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Effects of Oat β-Glucan and Barley β-Glucan on Fecal Characteristics, Intestinal Microflora, and Intestinal Bacterial Metabolites in Rats

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Supporting Information

ABSTRACT: The primary objective was to determine the beneficial effects of oat β-glucan (OG) and barley β-glucan (BG) on gut health. A total of 200 male Sprague−Dawley rats were divided into 5 groups of 40 rats each, control group (CON), low-dose OG-administered group (OGL), high-dose OG-administered group (OGH), low-dose BG-administered group (BGL), and high-dose BG-administered group (BGH). OGL and OGH were administered oat β-glucan by intragastric gavage at a dose of 0.35 g/kg of body weight (BW) and 0.70 g/kg of BW daily for 6 weeks, and BGL and BGH were administered barley β-glucan. The CON received normal saline. Intestinal-health-related indexes were analyzed at baseline, week 3, week 6, and week 7. Cereal β-glucan significantly influenced the fecal water content, pH value, ammonia levels, β-glucuronidase activity, azoreductase activity, and colonic short-chain fatty acid (SCFA) concentrations ($p < 0.05$). Moreover, the population of *Lactobacillus* and *Bifidobacterium* increased ($p < 0.05$), whereas the number of *Enterobacteriaceae* decreased ($p < 0.05$) in a dose-dependent manner during the period of cereal β-glucan administration. These results suggested that cereal β-glucan might exert favorable effects on improving intestinal functions and health but the gut-health-promoting effects of oat β-glucan were better than those of barley β-glucan.

KEYWORDS: *Cereal β-glucan, rat, intestinal microflora, β-glucuronidase, azoreductase*

INTRODUCTION

In healthy individuals, the intestinal flora is composed of 100 trillion viable bacteria, representing 100 or more different bacterial species. These organisms grow on the food components ingested and biocomponents secreted into the alimentary tract by the host, participating in extremely varied types of metabolism in the intestine. Among intestinal microflora, *Lactobacillus* and *Bifidobacterium* are considered beneficial microorganisms, which have positive effects on gut health by exhibiting antimicrobial activities that contribute to the gastrointestinal tract defense system of the host.[1] The intestinal microbiota shows marked stability and constancy but can be altered by many endogenous and exogenous factors. One of the most important intestinal microflora modifiers is the diet.[2]

Recently, there has been an increasing interest in the functional properties of dietary fiber. Dietary fiber is the edible part of plants or analogous carbohydrates that resists hydrolysis by alimentary tract enzymes.[3] Many studies have demonstrated that some dietary fiber could act as prebiotics, modulating the intestinal microbiota and improving the intestinal environment.[4−6] Therefore, it is possible to achieve an optimal intestinal microbiota and intestinal environment by the intake of a nutritionally well-balanced diet and some sources of dietary fibers.

One of the most important members of the dietary fiber family is cereal β-glucan, which widely exists in barley and oats. Cereal β-glucan is a kind of non-starch polysaccharide composed of (1 → 4)- and (1 → 3)-linked β-D-glucopyranosyl units in varying proportions. The β-glucan content of cereals varies considerably from 50 to 110 g/kg in barley and from 30 to 70 g/kg in oat.[7,8] Many studies have indicated the beneficial effect of β-glucan in hypercholesterolemia, abnormal glucose, and insulin response.[9−12] In addition, cereal β-glucan has been shown to have a potential immunomodulating effect and antineoplastic properties.[13]

Some studies *in vitro* and *in vivo* have demonstrated that cereal β-glucan and products containing cereal β-glucan can act as a substrate for microbial fermentation and selectively stimulate the growth and activity of a small number of beneficial bacteria, such as *Bifidobacterium* and *Lactobacillus*.[14−18] A comparison of effects of purified oat β-glucan and barley β-glucan on intestinal microbiota and fecal metabolites, however, remains deficient.

In the present study, we investigated the effects of different cereal-derived β-glucan on the fecal water content, pH, and colonic microbiota, especially bacterial metabolites in rats. Moreover, this research compared the prebiotic effectiveness of oat β-glucan and barley β-glucan from the perspective of the molecular weight and the level of insoluble β-glucan. The aim was to discuss the intestinal health-regulating mechanisms of different cereal-derived β-glucan, mainly from two aspects to introduce the beneficial bacterial metabolites of cereal β-glucan

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and the harmful bacterial metabolites of cereal β-glucan. The results of this study have relevance to the use of purified cereal β-glucan products as dietary supplements for human consumption.

MATERIALS AND METHODS

Chemicals. Amaranth, *p*-nitrophenyl-β-D-glucuronide, trypsin, and α-amylase were purchased from Sigma Chemical Company (St. Louis, MO). Other chemicals were analytical-reagent-grade.

Cereal β-Glucan. Cereal β-glucan samples used in the experiment were prepared by a two-step method of ethanol enzymatic and water from oat bran or naked barley according to the method by Shen et al.[19] Commercial oat bran or naked barley was comminuted, and the powder that passed through a 0.5 mm screen was suspended in 60% (v/v) ethanol at a final concentration of 0.05 mg/mL (w/v). Trypsin was added to the sample suspension (60 units/g of dry oat bran or barley flour) at pH 8.0, and the mixture was incubated at 50 °C for 40 min. The pH of the sample was then adjusted to 6.7; α-amylase was added to the mixture (35 units/100 mL of the mixture) and mixed; and the mixture was incubated for 30 min. The samples were then centrifuged (2700*g* for 15 min); the supernatant was decanted; and the precipitate was collected. The desiccated precipitate was then extracted with hot water (pH 10.0 at 80 °C) at a solid/liquid ratio of 1:10 for 60 min in a boiling water bath. The resulting solution was further centrifuged (2700*g* for 15 min) to collect the supernatant. Then, the supernatant was placed in a dialysis sac with a 7000 molecular-weight cutoff (MWCO), dialyzed against running water at 4 °C for 2 days, concentrated, and precipitated with 3 volumes of ethanol. The precipitate, collected after centrifugation (2700*g* for 15 min), was then freeze-dried.

The samples of oat β-glucan and barley β-glucan contained 90 and 87% total β-glucan, respectively. Insoluble β-glucan contents of the oat β-glucan sample and barley β-glucan sample, determined from the method by Aman and Graham,[20] were about 18.5 and 6.4%, respectively. The soluble β-glucan content was determined by subtracting insoluble from total β-glucan content. The average molecular weights of oat β-glucan and barley β-glucan samples, which were determined by gel chromatography on a column of Sepharose CL-4B, were about 1.33×10^5 and 1.0×10^4 Da, respectively.

Experimental Animals and Diets. A total of 200 male Sprague−Dawley rats (6 weeks of age, weighing 160.85 ± 4.74 g) were purchased from the Laboratory Animal Center of Henan Province (Zhengzhou, China) and were housed in a temperature-controlled animal room (20 ± 2 °C) with a 12 h light/dark cycle and humidity of 50 ± 5%. The animals were randomly selected and assigned to five groups (40 rats per group): the control group (CON), the low-dose OG-administered group (OGL), the high-dose OG-administered group (OGH), the low-dose BG-administered group (BGL), and the high-dose BG-administered group (BGH). The OGL and OGH groups were administered oat β-glucan by intragastric gavage at a dose of 0.35 g/kg of body weight (BW) and 0.70 g/kg of BW daily for 6 weeks, and BGL and BGH groups were administered barley β-glucan. The CON group was given a placebo (normal saline) for 6 weeks. The body weight was monitored every 2 weeks. During the experimental period, all rats had free access to the standard diet (Laboratory Animal Center of Henan Province) and water.

Measurement of Fecal pH and Water Content. The feces were collected at baseline, week 3, week 6, and week 7 (after stopping cereal β-glucan administration for 1 week). Then, the fresh fecal samples were diluted 10-fold (w/v) in distilled water and homogenized (Vortex-Genie 2, Service Science and Technology, Beijing, China), and the pH was measured with a pH meter (PHS-3C, Sheng Ci, Shanghai, China). The water content of fecal samples was measured by drying fecal samples in a drying oven (105 °C for 24 h). Fecal water content (%) was calculated by

$$\text{fecal water content (\%)} = \frac{W_{wet} - W_{dry}}{W_{wet}} \times 100$$

where W_{wet} and W_{dry} were the weights of the fecal sample before and after drying in the oven, respectively.

Microbial Analysis. Rats were sacrificed, and digesta samples were removed aseptically from the colon at baseline, week 3, week 6, and week 7 (n = 10). Approximately 1 g of the specimen was weighed and diluted in 9 mL of phosphate buffer (0.1 M, containing 0.5% cysteine). After homogenization, serial 10-fold dilutions of the homogenates were performed in phosphate buffer under an anaerobic environment (Model 1029 Forma Anaerobic System, Thermo Fisher Scientific, Waltham, MA). A total of 50 μL of each dilution was spread on selective media in triplicate: BBM agar for *Bifidobacterium*, LBS agar for *Lactobacillus*, MacConkey agar for *Enterobacteriaceae*.[21] Plates for *Bifidobacterium* and *Lactobacillus* were incubated anaerobically (Model 1029 Forma Anaerobic System, Thermo Fisher Scientific, Waltham, MA) at 37 °C for 48 h, whereas plates for *Enterobacteriaceae* were incubated aerobically (SPX-150, zy-lab, Beijing, China) at 37 °C for 24 h. Colony counts were obtained after incubation, and microbial data were expressed as $\log_{10}$ colony forming units (CFU) per gram of colonic content.

Short-Chain Fatty Acid (SCFA) Assays. At the end of the 6-week experimental period, the rats were autopsied and the colonic contents were collected and then stored at −70 °C prior to analysis. The frozen colonic digesta (0.1−0.2 g) was thoroughly mixed with 2 mL of normal saline solution followed by acidification with 1 mL of 50% H_2SO_4 solution. This solution was then extracted with 2 mL of ether, and 1 μL of extract was injected into the gas chromatograph.[22] An internal standard of 0.1% (v/v) geraniol in ethyl ether was added to all samples prior to injection. Standard solutions (acetate, propionate, and butyrate) were prepared by dilution of the individual compounds in ethyl ether to give a final concentration of 0.1% (v/v). A GC-14A gas chromatograph with a flame ionization detector (Shimadzu Corp, Kyoto, Japan) was fitted with a 30 m × 0.53 mm inner diameter fused silica capillary column (PEG-20 M stationary phase). Nitrogen at a column flow rate of 3 mL/min was used as a carrier gas. A column temperature of 80 °C and a detector temperature of 240 °C were used.

Fecal Ammoniacal Nitrogen Assays. Fecal samples were diluted with deionized water in the ratio of 1:5 (w/v), extracting for 60 min with shake extraction at a shaking rate of 150 rpm/min. Then, the fecal suspension samples were centrifuged at 13363*g* for 20 min at 4 °C, filtering the supernatant with a 0.45 μm filter membrane. The ammoniacal nitrogen of the supernatant was determined by an indophenol blue spectrophotometric method.

Determination of the Activities of Fecal Bacterial Enzymes. The fresh fecal samples were diluted (1:100, w/v) by 0.1 M potassium phosphate buffer (pH 7.5), flushed with 10% H_2, 5% CO_2, and 85% N_2 (Model 1029 Forma Anaerobic System, Thermo Fisher Scientific, Waltham, MA), and homogenized (Vortex-Genie 2, Service Science and Technology, Beijing, China) for 1 min. After centrifugation at 2000*g* for 2 min, the supernatant was stored in an anaerobic atmosphere and analyzed β-glucuronidase activity and azoreductase activity by the methods described by Haberer et al. and Lee et al., with slight modification.[23,24] All assays were performed at 37 °C and pH 7.5 in glass bottles with a rubber stopper. The compounds for each assay were pre-incubated for 10 min at 37 °C at an anaerobic incubator before introduction of the substrate.

The β-glucuronidase activity was assayed using 2 mL of a reaction mixture consisting of 0.8 mL of 2 mM *p*-nitrophenyl-β-D-glucuronide and 0.2 mL of the suspended fecal sample, incubated for 60 min at 37 °C, and then stopped by adding 1 mL of 0.5 N NaOH. The stopped reaction mixture was centrifuged at 3200*g* for 10 min. The enzyme activity was measured by monitoring absorbance at 405 nm by a spectrophotometer (Lab-Tech, Hopkinton, MA). The amount of *p*-nitrophenol produced by the enzyme reaction was calculated by comparison to a standard curve.

Azoreductase activity was assayed using 4 mL of a reaction mixture consisting of 2 mL of pre-reduced 0.1 M potassium phosphate buffer (pH 7.5) and 2 mL of the suspended fecal sample. The reaction was started by introduction of 0.1 mL of amaranth solution (final concentration of 0.33 mmol/L) and incubated for 60 min at 37 °C. The assay was sampled at 60 min by withdraw of 0.1 mL aliquotes of

the reaction mixture, and the reaction was terminated by mixing with 0.9 mL of 6% ice-cold trichloroacetic acid. The stopped reaction mixture was centrifuged at 3200*g* for 10 min. The enzyme activity was measured by monitoring absorbance at 540 nm by a spectrophotometer (Lab-Tech, Hopkinton, MA). The amount of amaranth used was calculated by comparison to a standard curve.

Statistical Analysis. All results were expressed as the mean ± standard deviation ($\bar{X} \pm SD$). The results obtained were subjected to the one-way analysis of variance (ANOVA). Duncan's new multiple range test was performed to determine the significant difference between samples using the SPSS 11.5 software (SPSS, Inc., Chicao, IL). The significant difference was set at $p < 0.05$.

RESULTS

Effect of Cereal β-Glucan on Body Weight. Overall, the weight gain in the control group was higher than that in the other groups (OGL, OGH, BGL, and BGH) ($p < 0.05$). The results suggested that both oat β-glucan and barley β-glucan might play a potential role in weight control or weight loss. Furthermore, the body weight of oat β-glucan oral-administration group was significantly lower than that of another group that received the same dose of barley β-glucan ($p < 0.05$) (Figure 1).

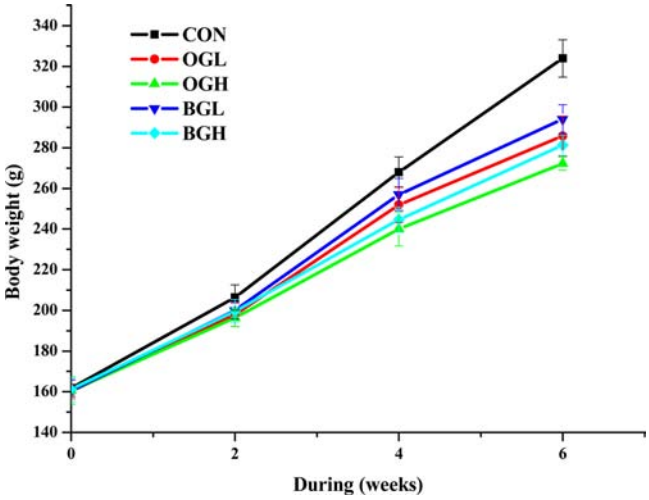

Figure 1. Changes in body weight in rats administered cereal β-glucan for 6 weeks. Values are presented as the mean ± SD ($n = 10$). Abbreviations: OG, oat β-glucan; BG, barley β-glucan; CON, control group; OGL, low-dose OG-administered group; OGH, high-dose OG-administered group; BGL, low-dose BG-administered group; and BGH, high-dose BG-administered group.

Effects of Cereal β-Glucan on Fecal pH Value and Fecal Water Content. Table 1 presents the effect of cereal β-glucan on fecal water contents and fecal pH values in rats. Throughout the experimental period, fecal pH values and fecal water contents in the control group had no obvious change, while changes in fecal pH and fecal water contents were observed in the other groups (OGL, OGH, BGL, and BGH). Noteworthy is that there was no remarkable difference in fecal pH values and fecal water contents among all groups at the baseline (week 0). However, fecal pH values for each cereal β-glucan group (OGL, OGH, BGL, and BGH) significantly decreased in comparison to the control group at week 3, week 6, and week 7 ($p < 0.05$). In addition, no significant difference was observed between the high dosage group and the low dose group. Oat β-glucan and barley β-glucan had a similar effect on lowering fecal pH values. As shown in Table 1, fecal water contents of each cereal β-glucan group (OGL, OGH, BGL, and BGH) were significantly higher than that of the control group at week 3 and week 6 ($p < 0.05$). After stopping cereal β-glucan administration for 1 week, fecal water contents of cereal β-glucan groups, besides BGL, were still higher than that of the control group ($p < 0.05$). The present result showed that oat β-glucan was more efficient in terms of increasing the fecal water content in rats than the same dose of barley β-glucan.

Effects of Cereal β-Glucan on Intestinal Microflora. Table 2 presents the effect of cereal β-glucan administration on the population of colonic *Enterobacteriaceae*, *Lactobacillus*, and *Bifidobacterium* in rats. The mean *Enterobacteriaceae*, *Lactobacillus*, and *Bifidobacterium* counts in the control group were relatively stable, and only a little change occurred throughout the experimental period. Moreover, the administered cereal β-glucan of rats not only had higher populations of *Bifidobacterium* and *Lactobacillus* in the colon but were also characterized by significant lower *Enterobacteriaceae* counts compared to the control group at week 3 ($p < 0.05$), week 6 ($p < 0.05$), and week 7 ($p < 0.05$). These results suggested that the cereal β-glucan administration could significantly improve the intestinal microbiota, and the influence of cereal β-glucan still existed after stopping cereal β-glucan administration. The results of the present experiment also showed that the effect of high doses of cereal β-glucan on modifying intestinal microbiota was better than that of low doses of cereal β-glucan. After 6 weeks of administration, the effect of oat β-glucan on improving intestinal flora was significantly better than that of barley β-glucan at the same dose ($p < 0.05$).

Effects of Cereal β-Glucan on Microbial Metabolites in Feces. Colonic SCFA tended to increase with the cereal β-glucan administration period, as shown in Table 3. There was a statistically significant increase in the total SCFA concentrations ($p < 0.05$) in the OGL group, OGH group, and BGH group compared to the control group, whereas no significant differences ($p > 0.05$) were observed in total SCFA concentrations in the BGL group relative to the control group after 6 weeks. Furthermore, rats in the high-dose group had higher colonic SCFA concentrations than those in the low-dose group ($p < 0.05$). The total SCFA concentrations in rats administered oat β-glucan tended to be higher than rats administered the same dosage of barley β-glucan, but the difference was not significant ($p > 0.05$).

The effects of cereal β-glucan administration on the fecal ammoniacal nitrogen levels are shown in Figure 2. At the baseline, there were no significant differences in the fecal ammoniacal nitrogen in rats among the 5 groups (CON, OGL, OGH, BGL, and BGH). During 6 weeks of administration, the fecal ammoniacal nitrogen levels of the control group rose slowly, while that of the cereal β-glucan groups (OGL, OGH, BGL, and BGH) reduced rapidly. Our results demonstrated that the administration of cereal β-glucan could significantly reduce the fecal ammoniacal nitrogen levels at week 3 ($p < 0.05$), week 6 ($p < 0.05$), and week 7 ($p < 0.05$), which would reduce the stimulation strength to intestinal cells. The positive effect was relative to doses; the higher the dose, the better the efficiency. Under the same dose, the effect of oat β-glucan was better than that of barley β-glucan.

Figures 3 and 4 present the effects of cereal β-glucan administration on the activities of β-glucuronidase and azoreductase in feces. Throughout the entire experiment, fecal β-glucuronidase activity of the control group tended to increase, while fecal β-glucuronidase activity of the cereal β-

Table 1. Effects of Cereal β-Glucan on Fecal Water Contents and Fecal pH Values of Rats[a]

	baseline	administration period		after administration
	week 0	week 3	week 6	week 7
		Fecal Water Content (%)		
CON	43.1 ± 1.5 a	45.0 ± 0.9 d	45.1 ± 0.8 d	44.9 ± 1.1 b
OGL	43.5 ± 1.3 a	51.6 ± 0.6 ab	52.3 ± 0.5 b	50.7 ± 0.9 a
OGH	43.2 ± 1.0 a	52.9 ± 0.7 a	53.7 ± 0.6 a	52.2 ± 1.6 a
BGL	43.2 ± 1.3 a	46.6 ± 0.6 c	48.2 ± 0.5 c	46.2 ± 0.9 b
BGH	43.3 ± 1.6 a	50.9 ± 0.6 b	52.6 ± 0.6 ab	51.6 ± 1.5 a
		Fecal pH Values		
CON	7.25 ± 0.15 a	7.24 ± 0.22 a	7.15 ± 0.18 a	7.20 ± 0.16 a
OGL	7.20 ± 0.13 a	6.29 ± 0.12 b	6.07 ± 0.17 b	6.29 ± 0.14 b
OGH	7.18 ± 0.23 a	6.15 ± 0.17 b	5.85 ± 0.17 b	6.13 ± 0.15 b
BGL	7.13 ± 0.14 a	6.40 ± 0.11 b	6.20 ± 0.14 b	6.31 ± 0.13 b
BGH	7.21 ± 0.16 a	6.24 ± 0.15 b	6.11 ± 0.16 b	6.24 ± 0.10 b

[a]7 weeks = after stopping cereal β-glucan administration for 1 week. Values (mean ± SD; n =10) in a column with different letters are significant (Duncan; $p < 0.05$). Abbreviations: OG, oat β-glucan; BG, barley β-glucan; CON, control group; OGL, low-dose OG-administered group; OGH, high-dose OG-administered group; BGL, low-dose BG-administered group; and BGH, high-dose BG-administered group.

Table 2. Effects of Cereal β-Glucan on the Population of *Enterobacteriaceae*, *Lactobacillus*, and *Bifidobacterium* in the Colonic Sample of Rats[a]

	baseline	during administration		after administration
	week 0	week 3	week 6	week 7
		Enterobacteriaceae		
CON	6.57 ± 0.11 a	6.67 ± 0.10 a	6.52 ± 0.06 a	6.33 ± 0.10 a
OGL	6.55 ± 0.13 a	6.33 ± 0.11 b	5.21 ± 0.04 c	5.31 ± 0.06 c
OGH	6.59 ± 0.08 a	6.10 ± 0.05 c	4.91 ± 0.10 d	5.17 ± 0.05 d
BGL	6.53 ± 0.07 a	6.43 ± 0.11 b	5.33 ± 0.06 b	5.44 ± 0.06 b
BGH	6.54 ± 0.13 a	6.31 ± 0.08 b	5.13 ± 0.05 c	5.25 ± 0.06 cd
		Lactobacillus		
CON	9.19 ± 0.07 a	9.13 ± 0.16 c	9.13 ± 0.16 d	9.18 ± 0.20 c
OGL	9.20 ± 0.08 a	9.56 ± 0.10 b	10.79 ± 0.16 ab	9.90 ± 0.26 ab
OGH	9.22 ± 0.12 a	10.10 ± 0.19 a	11.05 ± 0.19 a	10.21 ± 0.24 a
BGL	9.20 ± 0.16 a	9.43 ± 0.11 b	9.75 ± 0.15 c	9.59 ± 0.13 b
BGH	9.21 ± 0.13 a	9.59 ± 0.09 b	10.67 ± 0.15 b	9.86 ± 0.09 b
		Bifidobacterium		
CON	9.19 ± 0.14 a	9.19 ± 0.15 d	9.23 ± 0.11 d	9.23 ± 0.10 d
OGL	9.18 ± 0.12 a	9.53 ± 0.11 bc	10.96 ± 0.24 b	9.92 ± 0.16 b
OGH	9.22 ± 0.14 a	9.85 ± 0.09 a	11.47 ± 0.12 a	10.65 ± 0.19 a
BGL	9.23 ± 0.19 a	9.41 ± 0.10 c	9.73 ± 0.23 c	9.61 ± 0.10 c
BGH	9.21 ± 0.10 a	9.63 ± 0.11 b	10.64 ± 0.14 b	10.06 ± 0.24 b

[a]7 weeks = after stopping cereal β-glucan administration for 1 week. Values (mean ± SD; n = 10) in a column with different letters are significant (Duncan; $p < 0.05$). Abbreviations: OG, oat β-glucan; BG, barley β-glucan; CON, control group; OGL, low-dose OG-administered group; OGH, high-dose OG-administered group; BGL, low-dose BG-administered group; and BGH, high-dose BG-administered group.

glucan groups (OGL, OGH, BGL, and BGH) tended to decrease. These results suggested that oat β-glucan and barley β-glucan induced a significant decrease in β-glucuronidase activity in a dose-dependent manner ($p < 0.05$). However, the fecal bacterial β-glucuronidase activity was significantly lower in animals administered with oat β-glucan than in rats administered with the same dose of barley β-glucan ($p < 0.05$). During the experimental period, fecal azoreductase activities of the control group and cereal β-glucan groups (OGL, BGL, and BGH) tended to rise. Moreover, fecal azoreductase activities in the OGL group, BGL group, and BGH group had no significant variation in comparison to the control group over time ($p > 0.05$). However, fecal azoreductase activity in the OGH group significantly declined at week 6 and week 7 compared to the control group ($p < 0.05$).

DISCUSSION

It has been reported that cereal β-glucan has the ability to pass undigested through the gastrointestinal tract; therefore, it can act as a substrate for microbial fermentation and selectively stimulate the growth and activity of a small number of beneficial bacteria, such as *Bifidobacterium* and *Lactobacillus*, resulting in the formation of SCFAs.[3,25] However, it is worth noting that mixed linkage β-glucans present in cereals are unlikely to directly provide a carbon source to promote the proliferation of probiotic bacteria in the intestinal tract.[26] However, lactobacilli and bifidobacteria may be able to cross-feed on oligomers, resulting from the hydrolysis of β-glucans by other intestinal bacteria, such as *Bacteroides*, which grew using this polymer and had the ability to hydrolyze β-glucans.[27,28] Moreover, the fermentation products (SCFA) of cereal β-glucan resulted in a lower pH environment in the intestinal

Table 3. Effect of Cereal β-Glucan on the Total SCFA Concentrations and Relative Proportions of Acetate, Propionate, and Butyrate in the Colon of Rats at Week 6[a]

	acetate (%)	propionate (%)	butyrate (%)	total SCFA (mmol/g of colonic digesta)
CON	68.5	17.4	13.9	7.1 ± 0.6 a
OGL	58.5	21.1	20.2	14.9 ± 0.2 b
OGH	57.9	19.5	22.5	25.0 ± 0.7 c
BGL	65.5	19.1	15.2	11.0 ± 0.3 ab
BGH	59.7	19.0	21.2	20.6 ± 0.4 c

[a]Values (mean ± SD; n = 10) in a column with different letters are significant (Duncan; $p < 0.05$). Abbreviations: OG, oat β-glucan; BG, barley β-glucan; CON, control group; OGL, low-dose OG-administered group; OGH, high-dose OG-administered group; BGL, low-dose BG-administered group; BGH, high-dose BG-administered group; and SCFA, short-chain fatty acid.

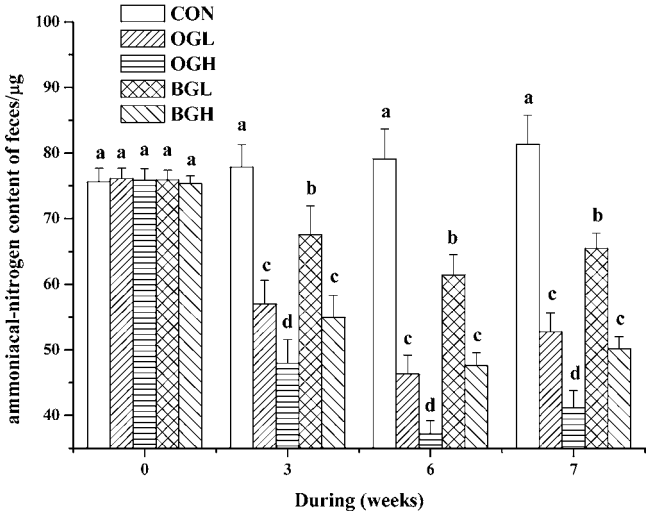

Figure 2. Effect of cereal β-glucan on ammoniacal nitrogen in feces of rats. Units, ammoniacal nitrogen content per gram of wet feces; 7 weeks, after stopping cereal β-glucan administration for 1 week. Values are presented as the mean ± SD (n = 10); within a given time, significant differences among groups are represented by different letters (Duncan; $p < 0.05$). Abbreviations: OG, oat β-glucan; BG, barley β-glucan; CON, control group; OGL, low-dose OG-administered group; OGH, high-dose OG-administered group; BGL, low-dose BG-administered group; and BGH, high-dose BG-administered group.

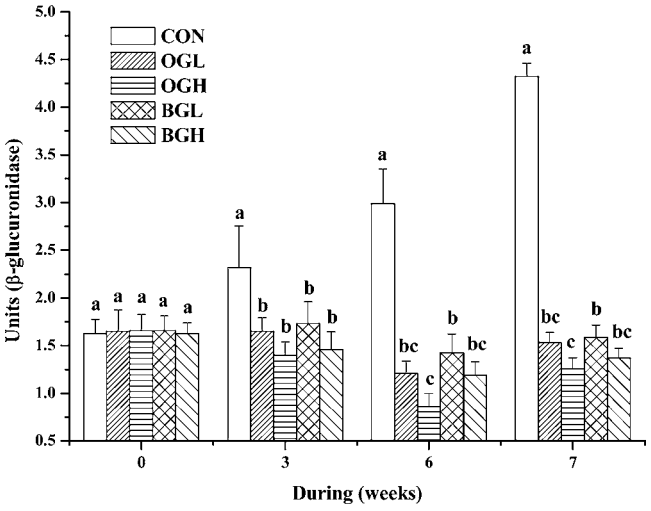

Figure 3. Effect of cereal β-glucan on β-glucuronidase activity in feces of rats. Units, millimoles of p-nitrophenyl-β-D-glucuronide metabolized per hour per gram of wet feces; 7 week, after stopping cereal β-glucan administration for 1 week. Values are presented as the mean ± SD (n = 10); within a given time, significant differences among groups are represented by different letters (Duncan; $p < 0.05$). Abbreviations: OG, oat β-glucan; BG, barley β-glucan; CON, control group; OGL, low-dose OG-administered group; OGH, high-dose OG-administered group; BGL, low-dose BG-administered group; and BGH, high-dose BG-administered group.

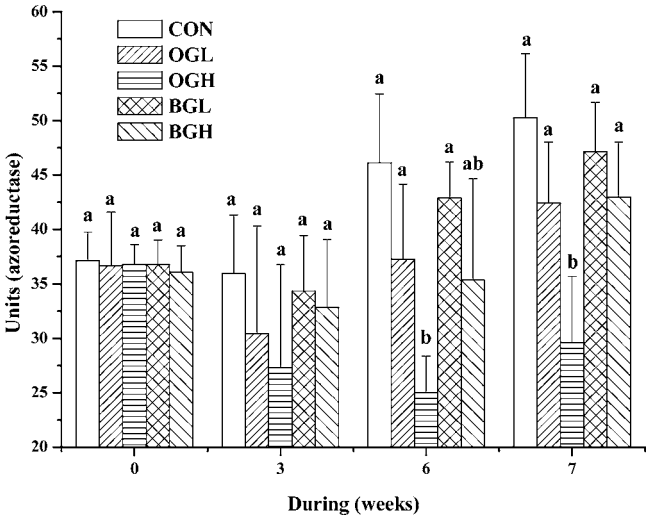

Figure 4. Effect of cereal β-glucan on azoreductase activity in feces of rats. Units, micromoles of amaranth metabolized per hour per gram of wet feces; 7 week, after stopping cereal β-glucan administration for 1 week. Values are presented as the mean ± SD (n = 10); within a given time, significant differences among groups are represented by different letters (Duncan; $p < 0.05$). Abbreviations: OG, oat β-glucan; BG, barley β-glucan; CON, control group; OGL, low-dose OG-administered group; OGH, high-dose OG-administered group; BGL, low-dose BG-administered group; and BGH, high-dose BG-administered group.

tract.[29,30] In the current study, total colonic SCFA concentrations were higher, while fecal pH was lower from ingesting oat β-glucan and barley β-glucan in comparison to the control group. The mildly acidic conditions in the human intestine play a role in inhibiting pathogen growth and influencing harmful enzyme activities in the human intestine.[31] Many studies subsequently demonstrated that the prebiotic effects of cereal β-glucan were related to the viscosity of β-glucan and may also be modulated by fecal bulking of non-fermentable polysaccharides in the large bowel of rats.[28,32] Additionally, previous research has reported that oat β-glucans have the function of regulating intestinal flora, and the effects are related to molecular weight and dosage; the lower the molecular weight and the higher the dosage, the better the effects.[33] However, this study revealed that cereal β-glucan administration exhibited benefits in terms of increased populations of *Bifidobacterium* and *Lactobacillus* and reduced populations of *Enterobacteriaceae* and indicated that oat β-glucan (high molecular weight) was more effective than barley β-glucan (low molecular weight) at the same dose. The reasons for these contradictory results may be due to the different sources of cereal β-glucans. In the oat β-glucan sample, as described above, 18.5% of β-glucan was insoluble, while in barley β-glucan, 6.4% of β-glucan was

insoluble. Water-soluble β-glucans are broken down in the small intestine, whereas the insoluble β-glucans can pass into the large intestine.[34] Therefore, it can be assumed that the higher level of insoluble β-glucan provided by the oat β-glucan samples in the current experiment passed undigested into the large intestine, where it acted as a prebiotic, stimulating the growth of the beneficial bifidobacteria and lactobacilli populations. A previous study that demonstrated that oat-based diets revealed higher ability to enhance beneficial microbial populations compared to barley-based diets confirmed the above inference.[35]

It is known that soluble fibers delay the gastric emptying rate, resulting in a decrease of food intake and, thus, inhibiting weight gain.[36] In contrast, insoluble fibers are of value in reducing weight gain; in that, they may influence food intake by decreasing the hunger feeling.[37−39] Moreover, colonic fermentation products of non-digestible carbohydrates (SCFA) affect proximal gut motility and especially gastric tone, resulting in retardation of gastric emptying.[40] In the present study, oat β-glucan exhibited higher efficacy in terms of inhibition of weight gain compared to barley β-glucan, which is probably because oat β-glucan samples contained more insoluble β-glucan.

The activities of intestinal microflora have a major impact upon the health of the host via the conversion of metabolites and interactions with host cells. Some genera of bacteria in gut have the ability to produce harmful enzymes, such as urease, β-glucuronidase, and azoreductase, which are associated with an increase in tumor growth rate.[41−43]

In the intestinal tract, about 20−25% of urea produced from amino acid degradation is hydrolyzed into ammonia by microbial urease. Ammonia is a toxic metabolite that may enter the bloodstream. High levels of ammonia are harmful to human health.[44,45] The present study demonstrated that administration of cereal β-glucan might reduce the fecal ammoniacal nitrogen concentration. This is consistent with other studies that reported a decrease in the daily excretion of ammonia in response to fermentable non-starch polysaccharides.[46−48]

Bacterial β-glucuronidase seems to play a critical role in the development of colon cancer, because of its wide substrate specificity and hydrolysis of many different glucuronides.[49,50] Azoreductase is related to the formation of aromatic amines, which can be converted to a N-hydroxy compound in tissues to form carcinogenic compounds.[51] The findings by Reddy et al. showed that oat bran could modify the environment of the human intestine by decreasing β-glucuronidase and azoreductase levels.[52] Similarly, Ryhaenen has shown that an oat-bran diet could decrease β-glucuronidase activity compared to the casein diet containing a small amount of fiber in rats.[53] However, Grasten et al. has demonstrated that an oat-bran diet increased the activity of fecal bacterial enzymes (β-glucosidase, β-glucuronidase, and urease) compared to the non-fiber diet.[6] In the present study, both oat β-glucan and barley β-glucan significantly decreased β-glucuronidase activity in feces compared to the control group and high-dose oat β-glucan could also decrease azoreductase activity in feces. The possible reasons were as follows: cereal β-glucans have the potential to act as a prebiotic and selectively promote the growth of *Bifidobacterium*, resulting in a decrease in both β-glucuronidase and azoreductase activity; in that, the activities of azoreductase and β-glucuronidase in *Bifidobacterium* species were almost invariably lower than those associated with other intestinal anaerobes.[54] In addition, the SCFAs produced during cereal β-glucan fermentation in the human intestine were potent inhibitors of intestinal harmful enzymes. Furthermore, some *Bifidobacterium* strains effectively inhibited harmful enzymes (β-glucosidase, β-glucuronidase, tryptophanase, and urease) and ammonia production of intestinal microflora.[55] However, further studies and clinical trials in human subjects are needed to confirm the effects of cereal β-glucan on intestinal harmful enzymes.

In conclusion, cereal β-glucans play an important role in improving the intestinal environment by promoting the beneficial microorganisms, acidifying intestinal tract, and decreasing intestinal inner harmful metabolites. In terms of health functionality, oat β-glucan provides an advantage over barley β-glucan; in that, oat β-glucan contains relatively more insoluble β-glucan.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Nutrient composition of the commercial standard diet for rats (Table S1). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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■ ABBREVIATIONS USED

OG, oat β-glucan; BG, barley β-glucan; CON, control group; OGL, low-dose OG-administered group; OGH, high-dose OG-administered group; BGL, low-dose BG-administered group; BGH, high-dose BG-administered group; BW, body weight; SCFA, short-chain fatty acid

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