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Stachyose-Enriched α-Galacto-oligosaccharides Regulate Gut Microbiota and Relieve Constipation in Mice

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ABSTRACT: This study probed the effects of Deshipu stachyose granules (DSG), a novel oligosaccharide preparation (55.3% stachyose, 25.8% raffinose, and 9.7% verbascose), on gut microbiota and constipation in mice. Mice were administered intragastrically without or with DSG (0.42, 0.83, and 2.49 g/kg bw), and feces were collected after 14 days of treatment and subjected to classical microbiological assays. Selective index (SI) and prebiotic index (PI) were incorporated to evaluate the prebiotic effect. DSG at 0.83 g/kg bw scored the highest SI and PI scores, thus supporting a strong prebiotic role. In addition, the impact of DSG (0.42, 0.83, and 1.68 g/kg bw) on defecation function of constipated mice was determined. Ink propulsion rate in the small intestine was significantly improved by DSG treatment. DSG supplementation also distinctly increased the weight and number of black feces within 5 h and evidently shortened the defecating time of first black feces, as compared with the constipation control mice. All of these findings indicate that DSG may promote the growth of beneficial intestinal bacteria and inhibit pathogenic bacteria and also facilitate intestinal peristalsis and fecal excretion, thereby enhancing intestinal health and relieving constipation.

KEYWORDS: *oligosaccharides, gut microbiota, bifidobacteria, lactobacilli, constipation*

INTRODUCTION

A vast number of microorganisms inhabit the mammalian gut, and their symbiotic and mutualistic relationship with the host (host−bacteria and bacteria−bacteria interactions) determines a complex and dynamic ecosystem.[1] The human gut contains a large variety of bacterial genera, species, and strains, which are either beneficial (e.g, *Bifidobacterium*, *Eubacterium*, and *Lactobacillus*) or detrimental (e.g, *Clostridium*, *Shigella*, and *Veillonella*) to the host's health.[2] It is widely acknowledged that the commensal gut microbiota plays vital roles, although not yet fully understood, in the normal digestive function of the host, maturation of human immunity, brain development, and natural defense mechanism against pathogenic bacteria.[3−5] A healthy gut microbiota (properly balanced bacterial groups) is normally required for human/animal health by maintaining host immune homeostasis and nutrient intake, as well as gut development.[4,5] Accordingly, a balanced gut microbiota composition confers benefits to the host, whereas gut microbiota imbalance may disturb the physiological homeostasis,[6] leading to various diseases such as inflammatory bowel diseases,[7] obesity,[8] colon cancer,[9] neonatal necrotizing enterocolitis,[10] irritable bowel syndrome,[11] and cardiovascular disease.[12]

Constipation, defined as fewer than three bowel movements per week together with symptoms such as pain or straining, is a common clinical problem in both developed and developing countries, afflicting as much as 34% of the population.[13] The relationship between constipation and gut microbiota is indivisible. Patients with chronic constipation generally had changes in the intestinal microbiota, which were characterized by a relative decrease in obligate bacteria and a parallel increase in potentially pathogenic microorganisms and fungi.[14,15] As a result, these alterations could alter the metabolic milieu of the colon with resultant changes in the concentration of physiologically active substances that may influence the motility and secretory function of the bowel.[15] However, the causes for constipation are often multifactorial, such as chronic illness, a low intake of dietary fiber or fluid, physical inactivity, side effects of medication, or symptoms of depression.[16] In this regard, pharmacologic treatment of constipation has been traditionally based on osmotic or secretory laxatives and bulking agents, and these therapies often fail or may have a short-lived efficacy and induce side effects such as bloating and abdominal cramps.[17] For these reasons, there is increasing interest in identifying novel dietary effective phytochemical agent capable of modulating gut microbiota and relieving constipation with few side effects.

Prebiotics are nondigestible food ingredients that selectively stimulate the growth and/or activity of specific species of gut bacteria, usually bifidobacteria and lactobacilli, with benefits to health.[18] It has been confirmed by previous studies that galacto-oligosaccharides and fructo-oligosaccharides can effectively promote the proliferation of specific members of intestinal microbiota (e.g., bifidobacteria).[19−21] The bifidogenic effects of galacto-oligosaccharides and fructo-oligosaccharides are structure-dependent, and bifidobacteria have relatively high amounts of β-galactosidase and β-fructosidase, which allowed these nondigestible oligosaccharides to be selectively fermented by bifidobacteria.[19,21,22] Unfortunately, there is extremely limited information about the role of stachyose-enriched galacto-oligosaccharides in intestinal bacteria. Deshipu stachyose

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granules (DSG) are a kind of health food approved by the China Food and Drug Administration, mainly consisting of nondigestible galacto-oligosaccharides, stachyose, raffinose, and verbascose. The objective of this research, therefore, was to evaluate the regulative effects of DSG on gut microbiota and bowel function in mice. In this study, we determined the effects of DSG on the number of intestinal bifidobacteria, lactobacilli, enteric bacilli, enterococci, and *Clostridium perfringens*. Furthermore, the efficacy in promoting defecation of DSG on constipated mice was also assessed in mice.

MATERIALS AND METHODS

Sample and Chemicals. Deshipu stachyose granules (DSG, approval no. G20040234 by the China Food and Drug Administration) with 5 g of recommended daily allowance (RDA) per person were the commercial α-galacto-oligosaccharides preparation derived from a new source, the edible roots of Chinese *Lycopus lucidus* Turcz.[23] DSG consisted of stachyose (55.3%), raffinose (25.8%), verbascose (9.7%), and sucrose (6.9%) by HPLC analysis with a refraction index detector[23] and was obtained from Xi'an Deshipu Bioindustry Co., Ltd. (Xi'an, China). Compound diphenoxylate (approval no. H32022716 by the China Food and Drug Administration) was bought from Changzhou Kangpu Pharmaceutical Co., Ltd. Verbascose, stachyose, raffinose, and sucrose (all >98%) were obtained from Sigma-Aldrich (St. Louis, MO, USA). BBL-agar, the selective medium for bifidobacteria, and bile esculin azide-agar for enterococci were prepared in our laboratory. MRS-agar for lactobacilli and TSC-agar for *C. perfringens* were obtained from Oxoid Ltd. (United Kingdom) and Merck KGaA (Germany), respectively. EMB-agar for enteric bacilli was purchased from Kaifeng City Institute of Medical Biology (China). All other chemicals were of the highest grade available.

Animals. Forty adult male BALB/c mice (16.8−19.1 g), purchased from Vital River Laboratory Animal Technology Co. Ltd. (Beijing), were used to assess the regulatory function on gut microbiota. One hundred adult male Kunming mice (18−22 g), purchased from the Experimental Animal Center of the Fourth Military Medical University (Xi'an, China), were used for the assessment of facilitating feces excretion function. The animals were housed in cages with free access to standard food and water in a standardized animal laboratory maintained at a temperature of 22−24 °C and a humidity of 50−55%. All of the experiments were approved by the Medical Research Committee on Animal Care and Use (SCXK11−00−0008), Disease Control Centre of Shaanxi Province, China.

Assessment for Regulating Gut Microbiota Function of DSG. The effect of DSG on mice intestinal microbiota was investigated in accordance with the literature with some modifications.[15,24,25]

Dosage Selection. A dose of 5 g/day (RDA) of DSG for per person (calculated as 60 kg of body weight (bw)) is equivalent to 0.083 g/d/kg bw for the human body. This study set three dose groups for mice, namely, 5, 10, and 30 times the RDA of DSG for humans. All mice were randomly divided into the following four groups with 10 mice in each group: control group and DSG-treated groups (0.42 g/kg bw of DSG for 5 times dosage, 0.83 g/kg bw for 10 times dosage, and 2.49 g/kg bw for 30 times dosage). DSG dissolved in sterilized water was administered by oral gavage once daily (9:00 a.m.) for 14 consecutive days. In the control group, mice were given sterilized water. The volume of gavage was 0.2 mL/10 g bw.

Fecal Microbiota. Freshly voided fecal samples, collected at 24 h before intervention and 24 h after the last oral gavage, were transferred into desiccated sterile tubes. Fecal samples collected were then dissolved in a sterile saline diluent of 1:10 to obtain homogeneous suspension, and 10-fold serial dilution was implemented. Inoculation was performed in selective media with appropriate dilutions. All inoculated selective media were incubated at a temperature of 37 °C. Specifically, BBL- and TSC-agar were cultured under anaerobic conditions (85% N_2, 10% H_2, and 5% CO_2) in a Bactron anaerobic chamber from Beijing A-Fit Biosciences Ltd. (Beijing, China) at 37 °C for 24 h.

Data Processing and Result Judgement. All experiments were conducted at least in independent triplicate, and data were expressed as the mean ± SD (standard deviation). The concentrations of fecal bacteria were log transformed and expressed as log 10 CFU per gram of wet feces. All statistical analyses were performed using analysis of variance (ANOVA) with SPSS 11.5 software. According to the procedures of ANOVA, homogeneity test of variances was first conducted, and then the F value was calculated if variances were homogeneous. If $F < 0.05$, differences between groups were not significant. If $F \geq 0.05$, $p \leq 0.05$, a paired t test would be applied to compare the differences between experimental groups and control group. For those data with abnormal distribution or with unequal variances, appropriate variable transformation should be performed to satisfy Gaussian distribution or get equal variances. Statistical analysis was carried out using the transformed data, but if the data still did not correspond to the requirement after variable transformation, a rank-sum test should be used for statistical analyses. The difference was considered significant if $p < 0.05$.

The differences of the fecal bacteria (bifidobacteria, lactobacilli, enteric bacilli, enterococci, and *C. perfringens*) concentration between pretest and post-test were compared. If the results met one of the following two criteria, we could determine that the tested sample had a positive effect on gut microbiota regulation: (A) After experimental treatment, fecal bifidobacteria and/or lactobacilli counts increase significantly, fecal enteric bacilli and enterococci counts have no evident changes, and fecal *C. perfringens* counts decrease or have no changes, as compared with the pretest results. (B) After experimental treatment, fecal bifidobacteria and/or lactobacilli counts increase significantly, fecal enteric bacilli and/or enterococci counts increase significantly, but the increased ratio of these is lower than that of fecal bifidobacteria and lactobacilli counts, and fecal *C. perfringens* counts decrease or show no changes, as compared with the pretest results.

Calculation of Selective Index (SI) and Prebiotic Index (PI). The equation used to estimate the SI values is as follows: SI = (Bif/total) − (Ent/total) + (Lac/total) − (Clos/total),[24] where Bif, Ent, Lac, Clos, and total are the numbers of bifidobacteria, enteric bacilli-enterococci, lactobacilli, *C. perfringens*, and total numbers of bacteria at the time of sampling, relative to their respective numbers at the time of inoculation, respectively. The equation assumes that an increase in the populations of bifidobacteria and/or lactobacilli is a positive effect, whereas an increase in enteric bacilli, enterococci, and/or *C. perfringens* is negative. The PI is defined as an increase in bifidobacteria, expressed as the absolute number of new bifidobacteria per gram (CFU/g) of feces divided by the daily dose (in grams) of prebiotic ingested.[25]

Assessment of Facilitating Fecal Excretion Function. The assay of facilitating fecal excretion function of DSG was performed using previously described methods with some modifications.[26]

Dosage Selection. One hundred Kunming mice were subjected to small intestine movement trial and defecation trial (50 mice each). For the two tests, mice were randomly divided into the following five groups with 10 mice in each group: control group, constipation control group, and DSG-treated groups (0.42, 0.84, and 1.68 g/kg bw of DSG for low, medium, and high dosages, respectively).

Establishment of Constipation Model in Mice. Compound diphenoxylate (5.0 mg/kg bw for small intestine movement trial and 10 mg/kg bw for defecation trial) was administered to mice to establish the constipation model. If the ink propulsion rate of mice was significantly declined, defecating time of first black feces was distinctly prolonged, and total weight and number of black feces within 5 h were both evidently decreased after diphenoxylate treatment, in comparison with control mice, it could be concluded that the constipation model of mice was established successfully.

Experimental Manipulation. Before the following two tests, DSG was given to the mice in DSG-treated groups by oral gavage once daily (9:00 a.m.) for 10 consecutive days. The volume by gavage was 0.2 mL/10 g bw. In the control group and constipation control group, the mice were given the same volume of distilled water.

Small Intestine Movement Trial. All of the mice were fasted overnight for 16 h. The next morning, mice in DSG-treated groups and constipation control group received 5.0 mg/kg bw of compound

diphenoxylate, whereas mice in the control group received the same volume of distilled water (0.2 mL/10 g bw). Thirty minutes after administration, DSG-treated mice were given ink together with the DSG application, whereas control mice and constipation control mice were just given ink (0.2 mL/10 g bw). After a 25 min interval, the mice were killed and laparotomized to collect the small intestinal segments of stomach to ileocecal junction. Ink was used as the indicator. The distance from the pylorus to the ileocecal junction was measured as the whole length of the small intestine, and the distance from the pylorus to the frontier of ink was measured as the migration distance of ink. The ink propulsion rate was calculated by using the following formula: ink propulsion rate (%) = migration distance of ink/whole length of small intestine × 100%.

Defecation Trial. With the exception of the application of 10.0 mg/kg bw diphenoxylate in mice, the previous processes on the mice mentioned above were exactly employed to evaluate the defecation characteristics. After given ink, each mouse was separately placed in a cage with compartments (the bottom of the cage was covered with blotting paper), normally receiving water and food. Timing began from providing mice with ink, and then defecating time of first black feces in each mouse and the weight and number of black feces within 5 h were recorded, respectively.

Data Processing. All experiments were conducted in independent triplicates, and data were expressed as the mean ± standard deviation (SD). Methods of statistical analysis were the same as the gut microbiota regulation experiment. The difference was considered significant if $p < 0.05$.

RESULTS

Effects of DSG on Body Weight of Mice. As shown in Table 1, the initial body weights of 0.42, 0.83, and 2.49 g/kg bw of DSG-treated mice (17.63−17.87 g) were not significantly different in comparison with that of mice in the control group (17.88 g, $p > 0.05$). After 14 days of intervention, the final body weight of control mice and those of the mice receiving DSG of 0.42, 0.83, and 2.49 g/kg bw all increased, remaining between 21.39 and 21.58 g. It was found that DSG-treated mice did not differ significantly in their final body weight from control mice ($p > 0.05$), suggesting that DSG had no significant effect on body weight gain of mice.

Table 1. Effect of DSG on Body Weight of Mice[a]

group (g/kg bw)	initial body wt (g)	p value	final body wt (g)	p value
0.00	17.88 ± 0.69		21.58 ± 0.83	
0.42	17.87 ± 0.51	0.969	21.39 ± 0.60	0.656
0.83	17.70 ± 0.61	0.482	21.55 ± 0.75	0.944
2.49	17.63 ± 0.44	0.331	21.40 ± 1.40	0.673

[a]Values are expressed as the means ± SD of 10 mice in each group. The differences between DSG-treated groups and the control group are considered statistically significant if $p < 0.05$.

Growth Promotion Effects of DSG on Intestinal Bifidobacteria and Lactobacilli. Difference of fecal bifidobacteria or lactobacilli concentrations (log 10 CFU/g wet feces) between pretest and post-test was assessed, respectively. As shown in Figure 1A, the increment in fecal bifidobacteria counts of mice treated with 0.42, 0.83, and 2.49 g/kg bw of DSG was 0.06, 0.18, and 0.63 CFU/g when compared to the pretest results, respectively ($p > 0.05$, $p > 0.05$, $p < 0.01$), whereas no change was observed in control mice (0 CFU/g), suggesting that DSG increased the number of bifidobacteria in feces. As depicted in Figure 1B, all three tested dosages of DSG raised the fecal lactobacilli level. DSG at 0.83 and 2.49 g/kg bw significantly elevated fecal lactobacilli concentration, with increments of 0.80 and 0.77 CFU/g from the pretest levels, respectively ($p < 0.01$, $p < 0.01$). In addition, it was also found that the number of fecal bifidobacteria was significantly greater ($p < 0.01$) in 2.49 g/kg bw DSG-treated mice than in the control group (Figure 1A). Similarly, DSG at 0.83 and 2.49 g/kg bw remarkably increased the number of fecal lactobacilli relative to control mice ($p < 0.01$, $p < 0.01$, Figure 1B), suggesting that DSG had the ability to promote the proliferation of lactobacilli, and 0.83 g/kg bw DSG showed a significant effect.

Inhibitory Effects of DSG on Intestinal Enteric Bacilli. When the same group was compared between pretest and post-test, fecal enteric bacilli concentration (log 10 CFU/g wet feces) was found to be declined by DSG (Figure 1C). DSG at the dosages of 0.42, 0.83, and 2.49 g/kg bw caused an decrease in the number of fecal enteric bacilli from 7.47, 7.32, and 7.39 to 7.43, 6.95, and 6.61 CFU/g, respectively ($p > 0.05$, $p > 0.05$, $p < 0.01$), whereas that of untreated control mice slightly rose from 7.40 to 7.48 CFU/g ($p > 0.05$). In comparison with the control group, mice receiving 0.42 and 0.83 g/kg bw DSG had no significant differences in fecal enteric bacilli level ($p > 0.05$), and only 2.49 g/kg bw DSG caused a significant decrease in fecal enteric bacilli level of mice ($p < 0.01$), implying that a high dose of DSG could inhibit the growth of intestinal enteric bacilli.

Quantitative Analysis of Fecal Enterococci and *C. perfringens*. Compared to the pretest results, detrimental fecal enterococci and *C. perfringens* concentrations (log 10 CFU/g wet feces) all decreased after DSG treatment (Figure 1D,E). DSG at the dose of 2.49 g/kg bw caused an evident fall in the level of fecal enterococci from 7.69 to 7.16 CFU/g ($p < 0.01$), whereas the decreases caused by 0.42 and 0.83 g/kg bw DSG were not significant ($p > 0.05$, Figure 1D). Besides, the decreases in fecal *C. perfringens* level caused by 0.42, 0.83, and 2.49 g/kg bw of DSG were all not distinct ($p > 0.05$, Figure 1E). As shown in Figure 1D,E, DSG-treated mice had no significant differences in fecal enterococci and *C. perfringens* population, in either pretest or post-test, relative to untreated control mice, respectively ($p > 0.05$). These results suggest that DSG has no significant influences on the population of intestinal enterococci and *C. perfringens*, which are considered as potentially pathogenic microorganisms.

Prebiotic Effect of DSG Evaluated with SI and PI Indices. To further evaluate the prebiotic effect of DSG, the SI and PI values were calculated. Dosages of 0.42, 0.83, and 2.49 g/kg bw DSG scored 0.86, 2.56, and 2.22 of SI, respectively, relative to −0.47 for control group. Similarly, the PI values of DSG at dosages of 0.42, 0.83, and 2.49 g/kg bw were 3.95×10^9, 1.15×10^{10}, and 1.06×10^{10}, respectively. It was noteworthy that 0.83 g/kg bw DSG scored the highest SI and PI scores, which demonstrated that the 10 times dose of DSG showed the best prebiotic effect.

Effect of DSG on Intestinal Transit in Constipated Mice. To establish the constipation model, mice were intragastrically administered with compound diphenoxylate. In comparison with control mice, the ink propulsion rate of constipated mice significantly declined ($p < 0.01$), defecating time of first black feces was distinctly prolonged ($p < 0.01$), and total weight and number of black feces within 5 h were both evidently decreased ($p < 0.01$, $p < 0.01$, Table 2), suggesting that the constipation model of mice was established successfully.

Figure 2 shows the ink propulsion rates in the small intestine of constipated control mice administered without or with

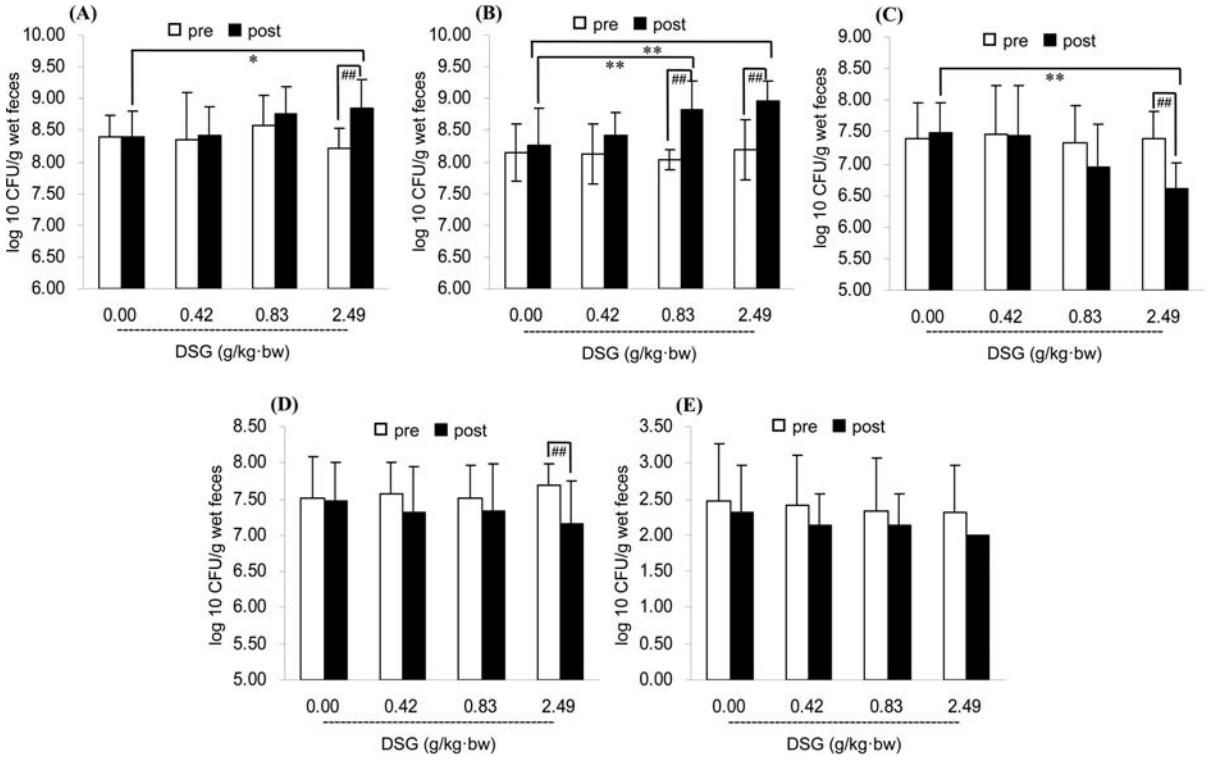

Figure 1. Effects of DSG on the number of intestinal bifidobacteria (A), lactobacilli (B), enteric bacilli (C), enterococci (D), and *Clostridium perfringens* (E). Mice were administered intragastrically without or with DSG at 0.42, 0.83, and 2.49 g/kg bw once daily for 14 consecutive days. The concentrations of fecal bacteria (log 10 CFU/g wet feces) were log transformed. Results are expressed as means ± SD (n = 10): (#) $p < 0.05$ and (##) $p < 0.01$, post-test vs pretest of the mice in the same groups; (∗) $p < 0.05$ and (∗∗) $p < 0.01$, relative to control mice.

Table 2. Comparison of Defecation Characteristics between Mice in the Constipation Group and Mice in the Untreated Control Group[a]

group	ink propulsion rate (%)	defecating time of first black feces (min)	wt of black feces within 5 h (mg)	no. of black feces within 5 h (pellet)
constipation group	36.54 ± 5.81	109.50 ± 6.74	30 ± 10	2.80 ± 0.79
control group	74.00 ± 10.87	77.60 ± 20.17	70 ± 30	5.80 ± 1.99
t value	9.607	4.743	4.196	4.434
p value	<0.01	<0.01	<0.01	<0.01

[a]Values are expressed as the means ± SD of 10 mice in each group.

different dosages of DSG. The average ink propulsion rates of low, medium, and high dose of DSG-treated mice (53.39, 54.29, and 52.52%) were significantly higher than that of constipation control mice (36.54%, $p < 0.01$, Figure 2). The results demonstrated that DSG could effectively expedite the advance speed of ink in the small intestine and apparently improve intestinal peristalsis and, interestingly, the medium dose of DSG was shown to exhibit a relatively better effect. In addition, the body weight gain of the mice subjected to the small intestine movement trial is represented in Table 3. The weight gain caused by DSG at low, medium, and high dose ranged from 6.40 and 6.60 g, which were less than that of constipation control mice (8.20 g), but the differences of weight gain between DSG-treated mice and constipation control mice were not remarkable ($F \geq 0.05$, $p \geq 0.05$, Table 3).

Improvement of DSG on Defecation Function in Constipated Mice. Defecation characteristics of constipated mice after administration of DSG are shown in Figure 3. DSG significantly shortened the defecating time of first black feces, with values of 85.60, 88.20, and 89.00 min for mice in the low-, medium-, and high-dose groups, respectively ($p < 0.01$, $p < 0.01$, $p < 0.01$), as compared to the constipation control mice (109.50 min, Figure 3A). In addition, constipation control mice excreted on average 31.0 mg of black feces within 5 h, whereas 0.42, 0.84, and 1.68 g/kg bw DSG-treated mice evacuated 62.0 mg ($p < 0.01$) and 53.1 and 56.3 mg ($p < 0.05$), respectively (Figure 3B). Furthermore, DSG at the three tested dosages all remarkably increased the number of black feces within 5 h, which were 5.20, 4.80, and 5.00 pellets for low-, medium-, and high-dose DSG-treated mice, respectively ($p < 0.01$, $p < 0.01$, $p < 0.01$), in comparison with constipation control mice (2.80 pellets, Figure 3C). These results clearly indicated that DSG could remarkably shorten defecation time and increase excrement amount of constipated mice, and the low dose of DSG also showed an obvious effect in promoting defecation. Similar to the small intestine movement trial, the body weight gain of mice receiving 0.42, 0.83, and 1.68 g/kg bw of DSG was between 5.20 and 5.50 g in the defecation trial, whereas the body weight gain of constipation control mice was 6.50 g (Table 3). The weight gain of DSG-treated mice did not differ significantly from that of constipation control mice ($F \geq 0.05$, $p \geq 0.05$), suggesting that DSG could decrease the body weight of constipated mice, but the influence was not significant.

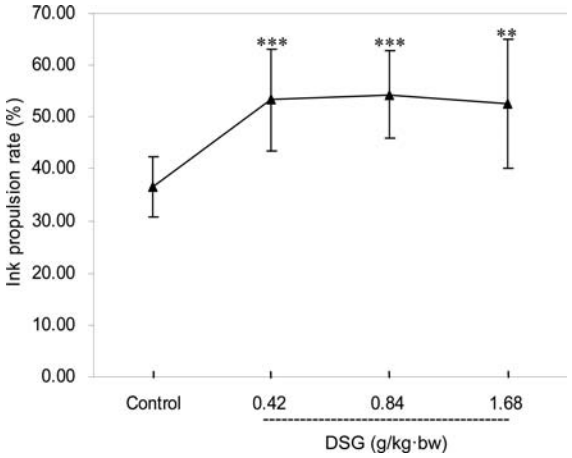

Figure 2. Ink propulsion rate in the small intestine of DSG-treated mice and constipation control mice. Mice were administered without or with DSG at 0.42, 0.83, and 1.68 g/kg bw by gavage for 14 consecutive days. Data in the figure represent the mean ± SD of 10 mice in each group. The asterisks indicate a statistically significant difference between DSG-treated groups and the constipation control group: (*) $p < 0.05$, (**) $p < 0.01$, (***) $p < 0.001$.

DISCUSSION

The present study was to elucidate the regulative effects of DSG, a mixture of novel stachyose-enriched nondigestible oligosaccharides, on gut microbiota and constipation in mice. Oligosaccharides, such as inulin, lactulose, and dietary fiber, have shown a strong prebiotic activity, mostly promoting the selection of lactobacilli and bifidobacteria.[27] Previous studies have demonstrated that galacto-oligosaccharides may stimulate selectively the growth of bifidobacteria and/or lactobacilli in infants,[28] healthy adults,[29] and elderly people.[30] Therefore, this study initially evaluated the influence of DSG on the number of intestinal bifidobacteria, lactobacilli, enteric bacilli, enterococci, and *C. perfringens*. To quantify the effects of DSG on intestinal microorganism, mice were supplemented intragastrically with DSG for 14 days, and the fecal samples were inoculated in selective media to count the number of bacteria. As a result, DSG treatment showed significant effects on mouse intestinal microbiota (Figure 1). The level of fecal bifidobacteria and lactobacilli in mice increased after DSG administration, with statistically significant and highly significant differences, respectively ($p < 0.05$, $p < 0.01$). Additionally, the decrease in the population of enteric bacilli caused by 2.49 g/kg bw DSG was remarkable ($p < 0.01$), and no significant changes were observed in the level of fecal enterococci and *C. perfringens* after DSG treatment ($p > 0.05$), suggesting that the results of intestinal microbiota regulation experiment of DSG are positive and DSG can influence the community structure of gut microbiota by stimulating the growth of beneficial intestinal bacteria, mainly bifidobacteria and lactobacilli, thereby improving the microorganism environment of intestinal tract and helping to maintain the health of the host. At the present time the SI or PI related to the prebiotic effect is in a simple form, reflecting the current definition of a prebiotic,[24,25] and thus SI and PI values were further introduced to quantitatively evaluate the prebiotic effects of DSG in the present study. It was found that 0.83 g/kg bw DSG scored the highest SI (2.56) and PI (1.15×10^{10}) values, indicating that the middle dose of DSG exhibited the best prebiotic effect.

DSG is an oligosaccharide mixture mainly consisting of stachyose, raffinose, and verbascose. Our earlier study discovered that the α-galacto-oligosaccharides preparation from the roots of *L. lucidus* Turcz., with a high stachyose content of 51.8%, 26.5% raffinose, and 10.1% verbascose, possessed the potential of humoral immune-enhancing activity and cellular immune function.[23] In the present study, we for the first time found that daily supplementation with DSG significantly increased the counts of bifidobacteria and lactobacilli, which was consistent with previous findings of fructo-oligosaccharides.[21] Furthermore, the SI score of 0.83 g/kg bw DSG (2.56) is slightly higher than that of fructo-oligosaccharides (2.31) obtained from static batch culture fermentation for 24 h.[24] These results suggest that the novel DSG may act as an excellent prebiotics resource.

The bifidobacteria and lactobacilli are among the generally recognized beneficial species with various health-promoting functions such as the production of short-chain fatty acids, which can acidify gut contents, stimulate intestinal peristalsis, and increase the humidity of the fecal bolus.[31,32] Furthermore, Xu et al. also reported the health benefits of bifidobacteria including modulation of intestinal microbiota, improvement of constipation, and positive effects on intestinal peristalsis.[31] On the basis of the fact that constipation could be influenced by the microbiota composition, we further tested the impact of DSG on bowel function of the constipated mice. To achieve this, we developed the mouse constipation model, which was successfully established by applying compound diphenoxylate. The results of the small intestine movement trial revealed that the ink propulsion rate of DSG-treated mice was significantly higher than that of constipation control mice (Figure 2, $p < 0.01$), suggesting that DSG could invigorate intestinal peristalsis and facilitate forward movement of small intestinal contents toward the colon. In the defecation trial, we compared the defecation characteristics of mice in DSG-treated groups and a constipation control group by measuring their defecating time of first black feces and weight and number of black feces within 5 h. The defecating time of first black feces of mice treated with DSG was conspicuously shorter than that of constipation control mice. In addition, the weight and number of black feces

Table 3. Effect of DSG on Body Weight of Constipated Mice in Small Intestine Movement Trial and Defecation Trial[a]

	small intestine movement trial			defecation trial		
group (g/kg bw)	initial body wt (g)	final body wt (g)	wt gain (g)	initial body wt (g)	final body wt (g)	wt gain (g)
0.42	18.10 ± 0.32	24.70 ± 1.25	6.60 ± 1.17	19.90 ± 0.74	25.10 ± 0.99	5.20 ± 0.92
0.84	18.10 ± 0.32	24.50 ± 1.78	6.40 ± 1.84	19.80 ± 0.63	25.30 ± 0.95	5.50 ± 0.85
1.68	18.10 ± 0.32	24.50 ± 2.07	6.40 ± 1.90	19.80 ± 0.63	25.20 ± 1.32	5.40 ± 1.58
model	18.00 ± 0.00	26.20 ± 1.75	8.20 ± 1.75	19.80 ± 0.79	26.30 ± 2.16	6.50 ± 2.17
		$F = 2.250$ $p = 0.079$			$F = 1.237$ $p = 0.309$	

[a]Values are expressed as the means ± SD of 10 mice in each group.

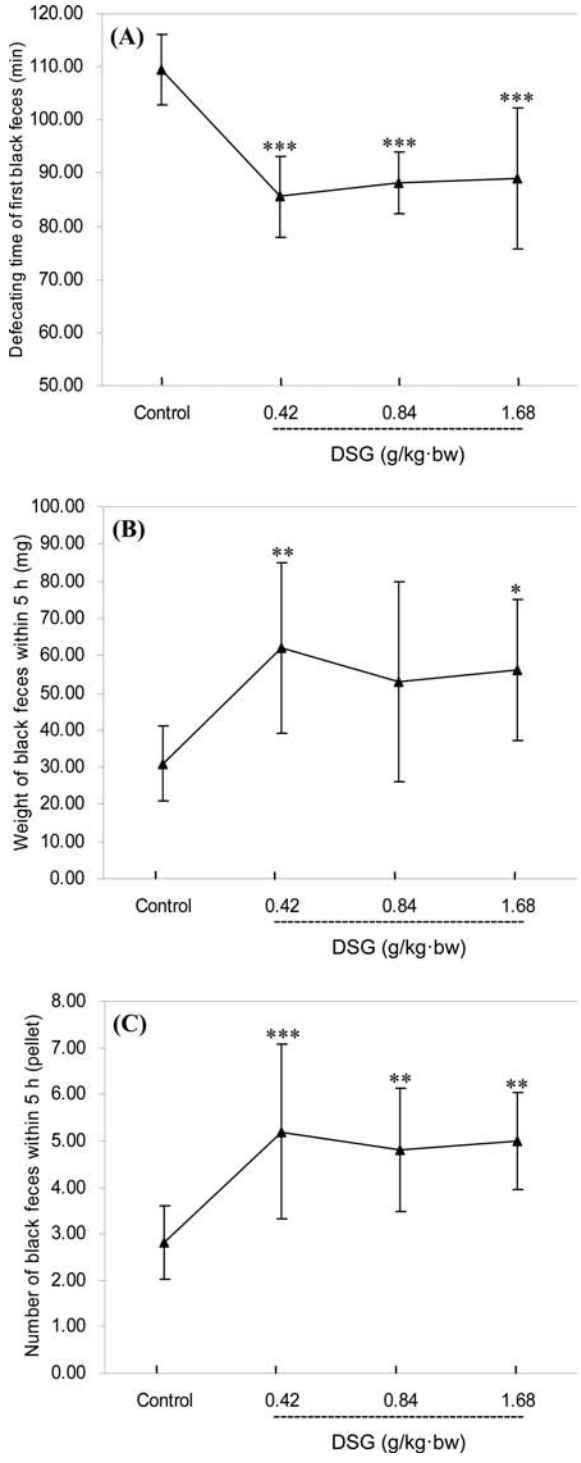

Figure 3. Effects of DSG on defecation function of constipated mice: (A) defecating time of first black feces; (B) weight of black feces within 5 h; (C) number of black feces within 5 h. Timing began from the operation of providing mice with ink. All values are expressed as mean ± SD (n = 10): (*) $p < 0.05$ and (**) $p < 0.01$ indicate that those groups differ significantly from the constipation control group.

within 5 h were both markedly increased by DSG administration (Figure 3). These findings demonstrated that DSG could remarkably shorten the dwell time of gut contents in the intestine and increase the stool bulk, thereby relieving the constipation. Similarly, Yen et al. documented that long-term supplementation of isomalto-oligosaccharides significantly increased the frequency of spontaneous defecation and fecal mass in constipated elderly people.[33] It has also been found that galacto-oligosaccharides seem to relieve constipation in most elderly people.[34] This anticonstipation mechanism of DSG could be partially due to the residual oligosaccharides in the intestinal tract and the increased beneficial intestinal bacteria, which increased the fecal mass and promoted the intestinal peristalsis.[33]

In conclusion, DSG, a novel stachyose-enriched α-galacto-oligosaccharide preparation, exerted a prebiotic and constipation alleviation effect, but had no significant effects on body weight of mice. The results of this study clearly showed that regular consumption of DSG was able to favorably modulate the composition of the intestinal microbiota with an increase in particular bifidobacteria and lactobacilli and a decrease in enteric bacilli. Furthermore, DSG exhibited strong effects on intestinal peristalsis promotion and bowel function improvement. Thus, we conclude that DSG may be used as dietary supplements to enhance human intestinal health and, simultaneously, this study also provides theoretical and experimental bases for the treatment of chronic or occasional constipation.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

DSG, Deshipu stachyose granules; RDA, recommended daily allowance; HPLC, high-performance liquid chromatography; bw, body weight; CFU, colony-forming units; SI, selective index; PI, prebiotic index

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