. "Cross-Talk between Akkermansia Muciniphila and Intestinal Epithelium Controls Diet-Induced Obesity." *Proc Natl Acad Sci U S A* 110, no. 22 (2013): 9066-71. https://doi.org/https://doi.org/10.1073/pnas.1219451110.
98. Terpou, A., and A. Papadaki. "Probiotics in Food Systems: Significance and Emerging Strategies Towards Improved Viability and Delivery of Enhanced Beneficial Value." *Nutrients* 11, no. 7 (2019): 1591. https://doi.org/https://doi.org/10.3390/nu11071591.
99. Walter, J., M. X. Maldonado-Gómez, et al. "To Engraft or Not to Engraft: An Ecological Framework for Gut Microbiome Modulation with Live Microbes." *Curr Opin Biotechnol* 49 (2018): 129-39. https://doi.org/https://doi.org/10.1016/j.copbio.2017.08.008.
100. Rose, C., A. Parker, et al. "The Characterization of Feces and Urine: A Review of the Literature to Inform Advanced Treatment Technology." *Critical reviews in environmental science and technology* 45, no. 17 (2015): 1827-79. https://doi.org/https://doi.org/10.1080/10643389.2014.1000761.
101. Hempel, K. A., and A. V. Sharma. "Collagenous and Lymphocytic Colitis." In *Statpearls*. Treasure Island (FL): StatPearls Publishing Copyright © 2021, StatPearls Publishing LLC., 2021.
102. Daulagala, A. C., and M. C. Bridges. "E-Cadherin Beyond Structure: A Signaling Hub in Colon Homeostasis and Disease." *International Journal of Molecular Sciences* 20, no. 11 (2019): 2756. https://doi.org/https://doi.org/10.3390/ijms20112756.
103. Singer, II, D. W. Kawka, et al. "Cyclooxygenase 2 Is Induced in Colonic Epithelial Cells in Inflammatory Bowel Disease." *Gastroenterology* 115, no. 2 (1998): 297-306. https://doi.org/https://doi.org/10.1016/s0016-5085(98)70196-9.
104. Kushkevych, I., D. Dordević, et al. "Hydrogen Sulfide as a Toxic Product in the Small-Large Intestine Axis and Its Role in Ibd Development." *Journal of Clinical Medicine* 8, no. 7 (2019): 1054. https://doi.org/https://doi.org/10.3390/jcm8071054.

105. Boets, E., L. Deroover, et al. "Quantification of in Vivo Colonic Short Chain Fatty Acid Production from Inulin." *Nutrients* 7, no. 11 (2015): 8916-29. https://doi.org/https://doi.org/10.3390/nu7115440.
106. Smith, S. A., S. A. Ogawa, et al. "Mitochondrial Dysfunction in Inflammatory Bowel Disease Alters Intestinal Epithelial Metabolism of Hepatic Acylcarnitines." *J Clin Invest* 131 (2020). https://doi.org/https://doi.org/10.1172/jci133371.
107. Bloemen, J. G., K. Venema, et al. "Short Chain Fatty Acids Exchange across the Gut and Liver in Humans Measured at Surgery." *Clin Nutr* 28, no. 6 (2009): 657-61. https://doi.org/https://doi.org/10.1016/j.clnu.2009.05.011.
108. Zhou, D., and J. G. Fan. "Microbial Metabolites in Non-Alcoholic Fatty Liver Disease." *World journal of gastroenterology* 25, no. 17 (2019): 2019-28. https://doi.org/https://doi.org/10.3748/wjg.v25.i17.2019.
109. Acharya, S., M. Timilshina, et al. "Mevalonate Promotes Differentiation of Regulatory T Cells." *Journal of Molecular Medicine* 97, no. 7 (2019): 927-36. https://doi.org/https://doi.org/10.1007/s00109-019-01784-y.
110. Ma, C., S. Wasti, et al. "The Gut Microbiome Stability Is Altered by Probiotic Ingestion and Improved by the Continuous Supplementation of Galactooligosaccharide." *Gut Microbes* 12, no. 1 (2020): 1785252. https://doi.org/https://doi.org/10.1080/19490976.2020.1785252.
111. Laukens, D., B. M. Brinkman, et al. "Heterogeneity of the Gut Microbiome in Mice: Guidelines for Optimizing Experimental Design." *FEMS Microbiol Rev* 40, no. 1 (2016): 117-32. https://doi.org/https://doi.org/10.1093/femsre/fuv036.
112. Robertson, S. J., P. Lemire, et al. "Comparison of Co-Housing and Littermate Methods for Microbiota Standardization in Mouse Models." *Cell Rep* 27, no. 6 (2019): 1910-19.e2. https://doi.org/https://doi.org/10.1016/j.celrep.2019.04.023.

## ACKNOWLEDGEMENT

First of all, I would like to say my thanks to Prof. Moonjae Cho for his close supervision and thoughtful guidance.

My thank with honor to Prof. Tatsuya Unno, who played a vital role in my first publication, his comments, and advice are always outstanding.

I want to mention with honor Prof. Somi Kim, who confers me the chance to study for my Master's at Jeju National University. Besides that, Prof. Kim always gives me thoughtful advice which helps me a lot in my Master's course.

Thank Dr. Young Mee Kim for being a dedicated technician and instructor. Big thanks to my senior Karthika Muthuramalingam for teaching me all the methods and techniques. My thanks to my labmates consist of Jyn Hyok Choi, Hye Rin Chong, and doctor Chae Min Cho for being there and supporting me with the lab work.

## APPENDIX A: List of publication

| Title | Journal | Date of publishment |
|---|---|---|
| Effects of β-Glucan, Probiotics, and Synbiotics on Obesity-Associated Colitis and Hepatic Manifestations in C57BL/6J Mice | European Journal of Nutrition | 2021-09-24 |
| Schizophyllum commune-derived β-glucan improves intestinal health demonstrating protective effects against constipation and common metabolic disorders | Unpublished | Unpublished |

## APPENDIX B: Conference presentation

| Conference | Type of presentation | Title |
|---|---|---|
| Busan 2020 International conference - KSBMB | Poster presentation | Effects of Dietary Treatments on Obesity-Associated Colitis and Hepatic Manifestations |
| Jeju 2021 International conference - KSABC | Poster presentation | Effects of β-glucan and Probiotics on Obesity-Associated Colitis and Hepatic Manifestations |

## DECLARATION

I, **Vuong Van Vu**, as a graduate student of Jeju National University, hereby declare that I have not committed any acts that may damage the credibility of my research. These include, but are not limited to falsification, a thesis written by someone else, distortion of research findings, or plagiarism. I affirm that my thesis entitled "***Schizophyllum commune* derived β-glucan improves gut healthiness**" submitted to Jeju National University, in partial fulfillment of the requirements for the award of the degree of **Master of Science** under the department of **Interdisciplinary Graduate Program in Advanced Convergence Technology and Science**. This thesis is a record of an original research work done and published by me during the period **March 2019 to August 2021** under the supervision and guidance of my thesis advisor **Prof. Moonjae Cho.**

**Vuong Van Vu**