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Effects of Konjac Glucomannan and Its Oligosaccharides on Improvement of Lactose Intolerance as Gut Prebiotics

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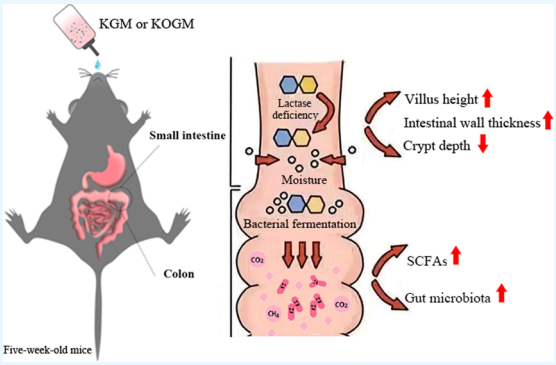
ABSTRACT: Lactose intolerance (LI) is a widespread health issue affecting almost 70% of the world population. This study evaluates the potential prebiotic benefits of konjac glucomannan (KGM) and konjac oligogalactomannan (KOGM) in improving LI. Colonic fermentation results indicate that lactase groups of healthy subjects showed lower pH, higher lactic acid content, and lactase activity in fermentation broth compared with LI subjects. Total short-chain fatty acid (SCFAs) content reached 1.71 mmol/L in healthy subjects, whereas it was 1.49 mmol/L in LI subjects. *In vivo* studies demonstrated that KGM and KOGM intake reduced total cholesterol (T-CHO) and triglyceride (TG) levels in the liver and significantly increased immunoglobulin G (lgG) and immunoglobulin A (lgA) values, while KOGM inclusion led to a significant 23.04% increase in serum free fatty acid (FFA) levels compared to the Blank group ($p < 0.05$). Furthermore, ileal tissue analysis revealed a marked increase in villus height and intestinal wall thickness ($p < 0.05$) as well as a decrease in crypt depth ($p < 0.05$). The composition and proportion of gut microbiota have improved with KGM and KOGM use, notably increasing the abundance of *Lactobacillus* and *Lachnospiraceae_NK4A136_group*, respectively ($p < 0.05$). Compared with the Blank group, *Lactobacillus* abundance increased by approximately 25.82% in the Drug group, 18.23% in the KGM group, and 8.67% in the KOGM group. These findings suggest that KGM and KOGM can be utilized as prebiotics to alleviate LI symptoms.



1. INTRODUCTION

Individuals with lactose intolerance (LI) experience difficulty in breaking down lactose, a sugar present in milk, due to the absence of the enzyme lactase, which is produced in the small intestine. As a result, lactose enters the colon, where it is metabolized by intestinal bacteria into lactic acid, disrupting the alkaline environment and prompting the secretion of alkaline digestive fluids to neutralize the acid. This alteration increases osmotic pressure in the intestine, leading to symptoms such as abdominal pain, bloating, colic, and diarrhea.[1] About 70% of the global population suffers from varying degrees of LI, underscoring its significance in public health.[2]

Studies have linked colonic fermentation with LI.[3−5] Common LI treatments include lactose-free (or low) milk powder, lactase supplementation, and probiotics.[4] Ahn et al.'s meta-analysis highlighted probiotic consumption's effectiveness in reducing LI symptoms like abdominal pain, diarrhea, and bloating among adults.[6] Zhong et al. discovered that supplementing *Bifidobacterium* capsules and *Lactobacilli*-rich yogurt for 2 weeks significantly increased lactose metabolism in LI patients, alleviating symptoms.[2] Microecological preparations containing lactic acid bacteria benefit LI treatment by producing *β*-galactosidase, which slows gastric emptying, prolongs intestinal transit, and maintains balanced gut microbiota.[8] Supplementation with various oligosaccharides can modify gut microbiota, increasing *Bifidobacteria* and/or lactic acid bacteria levels.[9] *Bifidobacterium* and *Lactobacillus* can ferment lactose, producing acid without generating gas, thereby avoiding an increase in osmotic pressure. Furthermore, they can enhance the absorption of SCFAs in the intestine, which is beneficial for alleviating LI symptoms.[4,7] Consequently, modifying colon bacteria through dietary intervention, especially by using prebiotics to enhance intestinal bacterial fermentation and produce probiotics, as well as metabolizing lactose, could be a novel and promising approach to treating LI.[10]

Konjac glucomannan (KGM) is a high molecular weight polysaccharide composed of glucose and mannan linked by *β*-1,4-glycosidic bonds, which is derived from konjac tubers.[11] KGM undergoes semidry enzymatic hydrolysis to produce konjac oligosaccharides (KOGM), which have lower molecular weight, viscosity, and higher solubility compared to KGM.[12,13] Both KGM and KOGM have been found to have prebiotic

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effects, promoting the growth of beneficial bacteria like *Bifidobacterium* and *Lactobacillus* and stimulating the production of SCFAs,[14] thus contributing to intestinal health regulation. *Bifidobacterium* has been observed to promote the proliferation of small intestinal villous epithelial cells and secrete lactase, thus synergistically maintaining the microecological balance in the intestine.[2,4] Our previous research suggested that KGM and KOGM may provide benefits for LI.[15] However, the in-depth mechanism of their functions remains to be elucidated.

To examine the role of colonic fermentation in LI and the potential prebiotic effects of KGM and KOGM on LI individuals, *in vitro* fermentation experiments using fecal samples from healthy and LI subjects and *in vivo* studies with mice have been conducted. These studies examined changes in pH, SCFAs, lactic acid content, and lactase activity during colonic fermentation as well as effects on body weight, organ index, serum and liver indicators, small intestine parameters, colon SCFAs content, and gut microbiota composition. This research aims to provide a scientific framework and guidance for developing new prebiotics for LI treatment, offering innovative strategies for addressing this condition.

2. MATERIALS AND METHODS

2.1. Materials. Five-week-old mice were chosen as subjects, based on previous research findings.[15] As 1 week was needed for adaptive feeding, 4 week-old specific pathogen-free (SPF) male KM mice (20 ± 2 g) were purchased from Hunan SJA Laboratory Animal Co., Ltd. (Hunan, China) with the license number SCXK (Xiang) 2019−0004.

Konjac flour containing 93% KGM content, with an average molecular weight of 952 kDa, was procured from Chongqing Kangjiake Co., Ltd. (Chongqing, China). β-mannanase (60,000 U/g) was purchased from Kunming Aikete Biotechnology Co., Ltd. (Yunnan, China). Various kits including lactic acid, protein quantitative determination, lactase, total protein (TP), triglyceride (TG), and total cholesterol (T-CHO) were purchased from the Nanjing Jiancheng Institute of Biotechnology (Jiangsu, China). Elisa kits of free fatty acid (FFA), immunoglobulin A (lgA), and immunoglobulin G (lgG) were sourced from Xiamen Huijia Biotechnology Co., Ltd. (Fujian, China). Siliankang Bifidobacterium four linked viable tablets were purchased from Hangzhou Yuanda Biopharmaceutical Co. Ltd. (Zhejiang, China). Additionally, a PBS phosphate buffer solution (0.01 M, pH 7.2−7.4) was obtained from Beijing Zhongshan Jinqiao Biotechnology Co., Ltd. (Beijing, China). All other reagents were assumed to be of analytical purity, unless otherwise specified.

2.2. Preparation of KOGM. The preparation of KOGM followed the laboratory method described by Zhang et al.[15] as follows: 20 g of KGM was mixed with 34 mL of citric acid sodium citrate buffer (pH = 6.0), and β-mannanase (1600 U/g) was added to the mixture. The combination was stirred and sealed with breathable glass paper and then incubated at 55 °C for 3.2 h. Subsequently, the sample was heated by using a MAGM-II microwave rapid sample preparation system (Shanghai Xinyi Company, China) at 700 W for 1 min until the center temperature reached 95 °C to deactivate the enzyme activity. The product was dried in a 45 °C vacuum drying oven. The dried sample was dissolved in 10 times (g: mL) of distilled water, stirred thoroughly, and centrifuged at 6000 rpm for 20 min (Eppendorf, Germany). The supernatant was collected and concentrated to 1/8−1/12 of the original volume by using rotary evaporation. Anhydrous ethanol was gradually added (9 times the volume of the solution), and the ethanol concentration was adjusted to 90%. After 1 h of alcohol precipitation, the mixture was centrifuged at 8000 rpm for 20 min to obtain the alcohol precipitate. Finally, the precipitate was dried at 40 °C, crushed, and sieved through a 160 mesh to obtain KOGM, with an average molecular weight of no more than 1.67 kDa.

2.3. Possible Role of Colon Fermentation in Human Lactose Dyspepsia. *2.3.1. Preparation of the Anaerobic Salt Solution.* A trace element solution and carbonate phosphate buffer solution were prepared according to the experimental method[16] and sterilized at 121 °C for 20 min the night before the experiment. Subsequently, the buffer was placed in an anaerobic environment until it became colorless, resulting in a prereduced anaerobic buffer. To remove oxygen, nitrogen was bubbled in, and 0.25 g/L L-cysteine hydrochloride was added and left overnight in the anaerobic chamber, obtaining an anaerobic salt solution.

2.3.2. Collection and Processing of Fecal Samples.[16] Fresh fecal samples were obtained from four healthy volunteers and four individuals with LI, and the samples were mixed equally in each group. The samples were divided into two groups: healthy subjects and LI subjects. Volunteers aged 22−28 who had not taken antibiotics in the last three months were recruited. Those with LI had to have a current or recent history of the disorder for at least one month and reported symptoms of LI after consuming traditional milk. Additionally, none of the volunteers had any dietary disorders or gastrointestinal diseases that could interfere with the study results.

2.3.3. In Vitro Simulated Large Intestine Fermentation.[17] A unified fecal sample was mechanically kneaded for 2 min. Then, 30 g of the sample was suspended in a solution of 270 mL of an anaerobic salt solution and purged with N_2 to remove O_2. Glass beads were added to the suspension and swirled for 3 min, followed by centrifugation at 3000 r/min for 1 min. Subsequently, 10 mL of this suspension was added to 10 mL of anaerobic salt solution, and the samples were divided into two groups: one with added lactose (Lactose group) and the other without lactose (Blank group). Both groups were incubated in a shaking bed at 37 °C under anaerobic conditions for 5 h.

2.3.4. Determination of the pH Value. At each time point (at 0, 0.5, 1, 2, 4, and 5 h) during the fermentation process, the supernatant was collected and the pH of the fermentation broth was measured using a PHS-3C pH meter (Shanghai Dapu Instrument Co., Ltd., China).

2.3.5. Determination of SCFAs Content. 1 mL of the sample (collected at 0, 0.5, 1, 2, 4, and 5 h) was mixed with 0.2 mL of copper sulfate (0.5%) and centrifuged at 4 °C and 4000 rpm for 5 min. The supernatant was analyzed for SCFA content using a GC-2010 Plus gas chromatograph (Shimadzu Corporation, Japan) equipped with an FFAP gas chromatography column of 30 m × 0.53 mm × 0.50 μm. Standard curves of valeric acid, isovaleric acid, butyric acid, isobutyric acid, propionic acid, and acetic acid (R^2 = 0.999) were used to calculate the total acid as the sum of various acids.

2.3.6. Determination of Lactic Acid Content. Samples were taken at 0, 0.5, 1, 2, 4, and 5 h during the cultivation period to measure the lactic acid concentration in the fermentation broth. The supernatant (0.02 mL) was tested using an LD test kit, followed by the determination of the absorbance values of each sample with the SYNERGYH1MG enzyme-linked immunosorbent assay (American Gene Biomedical Technology Co., Ltd.).

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Table 1. pH Values in Fermented Cultures Added with Lactose or Water at Different Times during Fermentation[a]

objects	groups	fermentation time (h)				
		0	1	2	4	5
feces of healthy subjects	blank group	7.77 ± 0.04^{Aa}	7.64 ± 0.00^{Ba}	7.56 ± 0.00^{Ca}	7.42 ± 0.01^{Da}	7.41 ± 0.01^{Da}
	lactose group	7.26 ± 0.01^{Ac}	6.78 ± 0.01^{Bd}	6.34 ± 0.04^{Cc}	5.47 ± 0.01^{Dc}	5.19 ± 0.04^{Ec}
feces of LI subjects	blank group	7.72 ± 0.01^{Aa}	7.59 ± 0.00^{ABb}	7.51 ± 0.00^{BCa}	7.40 ± 0.00^{CDa}	7.29 ± 0.13^{Da}
	lactose group	7.37 ± 0.03^{Ab}	6.83 ± 0.00^{Bc}	6.50 ± 0.01^{Cb}	5.88 ± 0.01^{Db}	5.59 ± 0.13^{Db}

[a]Note: Different capital letters after the same row of values indicate significant differences ($p < 0.05$) in pH values of the same group of samples at different times. Significant differences ($p < 0.05$) in pH values between different groups at the same time can be identified by lowercase letters following the same column of values.

2.3.7. Determination of Lactase Activity. Lactase activity in the fermentation broth was measured at 0, 0.5, 1, 2, 4, and 5 h using a lactase test kit. The Coomassie Brilliant Blue Method was utilized to determine the protein concentration of the fermentation broth quantitatively, with a range of 0.1−1.3 g/L. Proportional dilution of the sample before testing is necessary.

2.4. Metabolic Characteristics of KGM and KOGM in Mice. KM mice were housed in the SPF barrier animal laboratory at the School of Pharmacy, Southwest University (Chongqing, China), with a facility license number SYXK (Yu) 2020−0006. The controlled conditions of temperature (25 ± 0.5 °C) and relative humidity (50 ± 5%) were maintained on a 12 h light/dark cycle. After a week of adaptation, the mice were randomly divided into four groups ($n = 9$): Blank, Drug, KGM, and KOGM. The Drug group received Siliankang Bifidobacterium quadruplex live bacterial tablets (195 mg/kg), the KGM group received KGM (195 mg/kg), the KOGM group received KOGM (195 mg/kg), and the Blank group received an equivalent dosage of normal saline at 10:00 am daily. Throughout the experiment, parameters, such as weight changes, food and water intake, mental state, activity level, and fur luster, were monitored. After 28 days of gavage, the mice were euthanized, and their eyeballs were removed for serum extraction via centrifugation at 3000 rpm for 15 min, after which the serum was stored at −80 °C. Subsequently, the small intestine was removed, and lactase activity and other intestinal indicators were measured. The spleen and thymus were collected, cleaned of surface blood stains with ice physiological saline, and dried with filter paper to determine the organ mass. Moreover, a sterile collection of colon contents was performed to determine SCFAs and gut microbiota. All procedures involving animals adhered to the ARRIVE guidelines and were approved by the Laboratory Animal Welfare and Ethics Committee of Southwest University.

2.4.1. Determination of Small Intestine Lactase Activity in Mice. The measurement method is the same as described in Section 2.3.7.

2.4.2. Organ Index Measurement. The measurement of the organ index refers to the following equations:

$$\text{spleen index} = 10 \times \frac{\text{the weight of spleen(mg)}}{\text{mouse weight(g)}}$$

$$\text{thymus index} = 10 \times \frac{\text{the weight of thymus(mg)}}{\text{mouse weight(g)}}$$

2.4.3. Determination of Serum and Liver Indicators in Mice. The reagent kit's operating instructions were referred to when conducting the test. The serum biochemical indicators to be tested were TG, T-CHO, FFA, lgA, and lgG, and the liver indicators were TP, TG, and T-CHO.

2.4.4. Determination of Small Intestinal Indicators in Mice. The mouse ileum tissue was fixed with 4% paraformaldehyde and dehydrated by using an automated dehydrator. Subsequently, the tissue was embedded, sliced, and dewaxed with hematoxylin staining to water. After that, the slices were sealed with neutral gum and observed under a microscope using a BA 400 Three-Camera Digital Micro System (McAudi Industrial Group Co., Ltd., China). Finally, the chosen area was taken in pictures at 200× magnification to measure the villus height, crypt depth, and intestinal wall thickness.

2.4.5. Determination of SCFAs in the Colon of Mice. In a sterile environment, three portions of mouse colon contents were taken and mixed with a saturated sodium chloride solution in a ratio of 1:5 (m:v). The mixture was centrifuged at 10000 rpm for 10 min, and the supernatant was collected for SCFA determination using the same method as described in Section 2.3.5.

2.4.6. Determination of Microbial Diversity in the Colon of Mice. The MO BIO Laboratories' PowerSoil DNA Isolation Kit protocol, following the approach of Zou et al., was utilized to extract total bacterial DNA from the colons of mice.[18] After the purification and pooling of the chosen samples had been conducted, they were sequenced for bacterial rRNA genes on the Illumina HiSeq 2500 platform.

2.5. Data Analysis. The data were represented as mean ± standard deviation ($\bar{x} \pm s$) ($n = 3$) and statistically analyzed using SPSS 23.0 software, with Duncan's one-way ANOVA employed for comparison between data ($p < 0.05$). Mapping and analysis were conducted using Adobe Photoshop CS5, Origin Lab 2018, Mathur version v.1.30, and Image-Pro Plus 6.0 (Media Cybernetics).

3. RESULTS AND DISCUSSION

3.1. Role of Colon Fermentation in Human LI. *3.1.1. pH Measurement.* Table 1 indicates that the pH of each Lactose group significantly decreased ($p < 0.05$) within 4 h of fermentation. There was no significant difference ($p > 0.05$) in the pH between the Blank groups of the healthy and LI subjects. The pH value of the Lactose group in the healthy subjects was notably lower ($p < 0.05$) than that of LI subjects. It has been widely accepted that bacteria rely on oligosaccharides to produce SCFAs, which consequently decrease the pH level. However, the pH level of the intestinal content in mice is affected by multiple factors, including the rate of SCFA production, absorption of SCFAs by the intestinal mucosa, and the release of bicarbonate from the mucosal surface.[19,20] Lactase deficiency causes an increase in the fecal lactose content and disrupts the gut microbiota structure, leading to a decrease in the lactose fermentation ability. As a result, lactose cannot be fully converted into SCFA, and intermediate metabolites like lactate accumulate.[21] Consequently, the Lactose group of LI

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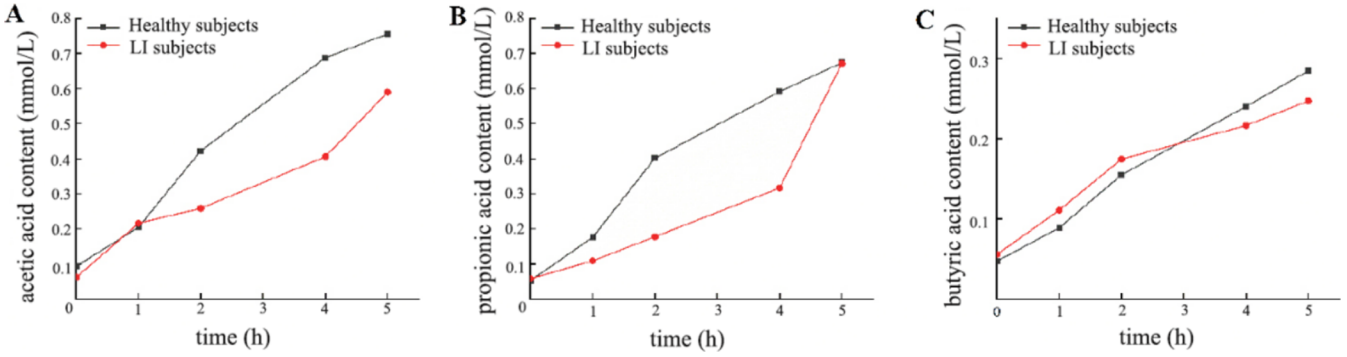

Figure 1. *In vitro* production of acetic acid (A), propionic acid (B), and butyric acid (C) by fecal bacteria of healthy and LI subjects.

subjects has a higher pH compared to the healthy subjects, possibly due to the reduced acid production ability resulting from the lack of lactase.

3.1.2. Determination of SCFA Content. Figure 1 shows that healthy subjects had a higher yield of acetic acid and propionic acid within 4 h, while at the end of the fifth hour, there was no significant difference between the two subjects in the concentrations of propionic acid ($p > 0.05$). Figure 1C shows that LI subjects' butyric acid content was higher than healthy subjects within the first 3 h and then decreased over time. The total SCFAs reached 1.71 mmol/L in healthy subjects, while in LI subjects it was 1.49 mmol/L. Due to a deficiency in lactase in LI subjects, there is a failure to fully convert lactose into SCFA.[21] This result also explains why the pH value of the Healthy group is lower.

3.1.3. Determination of Lactic Acid Content. During the 5 h fermentation period, the lactic acid contents in the fermentation cultures of healthy and LI subjects in the Lactose groups increased significantly ($p < 0.05$), as shown in Figure 2. Between 1 and 3 h, there was no significant difference in lactic acid contents between the Blank group of healthy subjects and LI subjects ($p > 0.05$). However, after 3 h of fermentation, the Blank group of healthy subjects had higher lactic acid contents compared to LI subjects ($p < 0.05$). Except for the 2 h point, there was no notable disparity in the rise of lactate levels between healthy subjects and LI subjects when lactose was introduced at different time points ($p > 0.05$).

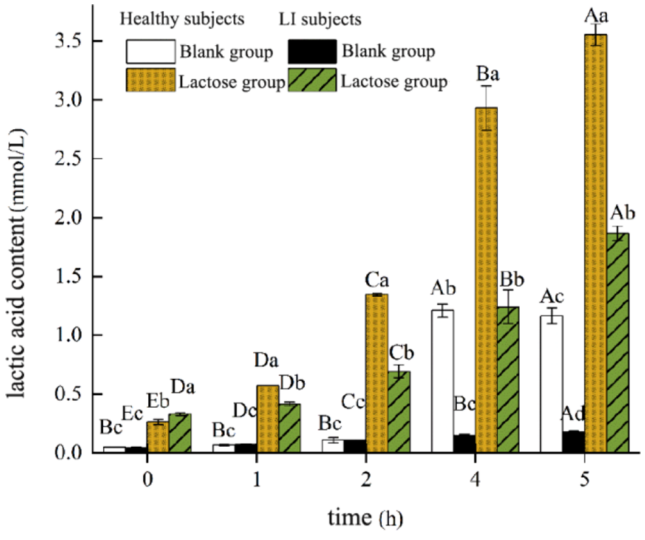

Figure 2. Lactic acid contents in fermented cultures added with lactose or water at different times during fermentation. Note: Different lowercase letters in the figure for different groups at the same time indicate significant differences ($p < 0.05$) in lactate contents among different groups at the same time. The different capital letters labeled at different times for the same group of samples indicate significant differences ($p < 0.05$) in lactate contents corresponding to different fermentation times.

3.1.4. Determination of Lactase Activity. Table 2 shows the changes in lactase activity of the Lactose group and Blank group at 0 and 5 h *in vitro* fermentation broth. It was observed that at 0 h, there was no significant difference between the Blank and Lactose groups in the healthy and LI subjects ($p > 0.05$). However, after 5 h of fermentation, the Lactose group in the healthy subjects exhibited the highest lactase activity, followed by the Lactose group in the LI subjects, with a statistically significant difference between the four groups ($p < 0.05$). Research has shown that premature infants may have limited lactase activity, leading to a lack of lactase secretion and resulting in LI. Lactase supplements can help break down lactose and alleviate symptoms of LI.[21] In this study, it was found that lactose could stimulate the production of more lactase in healthy individuals. The fermentation broth of LI subjects showed lower lactase activity compared with healthy subjects. This deficiency of lactase in the intestines of those with LI may be a contributing factor to the symptoms of LI.

Table 2. Changes of Lactase Activity in Fermented Cultures Added with Lactose or Water at Different Time during Fermentation[a]

		fermentation time/h	
objects	groups	0	5
feces of healthy subjects	blank group	0.00004 ± 0.00000^{b}	0.00040 ± 0.00020^{c}
	lactose group	0.00040 ± 0.00002^{a}	0.00087 ± 0.00005^{a}
feces of LI subjects	blank group	0.00004 ± 0.00000^{b}	0.00007 ± 0.00000^{d}
	lactose group	0.00038 ± 0.00003^{a}	0.00060 ± 0.00005^{b}

[a]Note: Data marked with different lowercase letters after the same column indicate a statistically significant difference ($p < 0.05$).

3.2. Metabolic Characteristics of KGM and KOGM in Mice. *3.2.1. Effects of KGM and KOGM on Mouse Body Weight.* Throughout the entire experiment, the mice maintained a healthy diet and lifestyle, and no changes in their behavior or activity were noted. There were no fatalities or illnesses, and the mice developed normally with glossy fur and

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good mental health. Neither constipation nor diarrhea was observed, and the feces of mice in each group exhibited a normal granular form, indicating their overall satisfactory physical condition.

Figure 3 illustrates the effects of KGM and KOGM on mouse body weight. As the number of feeding days increased, the body

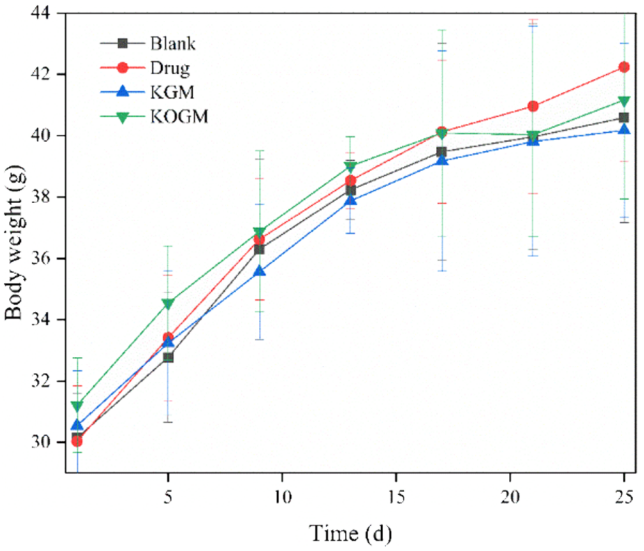

Figure 3. Effects of KGM and KOGM on the body weight of mice.

weight of each group also increased. After 14 days, the rate of body weight growth began to slow down, with the Drug group exhibiting a faster increase in body weight compared to the other groups. By day 25, the weight order of the mice was determined to be Drug group > KOGM group > Blank group > KGM group. While both the Drug and KOGM groups had an increase in weight compared to the Blank group, the weight gain decreased with the administration of KGM. This aligns with the result indicating that KGM can be used as a prebiotic for weight loss.[11] It is believed that KGM can promote satiety, resulting in reduced food consumption by the mice and thus control of weight gain. However, no significant variation in body weight was observed between the four groups, implying that neither KGM nor KOGM had a significant effect on their body weight.

3.2.2. Effects of KGM and KOGM on Lactase Activity in the Small Intestine of Mice. Table 3 reveals that after 4 of feeding,

Table 3. Intestinal Lactase Activity in Different Groups of Mice after 4 Weeks of Feeding[a]

groups	activity (U/mgprot)
blank	0.082 ± 0.002[a]
drug	0.110 ± 0.027[a]
KGM	0.079 ± 0.008[a]
KOGM	0.095 ± 0.026[a]

[a]Note: Letters in the same column of data may differ, indicating a significant difference between the data ($p < 0.05$).

there was no significant difference in the small intestine lactase activity of each group ($p > 0.05$), suggesting that KGM and KOGM could not directly promote lactase activity in the small intestine of mice in the short term. Consequently, their early relief effect on LI symptoms may be achieved through the fermentation of colonic bacteria.

3.2.3. Effects of KGM and KOGM on Spleen and Thymus Indexes of Mice. After 28 d of feeding, the spleen and thymus indexes of mice in the groups, except for the Blank group, experienced a remarkable rise ($p < 0.05$), as depicted in Figure 4. This suggests that KGM and KOGM can enhance the body's immune capacity to some extent.[22]

3.2.4. Effects of KGM and KOGM on Mouse Serum and Liver Indicators. 3.2.4.1. Serum Indicators. The data presented in Table 4 indicate that the concentration of TG in the serum of mice was significantly greater ($p < 0.05$) compared to the Blank group, while T-CHO was significantly reduced. This reduction in T-CHO is of great importance as the serum lipid, especially cholesterol, is a major risk factor for coronary heart disease.[23] Therefore, the significant reduction of T-CHO observed after KGM and KOGM intake has positive implications for health. Furthermore, the intake of KGM and KOGM increased the lgG and lgA values, demonstrating that KGM and KOGM could enhance the immune system of mice.

LI is linked to elevated levels of nonesterified FFA.[24] FFA are simple lipoproteins necessary for cellular energy metabolism in the body, mainly released from TG stored in adipose tissue. Research has indicated that increased FFA levels may act as a protective factor in the manifestation of symptoms in children with LI.[24] As demonstrated in Table 5, the inclusion of KOGM led to a significant 23.04% increase in serum FFA levels compared to the Blank group ($p < 0.05$), whereas both the Drug group and KGM group did not show a significant impact ($p > 0.05$).

3.2.4.2. Liver Indicators. Table 5 shows that the levels of TP, T-CHO, and TG were significantly lower in the Drug and KOGM groups compared to the Blank group. Notably, the consumption of KOGM considerably reduced ($p < 0.05$) TP, T-CHO, and TG in mice. On the other hand, KGM did not have a significant impact on liver lipids in mice, but it was able to effectively reduce serum lipid levels (Table 5). This suggests that KGM had a faster effect on T-CHO levels in the serum than on T-CHO levels in the liver. This is likely because the accumulation of T-CHO levels in the liver requires more time to be effectively reduced. Therefore, it can be concluded that the effect of KOGM on reducing liver cholesterol levels is more powerful than that of KGM.

3.2.5. Effects of KGM and KOGM on the Morphology and Structure of the Mouse Ileum. The physiological integrity of the small intestine, including its normal growth, development, and structural integrity, is essential for ensuring the full absorption of nutrients and maintaining the balance of intestinal microbiota.[24,25] Studies have indicated that lactase in mice is mainly present on the surface of the small intestine mucosa, with its highest activity occurring in the ileum.[26] Figure 5A displays histological observations of the ileum after HE staining, revealing a structurally intact crypt structure with villi arranged in a brush-like pattern. Additionally, the tissue structures of the Drug, KGM, and KOGM groups were all found to be complete and healthy. Figure 5B lists the villus height, crypt depth, and intestinal wall thickness of the ileal tissue in different groups. After 28 d of feeding, the ileal villus height of the Drug, KGM, and KOGM groups of mice was found to be significantly greater than that of the Blank group, while the crypt depth of KGM and KOGM groups was significantly lower than that of the Blank group, with a statistically significant difference being observed ($p < 0.05$). This suggests that KGM and KOGM could effectively increase the villus length of mice, reduce the crypt depth, and increase the villous gland ratio, thus improving the intestinal

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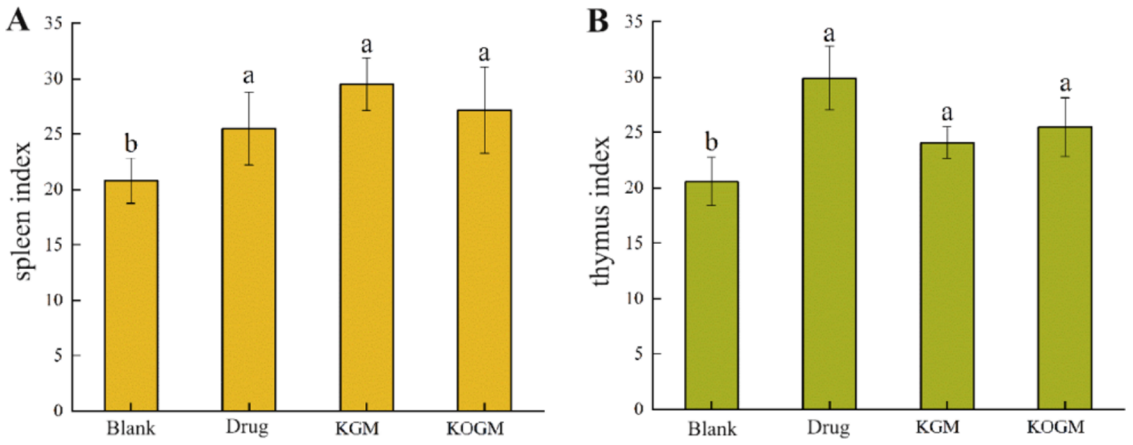

Figure 4. Effects of KGM and KOGM on the spleen index (A) and thymus index (B) in mice. Note: Lowercase letters indicate significant differences between data ($p < 0.05$).

Table 4. Effects of KGM and KOGM on Blood Serum[a]

	lipid metabolism indicators		immune indicators		
groups	TG (mmol/L)	T-CHO (mmol/L)	lgG (μg/mL)	lgA (μg/mL)	FFA (μmol/L)
blank	0.71 ± 0.09^{b}	5.54 ± 0.51^{a}	2.59 ± 0.26^{a}	0.65 ± 0.09^{a}	79.09 ± 13.14^{b}
drug	0.94 ± 0.07^{a}	5.03 ± 0.37^{b}	2.25 ± 0.21^{b}	0.46 ± 0.04^{b}	77.65 ± 5.93^{b}
KGM	0.92 ± 0.04^{a}	4.40 ± 0.14^{c}	1.90 ± 0.21^{c}	0.51 ± 0.07^{b}	75.36 ± 8.94^{b}
KOGM	0.86 ± 0.08^{a}	3.90 ± 0.24^{d}	2.62 ± 0.24^{a}	0.51 ± 0.08^{b}	97.31 ± 19.80^{a}

[a]Note: Variations in lowercase letters after the same column of values demonstrate a significant difference between the data ($p < 0.05$).

Table 5. Effects of KGM and KOGM on Liver Lipid Levels in Mice[a]

groups	TP (gprot/L)	T-CHO (mmol/g)	TG (mmol/g)
blank	0.89 ± 0.08^{bc}	3.12 ± 0.48^{a}	1.60 ± 0.23^{a}
drug	1.05 ± 0.13^{a}	2.44 ± 0.13^{b}	1.29 ± 0.30^{b}
KGM	0.93 ± 0.04^{b}	3.35 ± 0.25^{a}	1.41 ± 0.13^{ab}
KOGM	0.87 ± 0.04^{c}	2.71 ± 0.42^{b}	1.35 ± 0.22^{b}

[a]Note: Letters that differ after the same column of values demonstrate a significant distinction ($p < 0.05$).

absorption area and allowing the body to absorb and utilize the necessary nutrients. Additionally, the intestinal wall thickness of the Drug, KGM, and KOGM groups was remarkably greater ($p < 0.05$) than that of the Blank group, indicating that KGM and KOGM could effectively increase the intestinal wall thickness of mice, which affected the absorption function of the small intestine.[26,27] The length of villi and the depth of crypts are important indicators of intestinal digestion and absorption function and health status. The intake of KGM and KOGM could cause histological and physiological changes in the small intestine of mice, including increased villus length, reduced crypt depth, and increased intestinal wall thickness. This allowed for the absorption of amino acids, inorganic salts, and GLU from the digestive tract into the bloodstream, thus providing the body with various metabolic nutrients. Furthermore, this oscillation could impede the colonization of hazardous bacteria and stabilize the balance of the microbiota.[28,29]

3.2.6. Effects of KGM and KOGM on Colon SCFAs Content of Mice. The data presented in Figure 6 illustrate that the intake of KGM and KOGM significantly increased the SCFA concentration in the colon contents, particularly acetic acid, propionic acid, and butyric acid ($p < 0.05$). Studies have demonstrated that SCFAs generated by the gut microbiota can

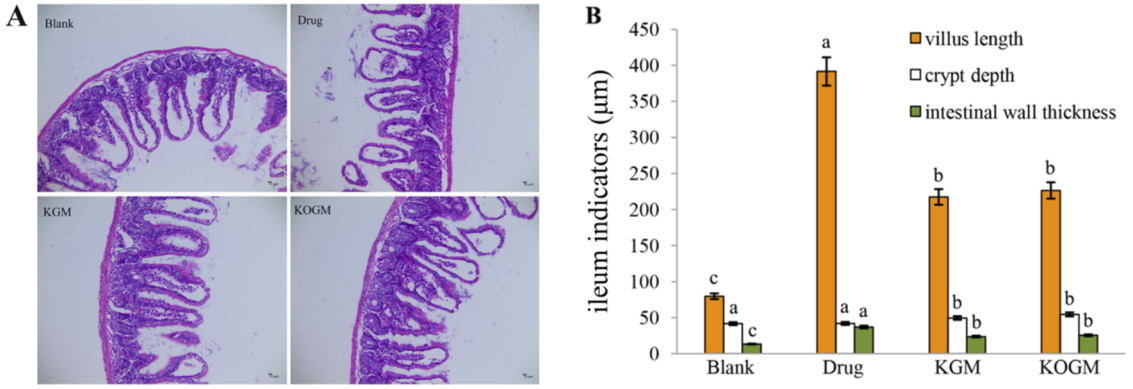

Figure 5. Representative histological sections of the ileum mucosa (A) and effects of KGM and KOGM on the ileum morphology in mice (B). Note: Different lowercase letters in panel (B) indicate significant differences between the two groups of the same indicator ($p < 0.05$).

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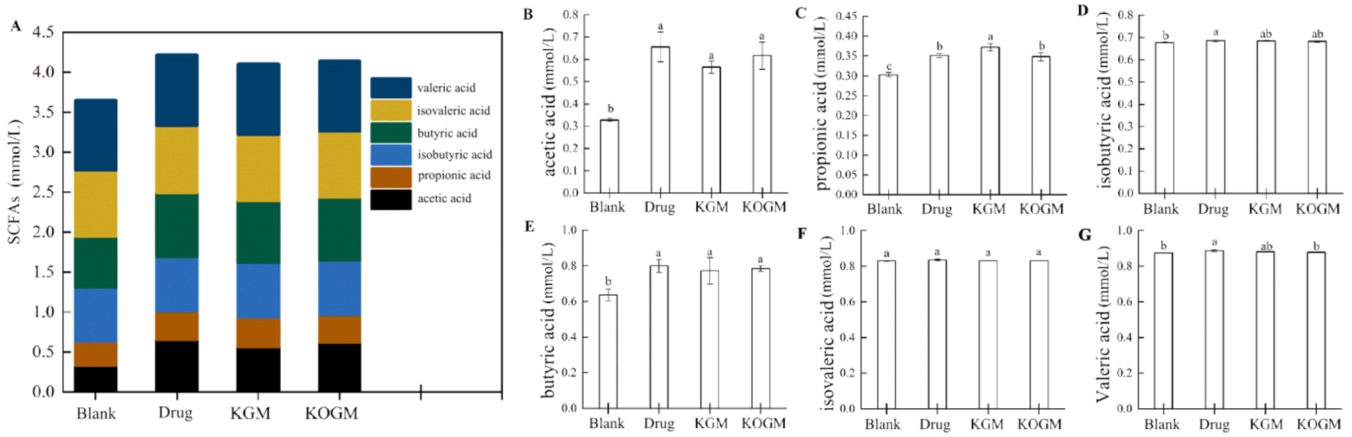

Figure 6. Effects of KGM and KOGM on the SCFAs content in feces of mice (A). The concentration of acetic acid (B), propionic acid (C), isobutyric acid (D), butyric acid (E), isovaleric acid (F), and valeric acid (G). Note: Lowercase letters were used to denote a statistically significant difference between data ($p < 0.05$).

be used as an indirect reflection of the activity of the gut microbiota. Acetic acid is primarily produced by the fermentation of *Bifidobacteria* and *Lactobacilli* in the fecal microbiota and can be oxidized and used by the brain, heart, and peripheral tissues.[30] Propionic acid, mainly produced by the *Bacteroides* strain of intestinal flora, has been found to have a significant impact on liver metabolism and cholesterol, reducing serum cholesterol levels and inhibiting liver cholesterol synthesis.[31] Butyric acid offers a range of advantages to the human body, including providing energy to the intestinal mucosa, protecting against colon diseases, regulating the growth and apoptosis of epithelial and immune cells, inhibiting colitis and colon cancer, possessing anti-inflammatory properties, and regulating oxidative stress.[32] KGM and KOGM exhibited the ability to enhance lactose fermentation in the cecum of mice, leading to the production of SCFAs and resulting in positive effects on the intestines.

3.2.7. Effects of KGM and KOGM on Mouse Colonic Microbiota. 3.2.7.1. Sequencing Data Statistics and Operational Taxonomic Units (OUTs) Analysis. A total of 953,078 pairs of sequencing sequences (Reads) were initially obtained, and after splicing and filtering, 861222 Clean tags were generated. Each sample had a minimum of 88987 Clean tags, with an average of 107,653 Clean tags, and the sample coverage rate was above 96%, indicating that the sequencing quantity of each sample was close to saturation. A similarity level of 97% enabled the determination of the number of OTUs for each sample, which totaled 386. The Blank, Drug, KGM, and KOGM groups had 349, 359, 366, and 353 OTUs, respectively. A Venn diagram (Figure 7) was used to compare the degree of overlap and similarity in the microbial composition between the four groups. It was found that there were 330 OTUs shared by all four groups, with the Blank, Drug, KGM, and KOGM groups containing 1, 6, 2, and 3 unique OTUs, respectively. The Drug and KGM groups exhibited the highest degree of overlap, indicating a strong correlation between the gut microbiota of mice administered KGM and the drug.

3.2.7.2. α Diversity Analysis of Microbial Community. The results of the dilution curve (Figure 8A) and Shannon index curve (Figure 8B) indicated that the representative sample sequence was sufficient and that the sequencing data volume was large enough to reveal a significant number of species in the community. Results from the Ace index (Figure 8C) and Chao1 index (Figure 8D) indicate that compared to the Blank group,

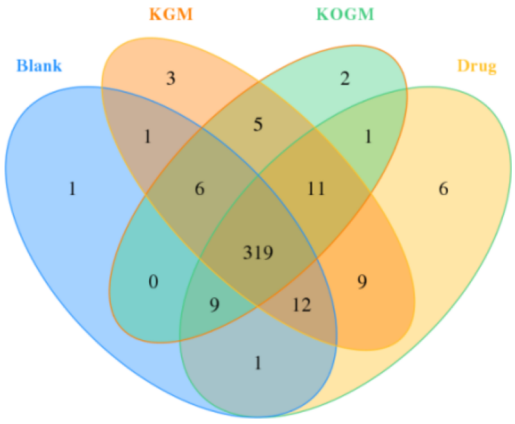

Figure 7. Venn analysis of intestinal flora in mice. Note: Different samples are represented by distinct colors, and the overlapping numbers between the different colored graphs indicate the number of common OTUs among the two samples.

the Drug and KGM groups had increased diversity of mouse colon microbiota, with no significant difference between the two groups ($p > 0.05$). Additionally, when the Simpson index (Figure 8E) and Shannon index (Figure 8F) were used to measure species diversity, compared to the Blank group, the Drug, KGM, and KOGM groups had smaller Simpson indexes and larger Shannon indexes, indicating that Drug, KGM, and KOGM treatment increased the community diversity of mouse colon samples, and the difference between groups was not significant ($p > 0.05$). This suggests that KGM and KOGM could increase the diversity of mouse colon microorganisms, with no notable difference compared to the Drug group ($p > 0.05$).

Analysis of mouse colon contents reveals that the microbiota was comprised of ten phyla, with Firmicutes and Bacteroides being the most abundant. This composition is illustrated in Figure 9A,B. Notably, the two phyla together accounted for around 80% of the relative abundance. In Figure 9A, it is observed that the Firmicutes in both the Drug group and KGM group significantly increased compared to the Blank group, while the Bacteroides decreased significantly. This consistent trend of change was the opposite in the KOGM group. *Lactobacillus acidophilus* and *Bacillus cereus* in the Drug group were found to effectively increase the levels of Firmicutes in the

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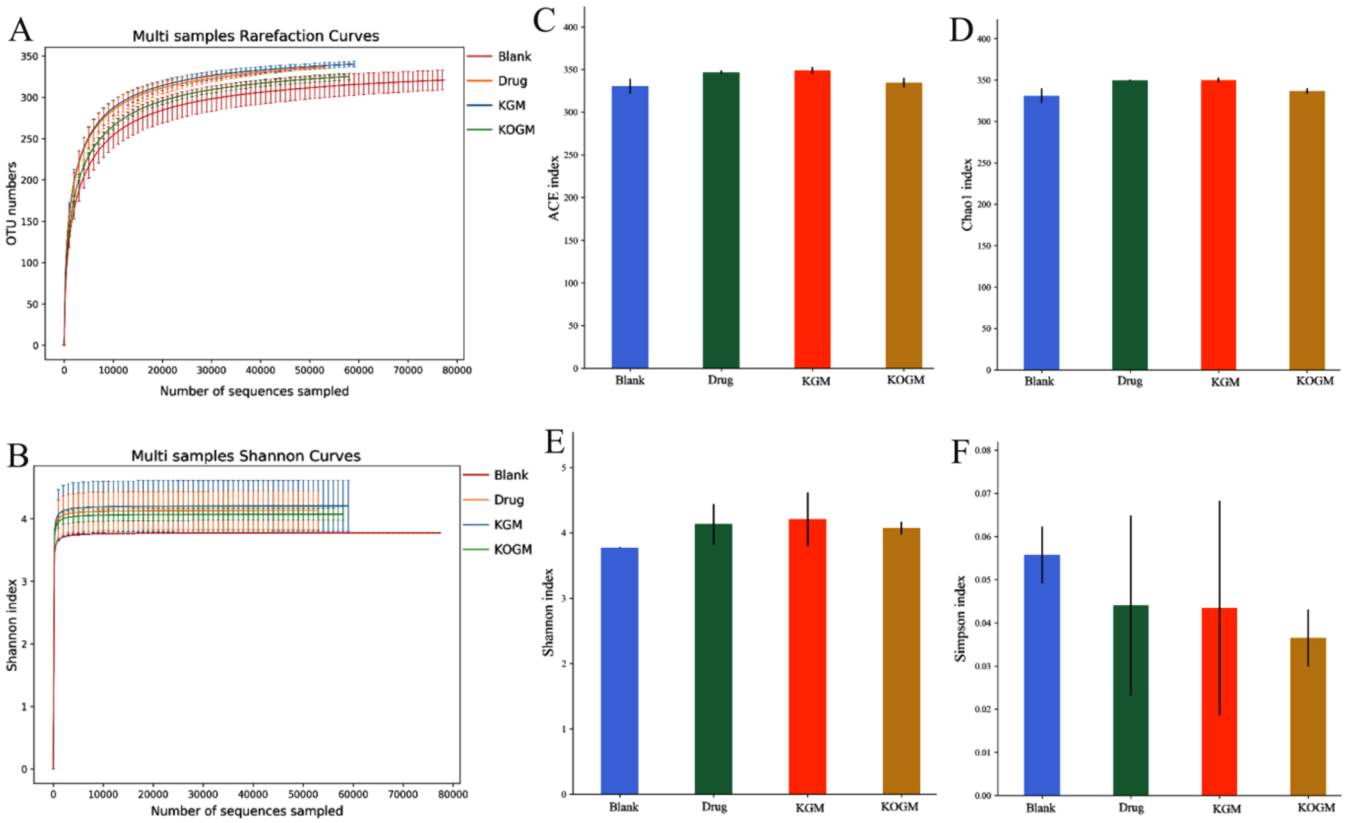

Figure 8. Rarefaction curves (A), Shannon index curves (B), and α diversity indexes of colon content of mice in different groups ((C−F) represent ACE, Chao1, Simpson, and Shannon indexes, respectively).

gut. Consumption of KGM is beneficial for health as it promotes the growth of beneficial bacteria such as *Bifidobacteria* and *Lactobacilli*, making it a high-quality prebiotic.[14] Diets high in fiber, such as KGM, can increase the abundance of Firmicutes and decrease the abundance of Bacteroidetes, leading to higher concentrations of SCFAs in the intestine. These findings are further supported by Figure 9C, which shows a significant increase in *Lactobacillus* in the Drug and KGM groups. KOGM, a functional oligosaccharide derived from KGM degradation, has a lower molecular weight and viscosity but higher solubility.[12,13] Studies have shown that increasing the intake of oligofructose can boost the number of *Bacteroides* in the gut.[14] Figure 9E also confirms this finding, as the relative abundance of *Bacteroides* increased significantly in the KOGM group while decreasing in the Drug and KGM groups.

Figure 9C−F reveals species distribution, indicating that *Lactobacillus* and *Lachnospiraceae NK4A136_group* are among the ten most abundant genera. In comparison to the Blank group, the abundance of *Lactobacillus* in the intestines of other groups of mice showed a significant increase. Specifically, the Drug group saw an increase of approximately 25.82%, the KGM group increased by 18.23%, and the KOGM group increased by 8.67%. Compared to the Blank group, the Drug, KGM, and KOGM groups significantly amplified the presence of *Lachnospiraceae NK4A136_group* ($p < 0.05$), whereas the levels of *Bacteroides* were significantly lower in the Drug and KGM groups ($p < 0.05$), with no substantial disparity between the KOGM and the Blank groups ($p > 0.05$). Furthermore, *Akkermansia* abundance was considerably reduced in the Drug, KGM, and KOGM groups ($p < 0.05$). The *Lactobacillus*, *Lachnospiraceae NK4A136_group*, and *Bacteroides* are beneficial for colonic fermentation and can reduce inflammation, making them ideal intestinal probiotics.[33,34] *Lachnospiraceae NK4A136_group* exhibits anti-inflammatory properties and promotes the repair of intestinal mucosa.[35] A KGM intervention executed by Zhang et al. to forestall loperamide-induced constipation in mice produced evidence of the microbiota being restored, mainly with the levels of *Lactobacillus* being notably improved.[36] Catarina et al. utilized β-mannanase to produce KOGMs with varying molecular weights through enzymatic hydrolysis and examined the impacts of KOGMs on the population dynamics of human fecal bacteria and the generation of SCFAs. The outcomes demonstrate that low molecular weight KOGM can raise the production of butyric acid in the colon, regulate the gut microbiota, and act as an intestinal prebiotic.[37] This is consistent with our previous research findings. Thus, the amelioration of LI through KGM and KOGM is achieved by raising the levels of *Lactobacillus* and *Lachnospiraceae NK4A136_group*, respectively.

4. CONCLUSIONS

This study has provided preliminary evidence that KGM and KOGM have the ability to hydrolyze lactose in the colon, resulting in increased production of intermediate and final metabolites of microorganisms, which may be associated with the occurrence of LI. Furthermore, these substances have been shown to enhance the immune system, mitigate illnesses related to cholesterol and triglycerides, and improve the morphology of the ileum tissue in mice, including increased villus height and intestinal wall thickness as well as decreased crypt depth. Additionally, KGM and KOGM can elevate the total acid concentration of SCFAs in colon contents, particularly acetic,

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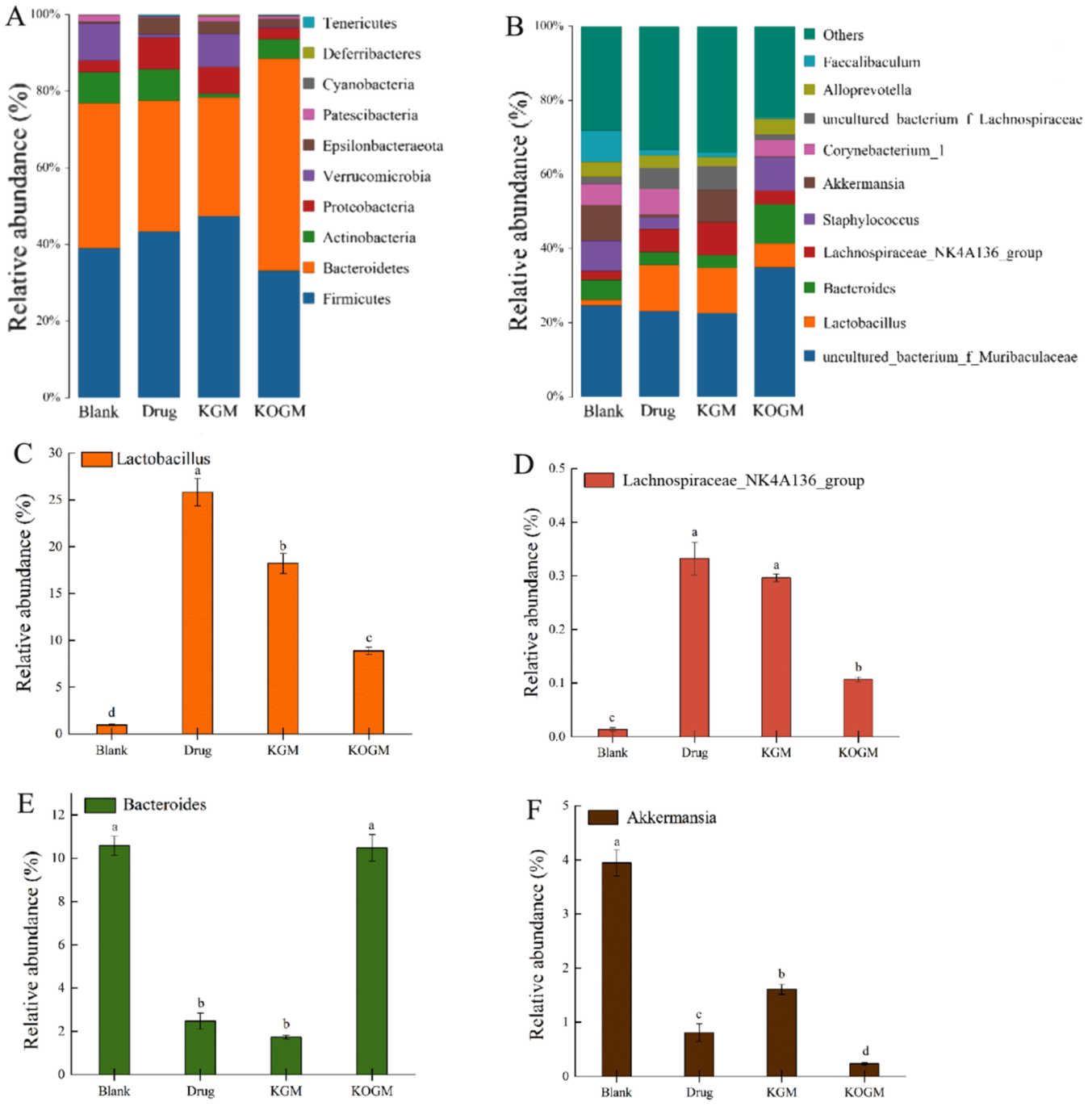

Figure 9. Different composition of gut flora in mice on the phylum level (A) and genus level (B). Different composition of gut flora in mice on genus level: The relative abundances of *Lactobacillus* (C), *Lachnospiraceae NK4A136_group* (D), *Bacteroides* (E), and *Akkermansia* (F).

propionic, and butyric acid. Moreover, they can improve colon fermentation by boosting the presence of beneficial gut microorganisms such as *Lactobacillus* and *Lachnospiraceae NK4A136_group*. In conclusion, the utilization of KGM and KOGM holds promise for creating a favorable intestinal environment, improving the metabolism of the mouse intestinal microbiota to produce SCFAs, promoting colon fermentation, and potentially serving as prebiotic products to alleviate LI.

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Author Contributions

L.D.: Conceptualization, data processing, analysis, writing, and submission. G.Z.: Gastrointestinal tissue analysis. D.Z.: Preparation of KOGM and animal experiments. Z.Z.: Conceptualization. Y.P.: Overall conception and revision of the manuscript. All authors have read and agreed to the published version of the manuscript.

Notes

The authors declare no competing financial interest.

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