IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

Prebiotic oligosaccharides from dragon fruits Alter gut motility in mice

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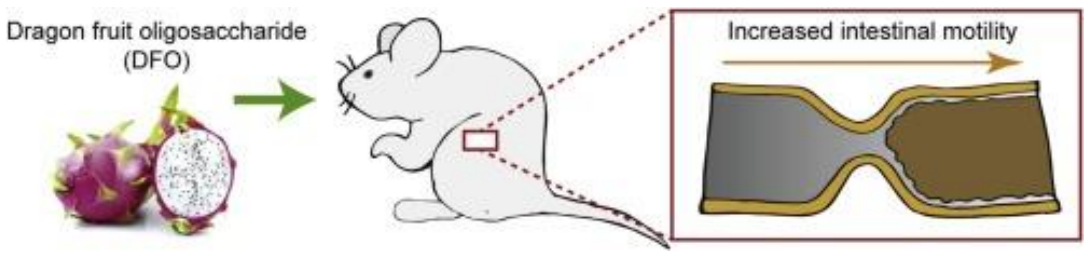
Abstract –

The prebiotic feature of dragon fruit oligosaccharide (DFO) enhances gut health by specifically stimulating the colonic bacteria. Intestinal motility may be impacted by altered microbial makeup. But no research has been done to Comprehend how DFO affects the motor processes of the gut. Thus, the purpose of this study In order to compare the effects of DFO and the prebiotic fructo-Probiotic bifidobacteria with oligosaccharide (FOS).The research's mice 100, 500, and 1000 mg/kg of DFO; 1000 mg/kg of FOS; or got pure water. 1 week of daily administration of 109 CFUBifidobacterium animalis and certain therapies for 2 Weeks. Analyses of gastrointestinal transits revealed smooth motility patterns. Colons' morphological structures and muscle (SM) contractions Wereassessed. FOS, 500, and 1000 mg/kg DFO administration significantly A higher rate of feces than the control group.In contrast to DFO groups, mice treated with FOS and bifidobacteria had shorter gut transit times and longer upper gut transit times. Temporal-spatial maps of DFO increased the number of colonic non-motility, as evidenced by colonic wall movements. Consistent with the results are propagation contractions and fecal pellet velocity.From teams that received FOS and bifidobacteria treatment. DFO likewise raised the SM contractions' amplitude and duration. Histological marks were visible.In all groups, there were normal epithelia, crypts, goblet cells, and SM thickness. In Conclusion: Without morphological changes, DFO increased colonic SM contractions. Worked as a bulk-forming laxative that was stimulating to enhance fecal Intestinal motility and output. Consequently, DFO as a dietary supplement could Address gastrointestinal motility issues and promote gut health.



1.Introduction

Inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), diarrhea, and constipation can all be brought on by an imbalance of the enteric microbiota, which also impacts other gastrointestinal (GI) processes, including motility. Microbial The GI tract's populations have been kept in a balanced state by Prebiotics as well as probiotics. Bifidobacteria and lactobacilli, two types of probiotics, are Living microorganisms that are advantageous to GI health in both humans and animals. Probiotics Reduce both diarrhea and constipation by controlling intestinal motility [6–8]. Probiotics have limitations for people with certain diseases, despite their advantages. Allergies or a pancreatitis attack.Probiotics are difficult to metabolize in some diets and can be destroyed by heat and acid [9, 10]. An alternate strategy for selectively altering the digestive tract's makeup and function Prebiotics are a type of dietary supplement known as microbiota. Prebiotics don't Easily absorbed dietary components that pass through the colon without being changed by Absorption and digestion. They could provide nutritious food for useful Organisms in the colon. Prebiotics not only encourage particular modifications to the They also trigger microbial growth by altering the GI microbiota's makeup and/or activities.Competition and lower bacterial populations that are unwanted. A major Short-chain fatty acids are by-product's of prebiotic fermentation in the colon. (SCFAs)

Abbreviations:

BW, body weight; CFU, colony-forming unit; DFO, dragon fruit oligosaccharide;DP, degree of polymerization; DW, distilled water; FOS, fructoligosaccharide; GI, Gastrointestinal; GIMM, gastrointestinal Motility Monitor; GOS, galacto Oligosaccharides; HE, Hematoxylin-eosin; HPLC, high performance liquidChromatography; IBD, inflammatory bowel disease; IBS, irritable bowel Syndrome; MW, molecular weight; PAS, periodic acid-Schiff; SCFAs, short chain Fatty acids;SM, smooth muscle that include acetate, propionate, and butyrate. SCFAs are energy sources for colonic epithelial cells and play roles in electrolyte Transport, cell differentiation, cell growth, and colonic motility. The best known Prebiotics are fructo-oligosaccharides (FOS), galacto-ol-gosaccharides (GOS),And inulin [15–18]. Other non-digestible oligo-saccharides, such as some Prebiotic-rich fruits and vegetables, have also been tested for prebiotic

Dragon fruit, or pitaya, is used to produce the refined substance known as dragon fruit oligosaccharide (DFO). This fruit has grown in popularity all over the world and is indigenous to Central and South America. Today, it is grown all over Southeast Asia. The food Is abundant in lycopene, vitamin E, vital fatty acids, beta-carotene, and Anti-inflammatory and antioxidant properties. Both red pitaya and white-fleshed pitaya Red pitaya with red flesh (Hylocereus polyrhizus) and Hylocereus undatus Reportedly contain DFO in both its meat and peel, according to reports. In an DFO, an artificial colon, resisted hydrolysis by synthetic gastric juice.And amylase, which encouraged the growth of bifidobacteria and lactobacilli.Despit the fact that DFO's prebiotic characteristics are abundantly obvious in vitro Study on DFO's prebiotic effects on There is no proof in an in vivo model of the prebiotic effects of DFO on GI functions, particularly intestinal motility. When treating intestinal motility disorders by balancing the intestinal flora It's helpful to understand the appropriate dosage for conditions like diarrhea and constipation.Length of prebiotic consumption. As a result, the current study's goal was to find out.The effects of DFO in vivo in male ICR mice. The mice received an addition of DFO for a week and a half. Interest-piqued responses included fecal output and intestinal Colonic motility patterns, transit, evacuation time, and colonic pellet propulsion Velocity, circular and longitudinal SM contractions in the proximal and distal colon,And morphological alterations in the colon.

2. Materials and methods

2.1. Animals

• The animals were housed four per cage and kept under standard Environmental conditions at 23 to 27 °C, with 50 to 55% humidity under A 12-hour light/12-hour dark cycle. They were fed standard commercial Food pellets (Perfect Companion Group Co., Ltd., Thailand) with filtered Water ad libitum.

2.2. Chemicals and equipment

The working solution was made fresh on the day of the experiment. The Gastrointestinal Motility Monitor (GIMM) System for ex vivo study of colonic Propulsive motility was purchased from Catamount Research and Development, St. Albans, VT.

2.3. Extraction and purification method of DFO (briefly)

Hylocereus undatus (Haw) Britt. And Rose, also known as the white-fleshed dragon fruit, was cultivated on and obtained from a certified organic, GAP (good agricultural practice) contract fruit farm in Hat Yai, Songkhla, Thailand. The product was Chosen from a single batch to guarantee chemical uniformity, The oligosaccharide content in particular. The fruits were cleaned and divided. Into portions of meat and peel. The flesh and peel portions were minced. Pieces, which have been coarsely powdered and extracted in a 50-L reactor using the pectinase enzyme. We examined the white-fleshed dragon fruit's sugar composition, which was primarily glucose.Using high-performance liquid chromatography, fructose and certain oligosaccharides Graphies (HPLC) in a prior investigation [25]. MW, or molecular weight, is low. Fraction, fructose and glucose, neither of which have prebiotic qualities, Physiologically eliminated by a two-step yeastculture of Saccharomyces cerevisiae). After that, centrifugation and filtration were used to remove the yeast cells. To create DFO, the pure DFO extract was evaporated and concentrated. powder. In order to maintain stability, the DFO powder was kept at 20 °C and used.every experiment. The mixed oligosaccharides' MW distribution was using mass spectrometry, it was verified. There were four parts to the numbers 716, 700, Relative percentages were 100, 68, 45, and 21 for 490 and 474 Da, respectively.Consequently, combined DFO's degree of polymerization (DP) is 3–4, which is in the similar to some FOSs in range. The nutritional profile of DFO is provided in Table S1 in the Supplement.

2.4 Experimental design, surgical procedure and tissue preparation

After a week of acclimatization, various gavage supplements were given to different animal groups as a daily dietary supplement. For one week, six groups received 0.2 mL of distilled water (DW) (vehicle control) or 100, 100, or 100 mg of DFO. 500 mg/kg, 1000 mg/kg, 1000 mg/kg of FOS, or 109 bifidobacteria CFU. For two weeks, either DW or 500 were added to three groups.With 1000 mg/kg FOS or mg/kg DFO. A description of the experiment Supplemental Fig. S1 provides the procedure over a period of 21 days. All mice had their fecal pellet outputs and food and water intakes recorded.Each day. The animal's colonic propulsive motility processes were Studied ex vivo. Animals were anesthetized by intraperitoneal injection of 70 mg/kg Thiopental sodium (Anesthal®) so the abdominal cavity could be rapidly dissected. The colon was sectioned whole from the cecum to the rectum and placed with contents in oxygenated (5% CO2 and 95% O2) ice-cold Krebs solution (pH 7.4 with an osmolality of 289–292 mmol/kg H2O). To study the effects of DFO on colonic propulsive motility, the whole colon was mounted horizontally in a 50 mL GIMM organ bath containing oxygenated Krebs solution at 37 °C. To study SM contractility, the colon was divided into two sections.The distal colon was divided into two sections, one 3 cm proximal to the rectum and the other 3 cm distal to the cecum. The two pieces were divided transversely into Size of 1 cm. 1 cm segments were used to examine longitudinal SM contraction.Longitudinally suspended in the organ bath. SM contraction in the circular direction, 1 The muscle layer and mesenteric boundary were opened in cm increments.Was split apart. The muscle layer was separated into a strip, and after that In a 20-mL organ bath, suspended such that contractions happened vertically.It includes a Krebs solution.

2.5. Fecal pellet output, gut transit and evacuation time a

Fecal pellets expelled over a six-hour period were counted and hourly averages were computed during treatments. Feces were weighed, dried at 100 °C for 30 minutes, then weighed again to. assess the amount of water present. The waste The formula for calculating water content (%) is ((wet weight – dry weight)/wet weight). 100%. The mice were given an Evan-blue dye to determine the overall intestinal transit time. Marker meal (0.1 mL (i.g.) of 5% Evan-blue in 1.5% methyl cellulose) and were Until the first blue pellet was discharged, seen every 10 minutes. Dispatch time Was assessed using a test for bead ejection. The glass bead was 3 mm in diameter. Via way of the anus 2 cm into the colon with a plastic needle lubricated with petroleum jelly Tip, and the amount of time it took for the bead to come out. Measurements were given to the mice.. The mice were given a meal of charcoal for the purpose of measuring upper gut transit, and 60 minutes later, charcoal transit (%) was determined by the distance of the meal of charcoal/total length of the 100% (small intestine).

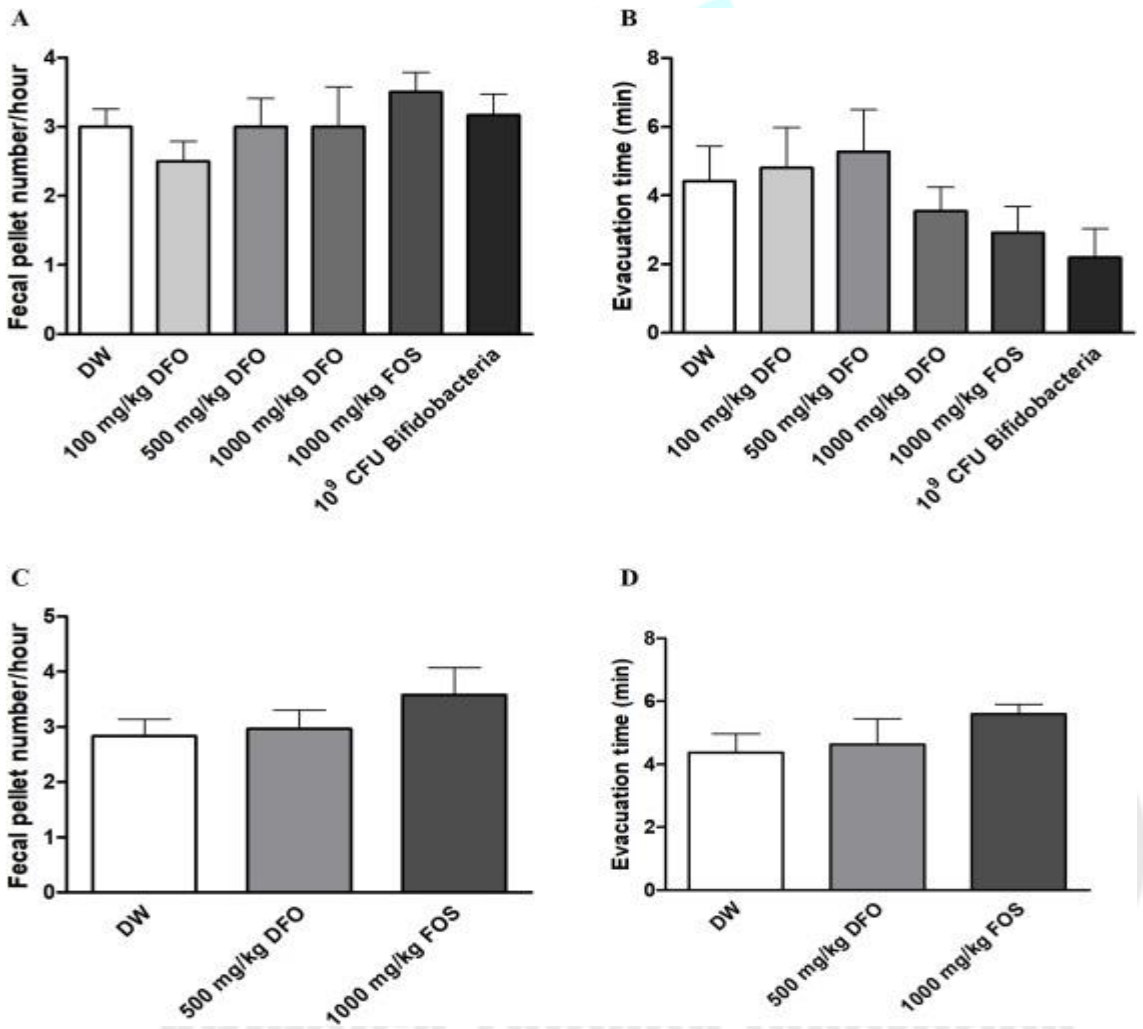


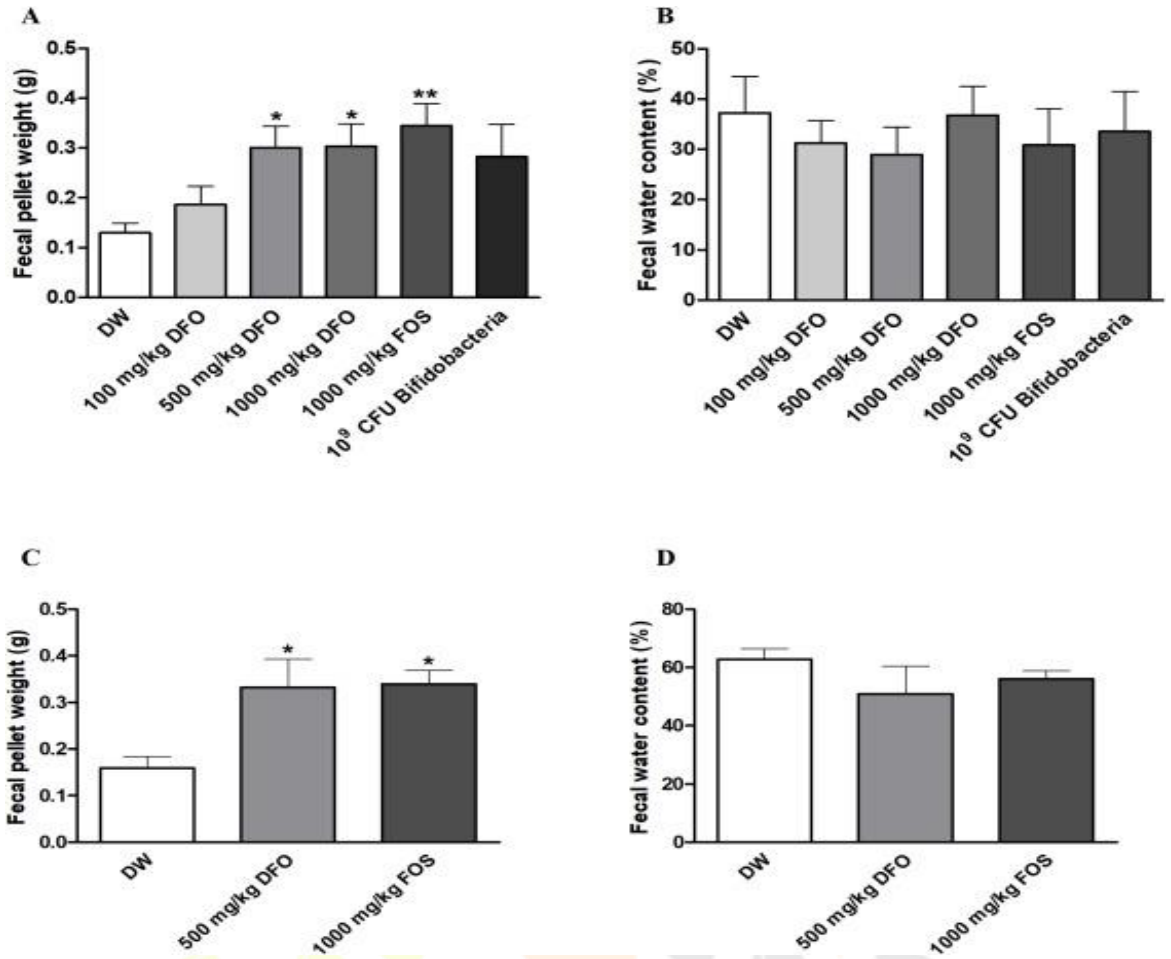
Fig. 1. Effects of one-week and two-week dietary supplementation with DFO on The number of fecal pellets and time of evacuation in mice. Mice were treated With (Aand B) 0.2 mL DW, DFO (100, 500, and 1000 mg/kg, p.o.), FOS (1000 Mg/kg, p.o.), and bifidobacteria (109 CFU, p.o.) for a week, then with (C and D) 0.2 mL DW,DFO (500 mg/kg, p.o.) and FOS (1000 mg/kg, p.o.) for two weeks. Each bar of the data represents means ± SEM (n = 4–6).

2.6. Measurement of ex vivo colonic motility

Colonic segments were silhouetted using luminance plates and GIMM organ baths lined with Sylgard that were continuously perfused with Krebs solution at a rate of 10 mL/min. Both the caudal and oral ends of the section were fastened in place.Its size. The portion was allowed to stabilize in Krebs before recording.Without flushing the fecal pellets, for 30 minutes. There was pellet movement.30 minutes were captured with a video camera attached to a PC that was running.GIMM software (two times per person). There were spatiotemporal maps of motility.Built from recordings made during separate runs. Every video Frame, the colonic segment's image was transformed into a silhouette. The Diameter was estimated and converted at each location throughout the full length Into a gray-scale.The relaxed diameters (intestinal dilation) were recorded as black, while the constricted diameters (intestinal contraction) were marked as white. The sum of the total number of spontaneous contractions was calculated as the number of The quantity of propagation contractions and non-propagation contractions Over 30 minutes. These contractions were referred to as non-propagation ontractions.It was ineffective in moving the pellet. The speed at which fecal pellets move Only in the propagation contraction that through the whole colon was determined.Pattern and was carried out in the GIMM utilizing the fecal pellet tracking method. Software that tracks the pellet from the oral end and dynamically darkens it Until the caudal end. The calculated and shown fecal particle velocity in Mm/second.

2.7. Measurement of in vitro smooth muscle contractility

The proximal end of the colonic segment was attached with a silk thread to an isometric force transducer (Model FT03, Grass, USA) and passively stretched under a load of 500 mg, while the distal end was tethered to an organ holder. The Mechanical activity's output signal, digitized and amplified by a bridge amplifier And PowerLab® System, which were saved on a computer for later study. Use the LabChart7 software. Signals were twice collected and examined. Each person. After the initial 30 minutes of equilibration, the spontaneous Five colonic SM contractions indicative of baseline activity were recorded.Min Carbachol (Tocris Bioscience, Bristol, UK) was used to promote contraction. Added cumulatively to the Krebs solution in the organ bath.These techniques were used and modified.Accordance with the techniques previously mentioned.Between concentrations, there was no washing as the concentrations increased from 0.1 to 1 to 10 M. the strength, length, and frequency of each contraction Colonic segment measurements were made. The average of peak-to-peak differences during a 5-minute period was used to establish the mean amplitude (in mg), which was then expressed as a Percent of the values measured with 1 M CCh (maximum) present Contraction). Both the frequency and the duration were stated as the average contraction and the number of contractions per minute (times/min) In a 5-minute period, times (in Seconds).



2. Effects of one-week and two-week dietary supplementation with DFO on Fecal pellet weight and fecal water content in mice. Mice were treated with (A And B)0.2 mL DW, DFO (100, 500, and 1000 mg/kg, p.o.), FOS (1000 mg/kg, p.o.), and bifidobacteria (109 CFU, p.o.) for a week, then (C and D) 0.2 mL DW, DFO (500 mg/kg, p.o.) and FOS (1000 mg/kg, p.o.) for two weeks. Each bar of The data represents means ± SEM (n = 6–10). *P < 0.05 and **P < 0.01 Compared to vehicle control group (DW).

2.8. Histological study

Colonic segments were embedded in paraffin, fixed in a 10% formalin solution, and sectioned every 7 m. The sections were rehydrated in serially graded ethanol after being deparaffinized with xylene. Periodic acid (PA) and hematoxylin-eosin (HE) According to the established protocols, Schiff (PAS) staining was carried out, and Light microscopy was used to examine tissues. The SM thickness was assessed using Picture J application.

2.9. Statistical analysis

Results from this investigation were presented as means ± SEM, with the number of animals (n) indicated in parenthesis. The statistical program GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, CA) was used to analyze the data.USA: California. Using one-way ANOVA, comparisons between various groups were done. The ANOVA test is followed by the Bonferroni post hoc analysis. The MINITAB Statistical Program was used to determine the sample size. Minitab 16 Analysis Package, Minitab Inc., Pennsylvania, USA The amount P 0.05 was considered significant for all statistical tests.

3. Results

3.1. Effects of DFO on body weight, food and water intakes, and fecal pellet Output

The BW, food intake, or water intake did not alter significantly. In mice whose diets were supplemented for one and two weeks with either the vehicle control or all dosages of DFO (Supplemental Figs. S2 and S3). Mean fecal pellet size Number did not substantially differ from that for all the DFO-treated groups.Group under control (Fig. 1A and C). However, substantial variations were discovered. In the mean moist weight of fecal pellets. The augmented group included 500 and The mean fecal pellet wet weight rose by 2.3 after a week of 1000 mg/kg DFO. The group that received 500 times more than the vehicle control Mg/kg DFO over two weeks, there was a two-fold rise in weight.. We came to the conclusion that DFO caused the alteration. The differences in fecal mass between the two DFO-treated groups provide credence to the prebiotic effects of DFO that were hypothesized in our prior study [25]. However, there were no significantly different fecal water contents in any of the (Figs. 2B and D) of the groups.

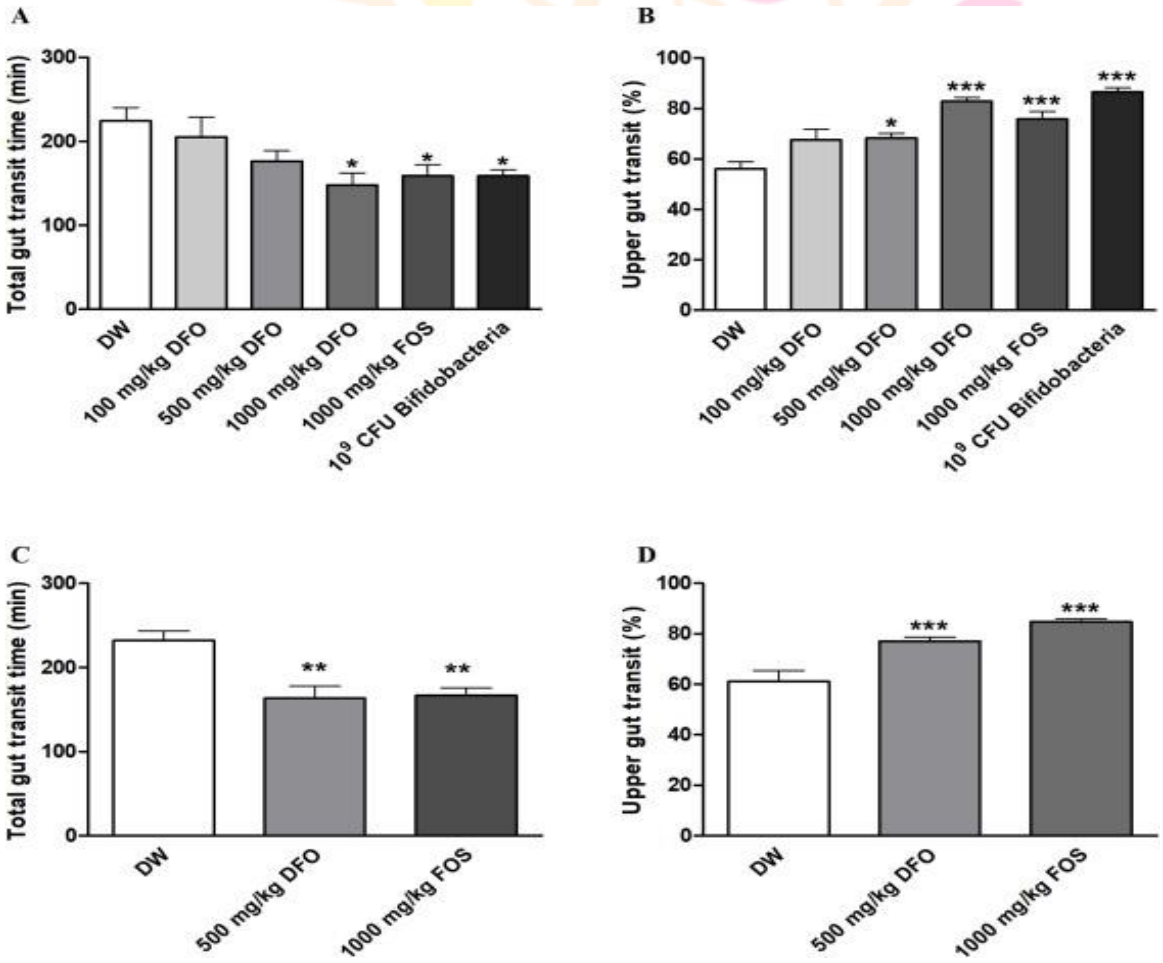


Fig. 3. Effects of one-week and two-week dietary supplementation with DFO on Evanblue total gut transit time and charcoal meal upper gut transit in mice. Mice were treated with (A and B) 0.2 mL DW, DFO (100, 500 and 1000 mg/kg, p.o.), FOS (1000 mg/kg, p.o.), and bifidobacteria (109 CFU, p.o.) for a week,then (C and D) 0.2 mL DW, DFO (500 mg/kg,p.o.) and FOS (1000 mg/kg, p.o.) for two weeks. (A and C) Each bar of the total gut transit time represents the mean of the total gut transit time (min) ± SEM (n = 4–6). (B and D)Each bar of the upper gut transit

represents the mean of the percentage of the small intestine length traveled by the charcoal plug ± SEM (n = 7–11). *P < 0.05,**P < 0.01 and***P < 0.001 compared to vehicle control group (DW).

3.2. Effects of DFO on evacuation time and gut transit

To illustrate how DFO affects the upper gut and overall gut transit time, marker meals were used. The vehicle control's overall gut transit time was around 230 minutes. Among the teams that received DFO at 1000 This time was shortened by 500 mg/kg for two weeks.30% roughly. The overall gut transit times seen in the groups treated with the reference prebiotic and the supplement were comparable to these shorter times. Probiotic standard (Fig. 3A and C). Upper gut transit is the percentage of 56.03 ± 2.80% of the small intestine length was passed through a charcoal meal.Among the mice given DW (vehicle control) supplements for a week and 61.01 4.40% of the mice who received two weeks of DW supplements. With the exception of the group that received DFO at a dose of 100 mg/kg for a week, all experimental groups experienced a considerable increase in these values (Fig. 3B and D). But in contrast to the Mice treated with one drug had slightly longer evacuation times compared to the control group. With DFO at 100 and 500 mg/kg for a week, but reduced in comparison to other groups For a week, supplements. Evacuation times did not differ noticeably.Comparing the mice given 500 mg/kg of DFO for treatment to the control group (Figs. 1B and D): Two weeks.

3.3. Effects of DFO on colonic motility

The fact that gut transit time decreased with DFO administration suggests that DFO may promote gut motility. There were two distinct motility patterns visible in the spatiotemporal maps. The propagation or peristaltic pattern was the initial one. Motility and contractions brought on by food pellets. In the 1000-treatment group Mg/kg DFO For a week, the pellets were propelled by aboral muscles at 0.6 mm/s (Fig. 4D). Propagation. When compared, this fecal particle velocity was significantly higher. The oversight group. In the meantime, giving 500 mg/kg DFO and 1000 Fecal pellet velocity was similarly raised by mg/kg FOS for two weeks, but not as significantly. (Fig. 5D).With the exception of a weeklong treatment with 1000 mg/kg FOS, the data revealed that the reference prebiotics and probiotics produced effects that were comparable to or even superior to those of DFO (Fig. 4D). The second motility pattern Consisting of brief bouts of shallow circular muscular contractions and Relaxation. Because the pellets were moved forward and backward by the contractions (segmentation or non-propagation contraction), the contraction velocity Not be computed. Compared to the vehicle's control, a sizable in-There was a decrease in the overall number of contractions (Fig. 4A) following Treatment for one week with 1000 mg/kg DFO.The non-propagation pattern (Fig. 4B) was where the contribution to this total increase was most obvious. This response matched the outcomes of the seven-day sessions with the (Figs. 4A and B) Prebiotic and probiotic references But the quantity of There was no discernible difference in propagation contractions (Fig. 4C). Likewise, two Therapy with 500 mg/kg DFO and 1000 mg/kg FOS for weeks resulted in a The total number of contractions and non-propagation has significantly increased. Contractions but not contractions of the propagation pattern (Fig. 5A-C).Supplemental Figs. S4 and S5 contain spatial and temporal maps of these intestinal motility patterns.

3.4. Effects of DFO supplementation for one week on proximal and distal Colonic circular and longitudinal SM contractions

The proximal colon segments from the control group's proximal colon segments had a mean amplitude of contraction of the circular SM of 30.27 ± 4.99%. The mean amplitude rose across every other group. For the group receiving 100 mg/kg DFO for a week of treatment, these increases ranged from 37.00 to 7.67%. 92.63 ± 5.16 after receiving 109 CFU of bifidobacteria for 7 days. After During a week of treatment with 500 and 1000 mg/kg DFO, the contraction. Significantly more amplitude was present than in the control group. This outcome Resembled the outcome for the prebiotic FOS (Fig. 6A). In Supplemental Fig. S6, representative traces of the proximal colonic

circular SM contractions in mice treated for 7 days with DW, DFO, FOS, and bifidbacteria are displayed. In the Segments of the proximal colon in longitudinal SM, mean contraction amplitude The control group was 36.27 ± 7.79%; however, following each 7-day course of therapy, the Mean contraction amplitudes increased. These augmentations were particularly Significant in the groups given 1000 mg/kg of DFO as a supplement (70.55 Bifidobacteria 109 CFU (79.21, 8.98%) and 8.31% were also present. 1000 mg/kg of medication FOS did not significantly increase the contraction's amplitude in longitudinal (Fig. 6A) SM.In Supplemental Fig. 7, representative traces of the proximal colonic longitudinal SM contractions in mice treated for 7 days are displayed. Both circular and linear spontaneous contractions have a similar mean frequency.Any experimental group's longitudinal SM proximal colon segments weren't Distinct from the control group in a substantial way (Fig. 6C). However, the periods Longitudinal SM were substantially longer in the treated groups in terms of contraction.That had bifidobacteria and 1000 mg/kg DFO than they were in the control group. Supplementation with 1000 mg/kg FOS, however, significantly raised the Solely in the circular SM for the duration of contraction (Fig. 6E). We also looked into The impact of DFO on the distal colonic SM's contraction (Supplemental Fig.S8 and S9).Only the group treated with bifidobacteria showed a significant increase in mean contraction amplitude in comparison to the control group in both circular and longitudinal SM segments (Fig. 6B). Likewise, the mean Both circular and longitudinal SM spontaneous contraction frequencies Exhibited no discernible difference in reaction to DFO and FOS compared to Fig. 6D shows the outcomes for the control groups. The length of contractions during Those who received 1000 mg/kg of substantially enhanced the size of the circular muscle DFO and bifidobacteria, although the length of the longitudinal contraction Muscle grew in the groups given 1000 mg/kg FOS and bifido treatments Organisms (Fig. 6F).

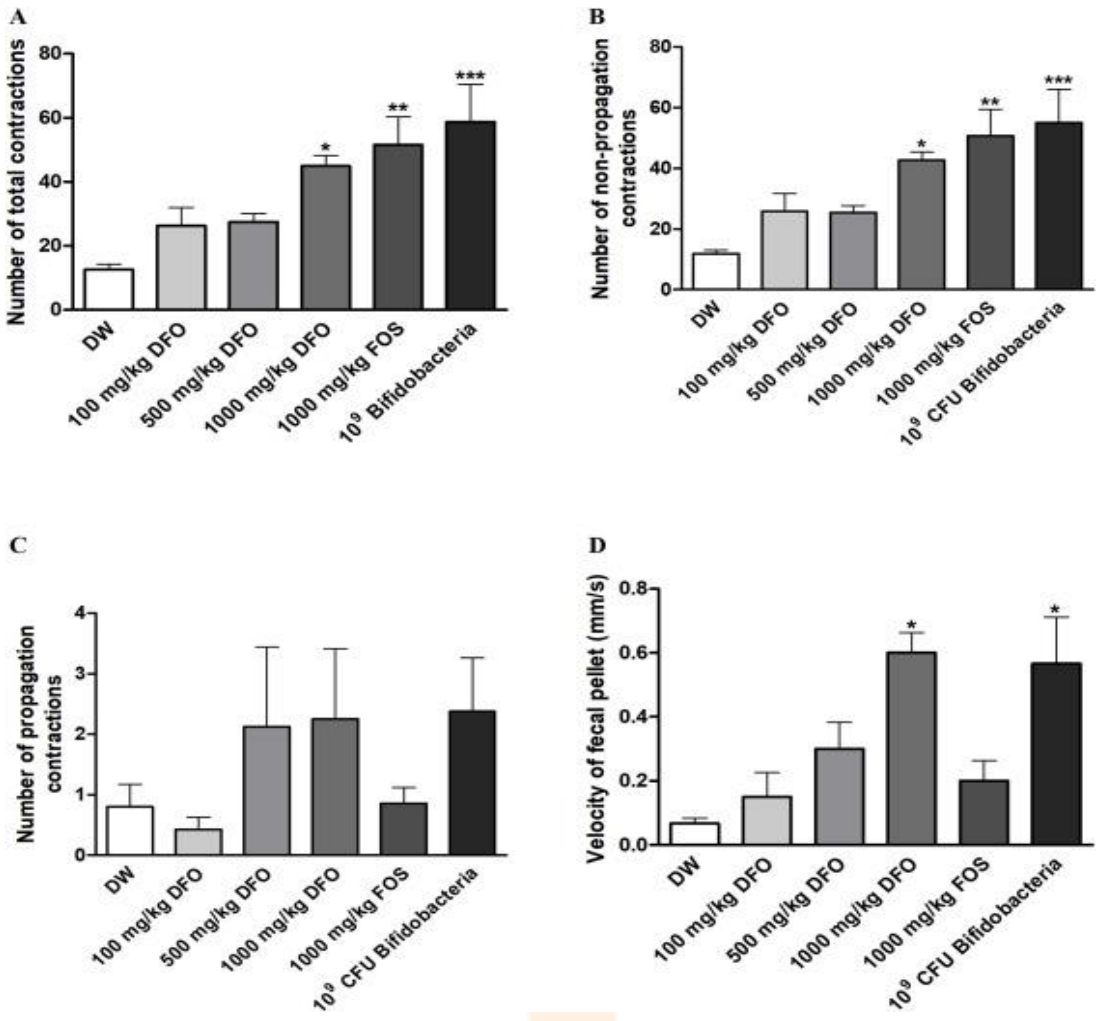


Fig. 4. Effects of a week of DFO dietary supplementation on the quantity of contractions lasting 30 minutes across the whole colon DFO (100, 500, and 1000 mg/kg, p.o.), FOS (1000 mg/kg, p.o.), and bifidobacteria (109 CFU, p.o.) were administered to mice for one week in 0.2 mL DW. (A) Total number of contractions; (B) Number of propagation contractions, (D) fecal velocity, and (n) number of non-propagation contractions Pellet movement throughout the entire colon. The n = 5 data are means SEM. P values are less than 0.05, 0.01, and ***.0.01in contrast to the control group 0.02(DW).

3.5. Effects of DFO supplementation for two weeks on proximal and distal Colonic circular and longitudinal SM contractions

The amplitude of proximal colonic contractions was considerably larger than the amplitude of the control group in both circular and longitudinal SM following treatment with 500 mg/kg of DFO and 1000 mg/kg of FOS for two weeks (Fig. 7A). Circular SM contractions occurred more frequently when DFO and FOS were present.Increased the length when compared to the control group (Fig. 7C) and also Longitudinal SM contractions (Fig. 7E). Reprographic evidence of the In Supplemental Figures S10 and S11, contractions of the proximal colonic circular and longitudinal SM of mice given DW, DFO, and FOS for 14 days are depicted.The amplitude of contractions in circular SM was substantially higher than the amplitude of linear SM contractions in the groups treated with 500 mg/kg DFO and 1000 mg/kg FOS in the distal colon.Control team. In longitudinal SM, there was no increase in contraction amplitude.Alteration (Fig. 7B). Contrary to the amplitude, no notable change was found. Following the administration of DFO and FOS, as determined by the frequency of contractions (Fig. 7D). the length of the

longitudinal SM contractions of DFO and FOS Compared to the control group, treated mice lived noticeably longer (Fig. 7F). The Typical signs of distal colonic circular contractions In Supplemental Fig., the longitudinal SM of mice given a 14-day treatment is displayed S12 and S13.

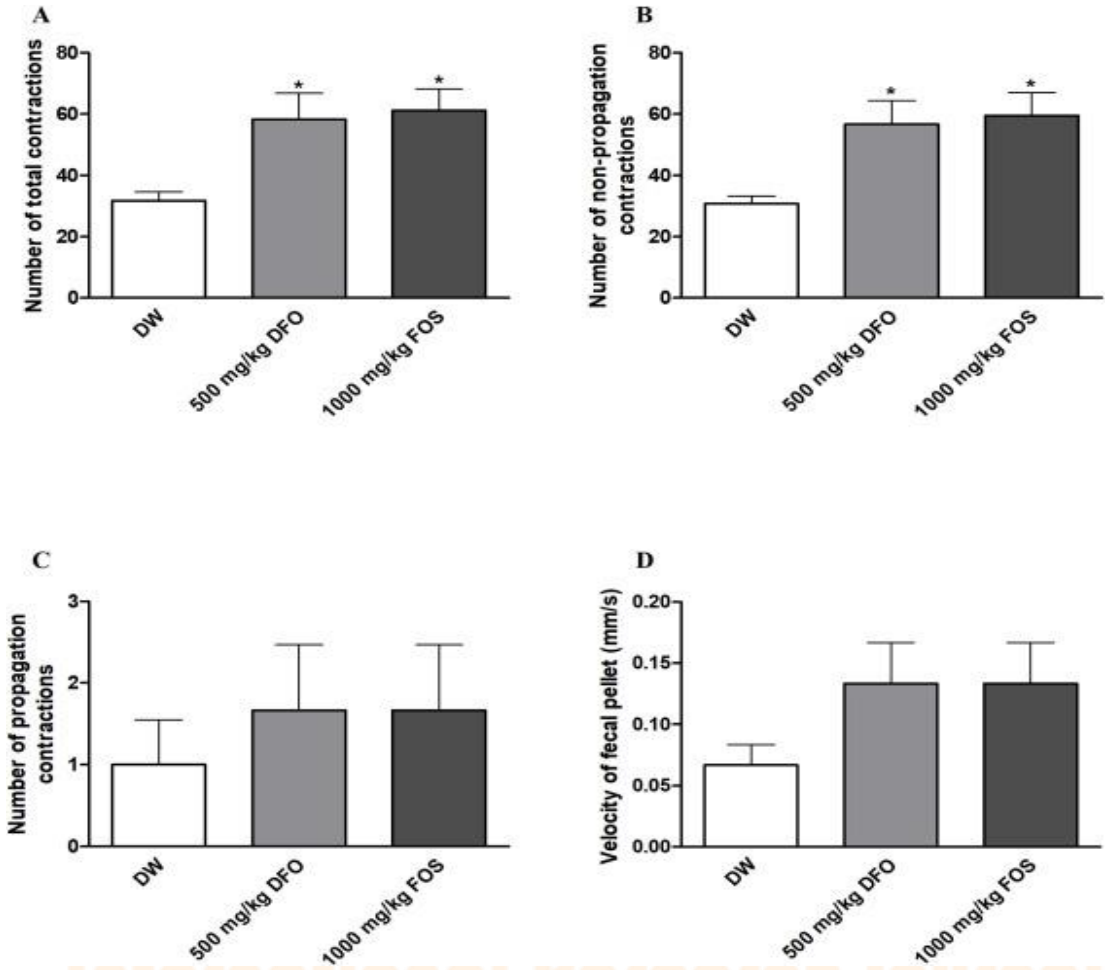


Fig.5 Effects of DFO dietary supplementation on the quantity of contractile responses for 30 min in the whole colon, as seen in Fig. 5 DFO (500 mg/kg, p.o.) and FOS (1000 mg/kg, p.o.) were administered to mice for two weeks in 0.2 mL DW. (A) Total number of contractions; (B) number of contractions that do not propagate; (C) The quantity of propagation contractions and (D) the propulsion speed of fecal pellets Over the entire colon. The n = 5–6 data are means SEM. *P 0.05 in comparison to Group under controvercy(DW).

3.6. Effects of DFO on colonic smooth muscle histology

The histological features of the intestinal wall were evaluated to ascertain whether DFO increased the thickness of the SM. There was no proof that consuming 500 mg/kg of DFO for two weeks had any adverse effects. The Intestinal mucosa was inflamed. The simple, squamous morphology The epithelium was healthy. The quantity of goblet cells in the mucosa Layer was comparable to the quantities found in the groups treated with FOS and DW.(Arrows in Fig. 8A) The muscle layer did not show any significant SM layer thickness in the DFO-treated group was different from the control group. Groups that received DW or FOS treatment (Fig. 8B).

4. Discussion

In the current study of mice, it was discovered that dietary supplementation with DFO sped up upper gut transit, which in turn sped up colon content transit and decreased overall gut transit time. This outcome is in line with the Findings from the control groups treated with bifidobacteria and FOS Supplementation with products containing Lactobacillus or short-chain FOS Additionally, Bifidobacterium species shortened intestinal transit time in adult humans. [33,34]. In malabsorptive situations, shorter intestinal transit times have been documented. With signs of diarrhea [35]. But in this research, the BW of DFO-treated Groups did not differ from the control group, and there were no diarrheal feces. Were noticed. DFO is a short chain that is not digestive, fermentable, or soluble.Carbohydrate. It is believed that about 50% of DFO reaches the consumer's colon.Gastric juice (2.5%), salivary and pancreatic amylases (16%), and small intestine brush-border enzymes (30%) hydrolyze the remaining material. This soluble fiber has a significant potential to store water in the colon, increasing fecal bulk. However, it is known what the DFO's water holding capacity is.Be lower than that of other fibers, including the fiber in wheat [36]. Wet feces pellets In this study, weight increased significantly in the DFO- and FOS-treated groups.Versus the control group. But the proportions of fecal water Material didn't alter all that much. These outcomes are in line with the A prior study's result that dogs' fecal water content was unaffected by FOS therapy [37].

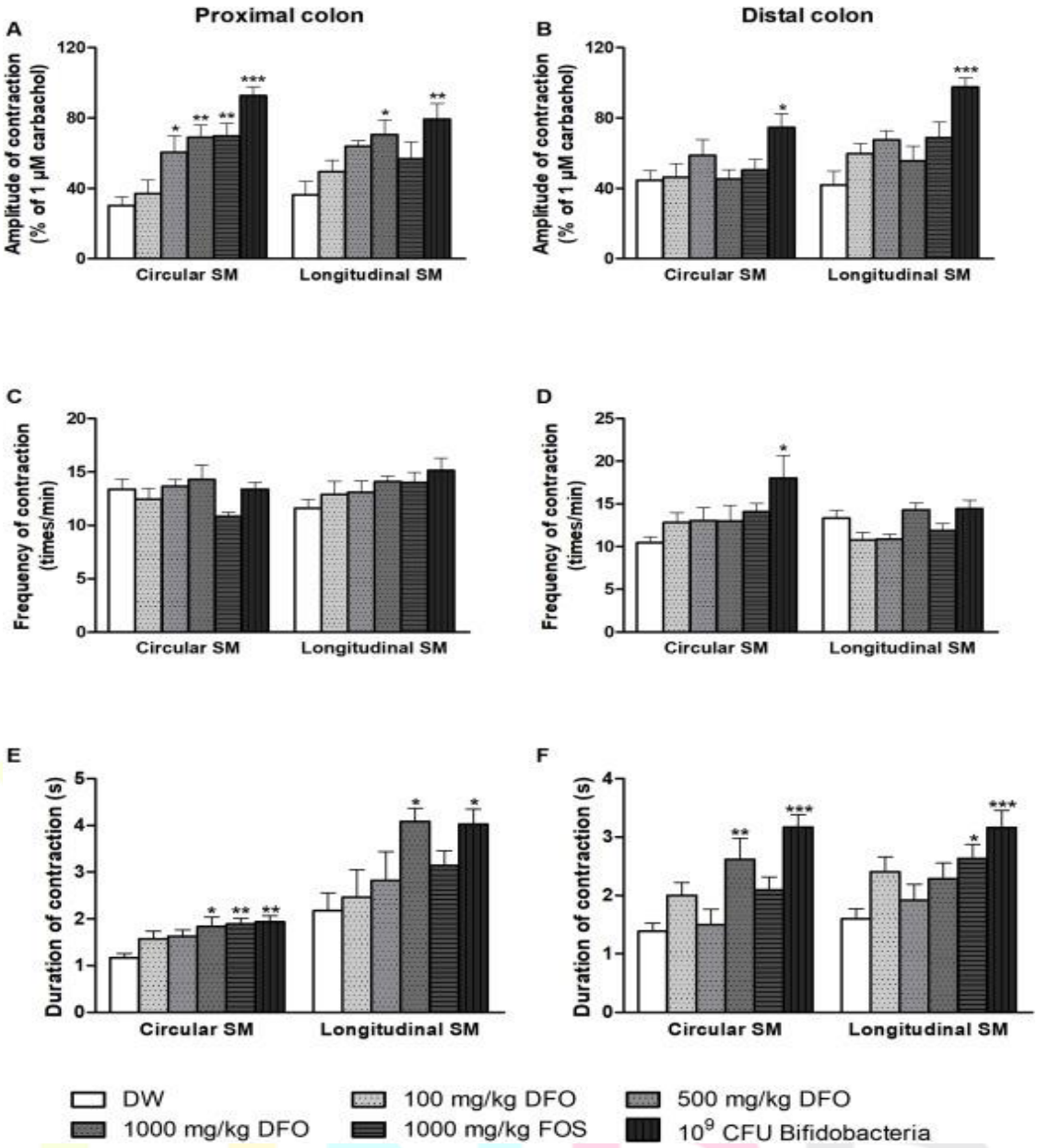


Fig. 6. Effects of a week of DFO supplementation on spontaneous (A, C and E) Proximal and (B, D and F) distal colonic circular and longitudinal SM Contractions in mice. Mice were treated with 0.2 mL DW, DFO (100, 500 and 1000 mg/kg, p.o.), FOS (1000 mg/kg, p.o.), and bifidobacteria (109 CFU, p.o.) For a week. Values are means ± SEM (n = 10) and are expressed as (A and B) a Percentage of the maximum amplitude of contraction, (C and D) Contractions/min, and (E and F) the duration of contractions in seconds. *P < 0.05, **P < 0.01 and ***P < 0.001 compared to vehicle control group (DW).

Increased colonic content sped up intestinal transit and enhanced peristaltic or propagation contractions. The colonic diameter was decreased by peristaltic contraction without occluding the lumen. Thus, The proximal colon was not completely emptied by these motions in a single sweep, although Small amounts of material were very gradually pushed into the distal colon.Even though the DFO-treated groups' propagation patterns were not statistically different.Unlike the control group, there was a trend toward a rise in After one week of observation, fecal pellet velocity dramatically increased. The administration of 1000 mg/kg DFO.In terms of the propagation pattern, the effect of DFO was equivalent to that of bifidobacteria but not to that of FOS, which only improved the non-propagation pattern. Natural-occurring stalling A significant catalyst for neurally mediated colonic propulsion was fecal pellets. Thus, It was conceivable to infer that colon peristaltic contractions were The

outcome of a motor pattern generator activated by distension and mediated by the Neural system of the intestine [41].

DFO may influence bowel motility by altering the colonic environment in addition to voluminously stretching the colonic wall. Studies carried out in vitro revealed that bacterial oligosaccharide fermentation boosted SCFA synthesis, The colonic pH is being lowered. As a result of the reduced pH, lactobacilli and Bifidobacteria and halted the expansion of dangerous bacteria [43]. Increasing Fermentation. By-products, including gas and SCFAs, could make stools more voluminous and Also promote intestinal motility [45]. There are numerous contradictory findings about the GI motility and the effects of prebiotics and probiotics Certain of these studies Some research revealed that these supplements improved intestinal motility, while others The converse was determined [46–48].Consumption of Lactobacillus reuteri decreased the amplitude of colonic contractions, which are necessary to cause phasic contractions in rats, at both constant and elevated luminal pressures [46]. On the other hand, taking fermented milk made with Lactobacillus Casei increased the pace of defecation and colonic propulsive contraction in pigs [47]. According to a different study, healthy newborns who were breastfed had smoother skin.Compared to infants fed bovine milk, who have more frequent feces. GOS And increased stool volume but decreased stool consistency with FOS supplementation. With what regularity? In line with earlier research, we discovered that DFO accelerated By raising intestinal motility, fecal pellet velocity, and moreover, Colonic contractions, particularly in a non propagation pattern,Contractions.

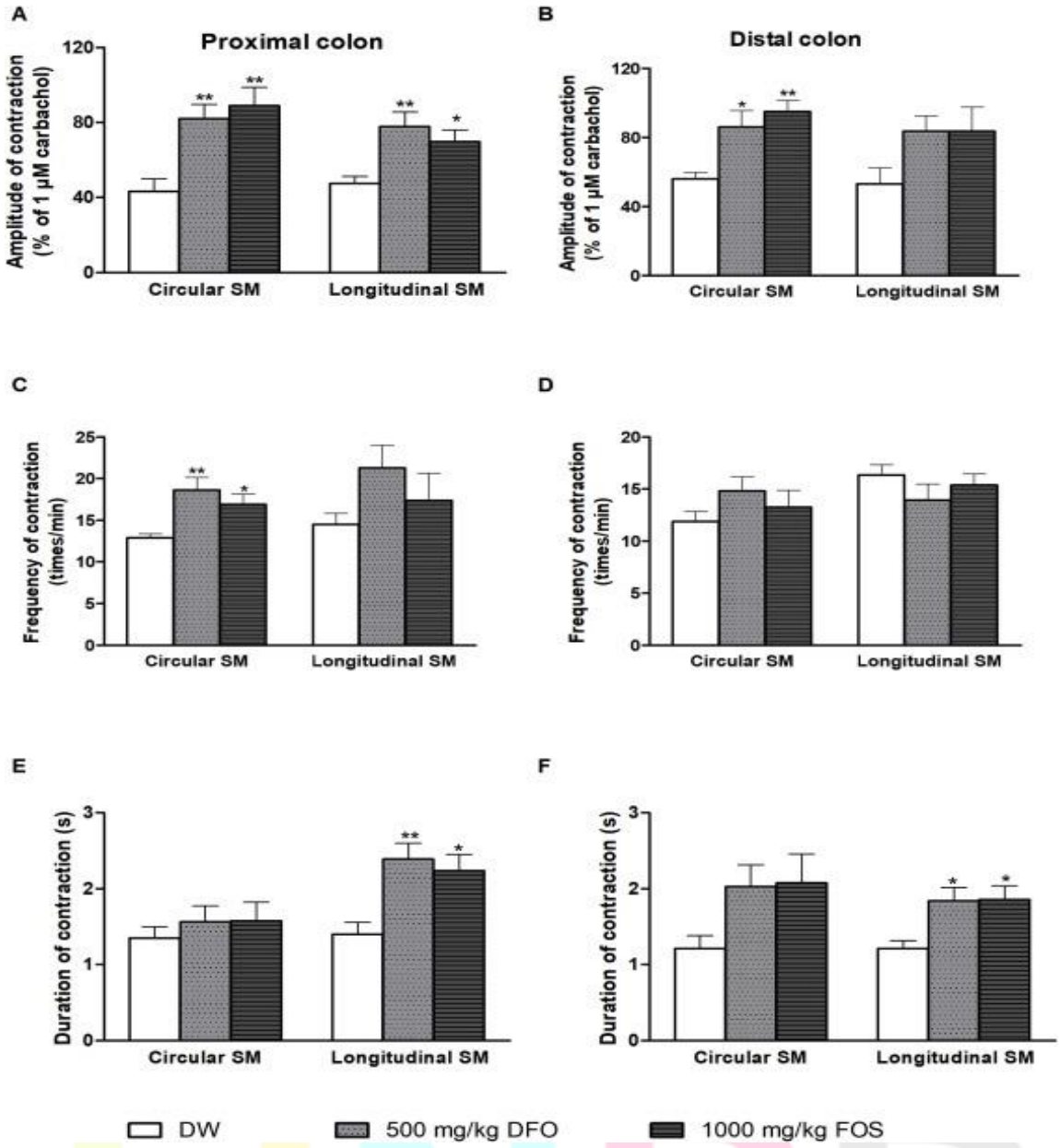


Fig. 7. Effects of food supplementation with DFO for two weeks on spontaneous circular and longitudinal contractions of the proximal and distal colons in mice (A, C, and E) DFO (500 mg/kg, p.o.), FOS (1000 mg/kg, p.o.), and 0.2 mL DW were administered to mice for two weeks. Values are averages of SEM (n = 8) and are presented as percentages of the greatest contraction's amplitude (A and B), contractions per minute (C and D), and contraction duration (E and F) Seconds. Compared to the vehicle control group (DW), *P 0.05 and **P 0.01 are significant.

Mixing and local circulation of materials are caused by non-propagation or segmenting contractions. This pattern may impede gastrointestinal transit in an effort to lessen the significant peristaltic contraction-inhibiting effects of DFO. Consequently, in this research, no Negative consequences of using DFO supplements, including diarrhea or malabsorption Were noticed. Internal anal sphincter normally closes the anal canal. Contraction. The internal sphincter relaxes when the rectum is enlarged by Reflex. The sensation of rectal distention indicates the need to urinate, Which the external anal sphincter prevents. The shortening of the Reflex stimulation through the dorsal roots of the spinal cord keeps the external sphincter in place. Sacral spinal cords, followed by DFO.We came to the

conclusion that DFO and the other treatments in this study had no effect on brain regulation of defecation since there was no discernible difference in evacuation times.

Constipation may result from the intestinal contents' delayed motility or transit. An increase in dietary fibers or prebiotics, an increase in colonic intraluminal bulk, and improved colonic transit all go hand in hand.Motility Prebiotics are said to boost health in a similar way, according to numerous studies. Similar to probiotics but are less expensive, safer, and simpler to include in the diet. However, consuming too many short-chain carbs can result in unfavorable Adverse symptoms such as watery stools, cramps, rumbling, bloating, and flatulence They are all brought about by gas production and some substances' osmotic effects. Products of fermentation. Fortunately, DFO doses of 1000 mg/kg/day or fewer were generally Mice in our study tolerated the substance well.

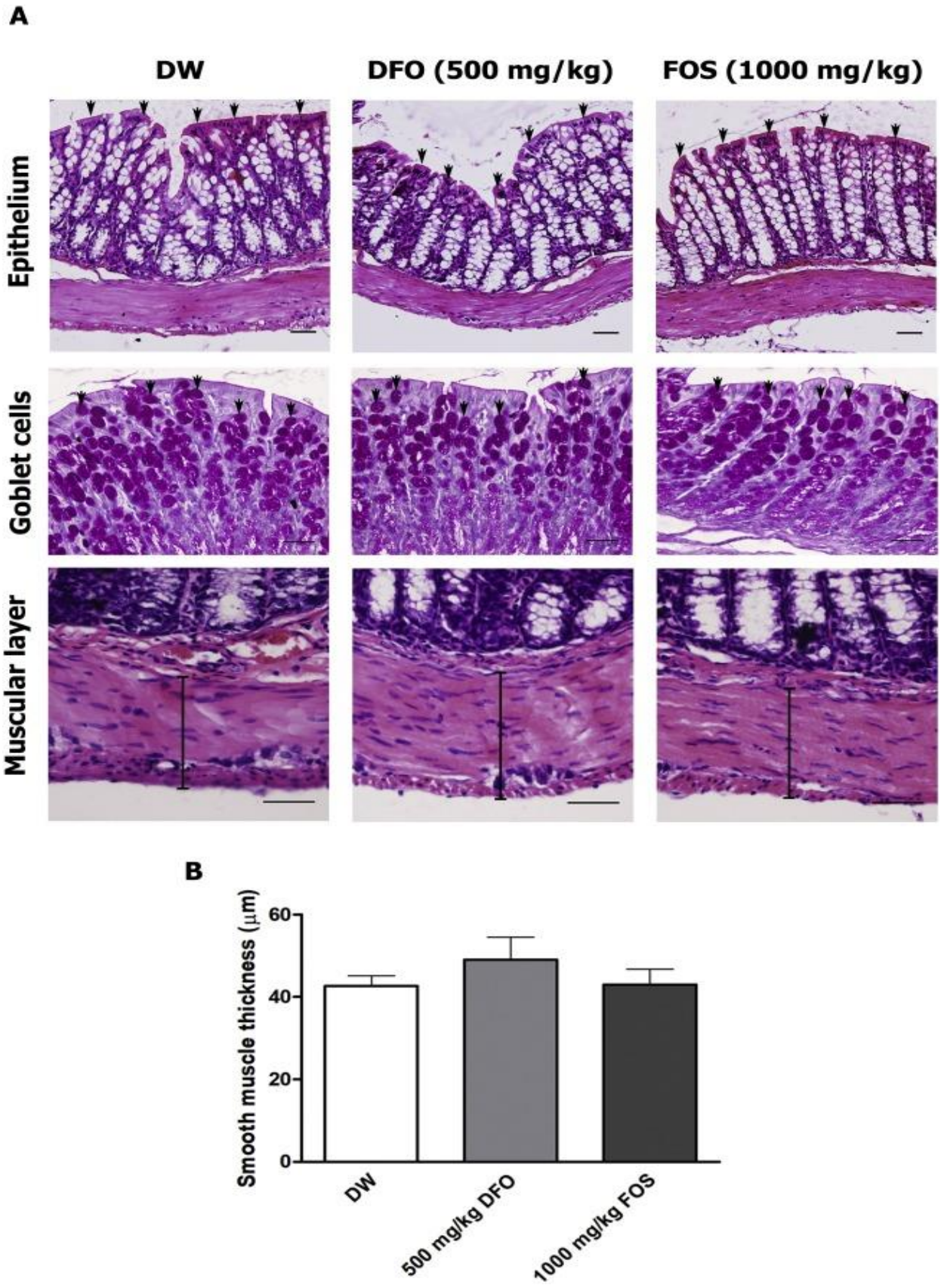


Fig. 8. Histological cross-section images (Conventional H&E and PAS staining) of (A) Mouse colon paraffin sections and (B) colonic SM thickness of DFO-treated mice. Mice were orally administered with 0.2 mL DW, DFO (500

mg/kg) or FOS (1000 mg/kg) for two weeks. Arrows in an upper row and a middle row in (A) Represent epithelium and number of goblet cells, respectively; a lower row Shows thickness of muscular layer; scale bar =20 µm.

Colonic SM contractions are set up to promote the best possible uptake of water and electrolytes, net aboral movement of contents, and storage and orderly expulsion of excrement. The muscularis layers exhibit two different motility patterns. Patterns: peristaltic contractions that are propagated entail the coordinated Contractions of the circular and longitudinal SM, as well as non-propagated Contractions for segmentation, primarily affecting the circular muscle layer. This A study revealed that a week of DFO treatment improved the force and duration Of contractions in the proximal colon's circular and longitudinal SM, although Simply lengthened the distal colon's circular SM contractions. After Following two weeks of DFO therapy, the frequency of circular SM contractions Had grown in the proximal colon but not the distal.The terminal ileum and proximal colon quickly digest short-chain carbohydrates, including DFO, to create SCFAs. SM in the proximal colon, then, Should be impacted substantially more in the distal colon than SM. Though, the Underlying processes that regulate how DFO affects colonic SM Contractions yet remain a mystery. According to a recent study, particular SCFAs, including As butyrate, colonic circular SM contractions were more frequently mediated by cholinergic Hurst and colleagues also observed that butyrate, acetate, and propionate were toxic to rats. The proximal and distal colons are affected differently by substances in the intestinal lumen.Contractions, and that the length of the chain affected these effects.The balance of SCFAs produced by the gut bacteria during the fermentation of non-digestible carbohydrates determined the overall impact of SCFAs on contraction. The happenings of The colonic SM cell's slow wave activity always affects contractions.Membrane. However, the contractions are started by an increase in potential. When slow waves cross the electrical threshold, activity starts to happen. Thus, The maximal frequency of contractions is determined by the slow wave frequency. The.force And the frequency and length of muscle contractions are connected. Intensity and Frequency of potential spikes. The likelihood of the spike occurring substantially depended on Gradual effects on local chemical agents and hormonal and neuronal activity The waves were very predictable. According to our findings, the impacts of DFO may control the amount of spiking, but less so the slow wave threshold. DFO thus primarily impacts contraction strength and duration rather than frequency. Intestinal circular and longitudinal SM that is coordinated Through peristalsis, contractions cause the caudal propulsion of luminal contents.SCFAs produced by the bifidogenic action and distention resulting from The osmotic action may impact stretch receptors and free fatty acids at the Epithelia in the gut. They activate CGRP by stimulating 5-hydroxytryptamine. Comprising motor neurons, a set of interneurons, and neurons Substance P, tachykinin, or acetylcholine all respond to the luminal stimulation. Circular SM contractions and longitudinal SM relaxation are brought on by these neurotransmitters. Nitric oxide and vasoactive intestinal peptide are delivered to the Longitudinal SM, circular SM stimulation, and luminal stimuli Contractions. Some research has demonstrated that SCFAs or prebiotic supplements Potentially alter the colon's morphology. For instance, butyrate had trophic Colonocytes are affected. However, DFO did not demonstrate trophic levels in this investigation.Neither alter the gut wall nor the thickness of the SM. Previous research also Revealed a high-fiber diet boosted mucin-producing cells' numbers and secretory activity. Rat colonic goblet cells that secrete.On the other hand, neither the quantity of goblet cells nor the epithelium was affected by DFO in our investigation. Since intestinal mucin in the colonic mucosa protects cells from a range of luminal dangers, And since the quantity of goblet cells changed during intestinal infections Based on histomorphological scores for overall intestinal inflammatory criteria, Consumption did not cause modifications to the architecture of the epithelium or the mucosa Of DFO . These results might attest to the safety of this substance. These findings imply that in addition to advantageous prebiotic DFO also functions as a bulk-forming laxative, absorbing water from An osmotic action on the intestinal lumen to increase fecal bulk A laxative stimulant that makes mice's intestines move more quickly.We also demonstrated a link between ingesting DFO and changes in colonic SM contractility. These results demonstrate that DFO may be an appropriate dietary supplement. Products that are laxatives, prebiotics,

probiotics, and symbiotics DFO could potentially A promising nutritional treatment for gastrointestinal motility issues like constipation Also IBS. However, more research is necessary to determine the underlying mechanisms causing GI motility alterations brought on by nutrition or the gut Bacteria.

Disclosure

Throughout the course of the investigation, none of the authors experienced any conflicts of interest.

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