Final Report

Project Title: The effects of dragon fruit oligosaccharide on motility of mouse proximal and distal colon

By Pissared Khuituan

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Department of Physiology, Faculty of Science,

Prince of Songkla University

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บทคัดย่อ

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ชื่อโครงการ: ผลของโอลิโกแซคคาไรด์จากแก้วมังกรต่อการเคลื่อนไหวของลำไส้ใหญ่ส่วนต้นและส่วนปลายในหนูถีบจักร

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บทคัดย่อ:

โอลิโกแซคคาไรด์จากแก้วมังกร (ดีเอฟโอ) มีคุณสมบัติเป็นพรีไบโอติกส์ ช่วยให้การทำงานของระบบทางเดินอาหารดีขึ้น โดยกระตุ้นการเจริญของแบคทีเรียที่เป็นประโยชน์ในลำไส้ใหญ่ การเปลี่ยนแปลงชนิดของแบคทีเรียอาจส่งผลต่อการเคลื่อนไหวของลำไส้ อย่างไรก็ตามยังไม่มีการศึกษาผลของดีเอฟโอต่อการทำงานของระบบประสาทสั่งการของทางเดินอาหาร ดังนั้นงานวิจัยนี้จึงมีวัตถุประสงค์เพื่อศึกษาผลของดีเอฟโอต่อการเคลื่อนที่ของก้อนอุจจาระและรูปแบบการเคลื่อนไหวของลำไส้ใหญ่ และศึกษาโครงสร้างและการหดตัวของกล้ามเนื้อเรียบลำไส้ใหญ่ของหนูถีบจักร หนูที่ได้รับพรีไบโอติกส์อ้างอิง (ฟรุกโตโอลิโกแซคคาไรด์ (เอฟโอเอส)) ขนาด 1000 มก/กก เป็นเวลา 1 และ 2 สัปดาห์จะมีน้ำหนักของอุจจาระเพิ่มขึ้นเมื่อเปรียบเทียบกับกลุ่มควบคุมเช่นเดียวกับหนูที่ได้รับดีเอฟโอขนาด 500 และ 1000 มก/กก นอกจากนี้หนูที่ได้รับเอฟโอเอสและโพรไบโอติกส์อ้างอิง (บิฟิโดแบคทีเรีย) ยังมีระยะเวลาการขนส่งกากอาหารที่ลดลง และมีระยะทางการเคลื่อนที่ของกากอาหารที่เพิ่มขึ้นเช่นเดียวกับหนูที่ได้รับดีเอฟโอ การศึกษาแผนที่ช่วงเวลาในเชิงพื้นที่ของการเคลื่อนไหวของผนังลำไส้ใหญ่ที่ถูกบันทึกด้วยกล้องวิดีโอพบว่า ดีเอฟโอเพิ่มจำนวนการหดตัวของลำไส้ใหญ่ โดยเฉพาะชนิดการเคลื่อนที่แบบไม่ไปข้างหน้า และยังเพิ่มความเร็วของการเคลื่อนที่ของอุจจาระในลำไส้ใหญ่เช่นเดียวกับผลของเอฟโอเอสและบิฟิโดแบคทีเรีย นอกจากนี้ดีเอฟโอยังเพิ่มความแรงและช่วงเวลาการหดตัวของกล้ามเนื้อเรียบทั้งที่เรียงตัวแบบวงกลมและตามยาวของลำไส้ใหญ่ส่วนต้นและปลายโดยใช้วิธีการวัดแรงดึงตัวในอุปกรณ์ใส่เนื้อเยื่อนอกร่างกาย สำหรับการย้อมเนื้อเยื่อพบว่าเยื่อบุผนังลำไส้ คริปส์ เซลล์กลอเบล็ต และความหนาของชั้นกล้ามเนื้อเรียบ มีลักษณะเหมือนกันทุกกลุ่มการทดลอง จากการทดลองทั้งหมดสรุปได้ว่า ดีเอฟโอทำงานเป็นยาระบายชนิดที่ทำให้อุจจาระเกาะตัวเป็นก้อน และชนิดกระตุ้นการเคลื่อนตัวของลำไส้ ดีเอฟโอยังเพิ่มการหดตัวของกล้ามเนื้อเรียบลำไส้ใหญ่โดยไม่เปลี่ยนแปลงลักษณะทางสัณฐานวิทยาอีกด้วย

คำหลัก:

แก้วมังกร; คาร์โบไฮเดรตสายสั้น; กล้ามเนื้อเรียบลำไส้ใหญ่; ยาระบาย

Abstract

Project Code: MRG5980042

Project Title: The effects of dragon fruit oligosaccharide on motility of mouse proximal and distal colon

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Abstract:

Dragon fruit oligosaccharide (DFO) has prebiotic properties which improve gut health by selectively stimulating the colonic microbiota. Altering microbiota composition may affect intestinal motility; however, there is no study on effects of DFO on gut motor functions. Thus, this research aimed to investigate the effects of DFO ingestion on fecal pellet propulsions and spontaneous motility patterns in the isolated mouse colon, and to examine the morphology and physiology of colonic smooth muscle (SM). Administration of 1000 mg/kg prebiotic reference (fructo-oligosaccharide, FOS) for one and two weeks to adult mice significantly increased fecal pellet weight when compared to vehicle control. Similarly to the positive control, fecal pellet weight of 500 and 1000 mg/kg DFO-treated mice were significantly increased. Moreover, mice treated with FOS and probiotic bifidobacteria significantly reduced the transit time and increased the distance of upper gut transit which was comparable to DFO. Spatiotemporal map of whole colonic wall motions which recorded with a video camera showed that DFO significantly increased the number of total colonic contractions, especially non-propagation pattern, and velocity of fecal pellet movement through the colon, consistent with the results from FOS- and bifidobacteria-treated groups. In addition, DFO increased the amplitude and duration of contractions of proximal and distal colonic circular and longitudinal SM as determined by *in vitro* tension measurement in an organ bath. Histological studies by hematoxylin-eosin and periodic acid-Schiff staining showed normal morphology of epithelium, crypts, goblet cells, and also the thickness of SM in all groups. As a result, it has been defined that DFO acts as a bulk laxative which increases fecal output, and a stimulant laxative which increases intestinal motility in mice. DFO ingestion also increased the contraction of colonic SM without changing in the morphology.

Keywords:

dragon fruit; short-chain carbohydrate; colonic smooth muscle; laxative

Executive summary

A dragon fruit is an interested agricultural product in Thailand. It is originally native to Mexico and widely distributed to Central America and to other parts of the world. This fruit is known under several commercial and native names, but a pitaya or a pitahaya prevails all around. There are two or three famous varieties of the dragon fruit in Thailand, i.e., a red pitaya with white-flesh (*Hylocereus undatus* (Haw.)), a red pitaya with red-flesh (*Hylocereus polyrhizus*), and a yellow pitaya (*Hylocereus megalanthus*). The dragon fruit is found to be rich in many nutrients, such as, β-carotene, lycopene, vitamin E and essential fatty acids, and also has antioxidant activity. Thus, the dragon fruit has potential for use as a source of functional ingredients to provide nutrients that may prevent nutrition-related diseases and improve physical and mental well-being of the consumers. Moreover, both flesh and peel of the dragon fruit have been reported as a source of glucose, fructose and non-digestible oligosaccharides which has prebiotic properties.

Prebiotics, such as, fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), lactulose, and inulin, are non-digestible food ingredients that selectively stimulate the growth and/or activities of specific microbiota in the gastrointestinal (GI) tract, usually bifidobacteria and lactobacilli, and inhibit the growth and/or activities of undesirable microbiota, such as, *Salmonella* sp. and *Escherichia coli*. Gut microbiota plays a role in metabolic activities by extracting energy from non-digestible dietary. When non-digestible carbohydrates are fermented by the microbiota, they form short-chain fatty acids (SCFAs), mainly acetate, propionate, and butyrate. These SCFAs are the main energy source of the colonocytes and play roles in electrolyte transport, cell differentiation and growth, and motility of the colon. In addition, the gut microbiota participates in the homeostasis and protecting the host against pathogens by mechanisms, such as, colonization resistance and production of antimicrobial compounds.

Alteration in the gut microbial diversity can be observed as a result of variations on the dietary input, pathological conditions including constipation and colitis, and antibiotic treatments. The imbalance among beneficial and undesirable gut microbiota affects various GI functions including intestinal motility and causes several GI diseases, e.g., colitis, diarrhea, irritable bowel syndrome (IBS), inflammatory bowel diseases (IBDs), and constipation. To rebalance these microorganisms, intake of probiotics, prebiotics or symbiotic is recommended. These ingredients can modulate a healthy gut microbiota and favor the production of SCFAs that have a positive effect on intestinal motility.

The objectives of this project were to investigate effects of prebiotic dragon fruit oligosaccharides (DFO) on fecal output, intestinal transit and evacuation times, patterns and velocity of fecal pellet propulsion, proximal and distal colonic circular and longitudinal smooth muscle (SM) contractions and the colonic morphological changes of mice. This study was approved and guided by the Animals Ethic Committee of the Prince of Songkla University, Thailand (Project license number MOE0521.11/799). After a week of acclimatization, male ICR mice (6-7 weeks old) were randomly divided into six groups and fed with 0.2 mL distilled water (DW), 100, 500 and 1000 mg/kg DFO, 1000 mg/kg FOS, or 10^9 CFU bifidobacteria daily for one or two weeks. Body weight (BW), food and water intakes, and fecal pellet output of all mice were recorded every day during treatments. After treatments, the fecal pellet number was counted, weighted, and dried. To measure the total gut transit time, mice received Evan-blue and the fecal pellet was observed until the first blue pellet was expelled. To measure the evacuation time, the bead expulsion from the anus was recorded. One hour before anesthetization, mice received charcoal meal and the charcoal distance was measured to determine the upper gut transit. The colon with contents was removed and placed in an oxygenated ice-cold Krebs solution. To study the effects of DFO on colonic propulsive motility, whole colonic segment was mounted horizontally in a gastrointestinal motility monitor (GIMM) organ bath. The spatiotemporal map of colonic wall motions was recorded with a video camera and analyses by GIMM software. To study the SM contractions, segments of proximal and distal colon were separated, cut into small segments, and suspended in an organ bath. Signal output of the contractions was sent to isometric force transducers, amplified and digitized via Bridge Amp and PowerLab® System, and analyses by LabChart7 program. In the histological study, hematoxylin-eosin and Periodic acid–Schiff staining were performed.

The results of this study showed that ingestion of DFO increased the upper gut transit which reduced the time of the content to the colon and also reduced the total gut transit time. There were some previous studies reported that rapid intestinal transit was seen in malabsorptive states and diarrhea symptoms. However, the BW of DFO-treated group was not changed when compared to control group, and there were not shown the diarrheal feces characteristics. Fecal pellet wet weight in DFO- and FOS-treated groups significantly increased when compared to control. Conversely, the percentage of fecal water content was not significant different in all groups. The propagation pattern of the DFO-treated group was not significant different when compared to control, but showed increasing in trend. In addition, the velocity of fecal pellet movement through the entire colon significantly increased in one week of 1000 mg/kg DFO treatment when compared to control. These results suggested that peristaltic contractions in the colon result from a natural fecal pellets distension-triggered motor pattern generator mediated by enteric nervous system. In addition, increasing fermentation by-products, such as, gas and SCFAs, could increase the stool bulk and stimulate the gut motility. DFO also increased the non-propagation pattern. This pattern may slow the gut transit to abolish the high effect of DFO on peristaltic contraction, finally, there were no adverse effects, e.g., diarrhea and malabsorption, of DFO supplementation. There were no significant different of evacuation time which means that the prebiotic DFO may not affect the neural control of the last process of the defecation.

Moreover, DFO treatment for a week increased force and duration of contraction of both circular and longitudinal SM in proximal colon, but only increased the duration of circular SM contraction in the distal colon. For two weeks treatment, DFO could increase the spontaneous contraction frequency of circular SM in the proximal, but not in the distal colon. DFO is a short-chain carbohydrate which is very rapid fermented in the terminal ileum and proximal colon to produce SCFAs, therefore the proximal colonic SM should be affected much more than the SM in the distal colon. In addition, there were some studies reported the effect of prebiotic supplement could change the colonic structure by gut microbiota and SCFAs effects. However, our study showed no effect of DFO on epithelium, goblet cell number, and SM thickness.

Taken together, these data suggested that in addition to be a prebiotic, DFO also acts as a bulk laxative which increases fecal mass as well as being a stimulant laxative which increases intestinal motility. We also showed an association between DFO ingestion and alteration in the colonic SM contractions. According to these findings, it seems that DFO may be suitable for inclusion as food supplements in a wide variety of food products, e.g., prebiotic/probiotic/synbiotic products, laxative product, and may be a promising nutritional therapy for GI motility disorders, such as, constipation and IBS. Nevertheless, further investigation is required to identify the underlying mechanisms responsible for diet- or gut bacteria-induced changes in GI motility.

The effects of dragon fruit oligosaccharide on motility of mouse proximal and distal colon

1. Introduction to Research

The imbalance of adverse and beneficial enteric microbiota affects various gastrointestinal (GI) functions including GI motility (1), resulting in inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), diarrhea, or constipation (2, 3). To maintain these microbial populations in a normal state, intake of probiotics and prebiotics is extremely helpful (4, 5). Probiotics, such as bifidobacteria and lactobacilli, are live valuable microbes that are good to human and animal health, especially in the GI tract. It is well known that these bacteria have the effects on modulating intestinal motility with reductions in both diarrhea and constipation (6, 7, 8). Although ingestion of probiotics has excellent advantages, there are some limitations in critical patients, such as patients with acute pancreatitis or allergy (9, 10), and they may be destroyed easily by heat and acid and difficult to handle in some foodstuffs. The second approach to selectively modify the composition and activity of intestinal microbiota is to supply the microbiota which already present in the colon with prebiotics.

Prebiotics are non-digestible food ingredients that enter the colon without alteration by the digestion and absorption, serving as a nutrient source for the beneficial bacteria living in the colon. Prebiotics not only selectively allow specific changes in the compositions and/or activities of the GI microbiota, they also induce microbial competition and reduce the populations of undesirable microbiota (11, 12). The major products of prebiotic fermentation in the colon are short chain fatty acids (SCFAs), mainly acetate, propionate and butyrate. SCFAs are the energy sources of colonic epithelial cells and play roles in electrolyte transport, cell differentiation, cell growth, and motility of the colon (13, 14). The best known prebiotics is fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS) and inulin (15– 18), and other non-digestible oligosaccharides have also been tested for their prebiotic properties, especially prebiotic-rich fruit and vegetables.

Dragon fruit oligosaccharide (DFO) is extracted and purified from dragon fruit or pitaya. Dragon fruit is an agricultural product which is native to Central and South America (19, 20) and has been interested in many countries such as Vietnam, Singapore, China, Philippines, Malaysia and Thailand (21). It is found to be rich in various nutrients, such as β-carotene, lycopene, vitamin E and essential fatty acids (22, 23) and also has antioxidant and anti-inflammatory activities (24). Both flesh and peel of red pitaya with white-flesh (*Hylocereus undatus* (Haw.)) and red pitaya with red-flesh (*Hylocereus polyrhizus*) have been reported as a source of DFO (25, 26). DFO is resistant to hydrolysis by artificial human gastric juice and α-amylase, and stimulates the growth of lactobacilli and bifidobacteria in the artificial colon (25, 27). Even though, prebiotic properties in an *in vitro* studies of DFO are quite known, there is no study the prebiotic effects of DFO on GI functions, especially intestinal motility.

Thus, the aim of the present study was to investigate the *in vivo* effects of oral administration of DFO for one to two weeks on fecal output, intestinal transit time, evacuation time, pattern of colonic motility, velocity of the colonic pellet propulsion, proximal and distal colonic circular and longitudinal smooth muscle (SM) contractions, and the colonic morphological changes of male ICR mice. Knowing the effective doses and durations of prebiotic DFO intake on intestinal motility may very useful to improve the imbalance of intestinal microbiota ecosystem, and helping the intestinal motility disorder such as constipation and diarrhea.

2. Literature review

2.1 Intestinal microbiota

Microbiota (formerly called microflora) is an ensemble of commensal, symbiotic and pathogenic microorganisms that resides in different parts of the body, such as in the skin (skin microbiota), in the mount (oral microbiota), in the vagina (vaginal microbiota) and also in the intestine (intestinal microbiota). The human GI tract is colonized by a large number of microbiota which is ten times higher than eukaryotic cells in the human body (28). Intestinal microbiota supports a variety of metabolic, nutritional, physiological and immunological functions in the human body. The distribution of microbiota varies according to the GI parts, low concentrations in the stomach and duodenum, higher concentrations in the jejunum and ileum and the highest concentrations in the colon (29). In the colon, the microbiota consists of about 1000 species, the most common genus is anaerobic bacteria including *Bifidobacterium*, *Lactobacillus*, *Clostridium*, *Bacteroides* and *Eubacterium*, and are also found aerobic bacteria, such as, *Escherichia*, *Enterococcus*, *Streptococcus* and *Klebsiella* (30, 31).

The intestinal microbiota plays a role in metabolic activities by extracting energy from non-digestible dietary. In the colon, when non-digestible carbohydrates such as fibers and oligosaccharides are fermented by the microbiota, they form SCFAs, mainly acetate, propionate and butyrate. These SCFAs are the main energy source of the colonocytes. Although the fermentation of peptides and proteins also produce SCFAs, it generates a number of potentially toxic substances, including ammonia, amines, phenols, thiols and indols (32). Therefore, SCFAs production through non-digestible carbohydrates fermentation is more powerful because it does not produce toxic products that can damage the intestinal epithelium. The metabolic activities of microbiota also produce other important nutrients, such as, vitamin K, vitamin B_{12}, folic acid and amino acids, which humans are unable to produce themselves (33). Not only used the SCFAs as an energy source by colonic epithelial cells, but also play roles in electrolyte transport, cell differentiation and growth, and motility of proximal and distal colon (13, 14). The production of SCFAs is greatest in the cecum and proximal colon, where most of the microbiota bacteria reside and where most non-digested material is held in the colon. As material moves caudally, the SCFAs concentration decreases due to their uptake and utilization as a nutrient source for colonocytes. Butyrate is considered to be the primary nutritive SCFAs for colonocytes (34).

In addition, the intestinal microbiota participates in the homeostasis and protecting the host against pathogens by mechanisms such as colonization resistance and production of antimicrobial compounds. Furthermore, the intestinal microbiota is involved in the development, maturation and maintenance of the GI sensory and motor functions, the intestinal barrier and the mucosal immune system (35–38).

Intestinal microbial colonization begins immediately after birth which is introduced through the secretion of the birth canal as well as through breast milk (39) and becomes more complex with increasing in age, with a high degree of variability among human individuals. Alteration in the intestinal microbial diversity can be observed as a result of variations on the dietary input, pathological conditions including constipation and colitis, and antibiotics treatment (40, 41). The imbalance among beneficial and undesirable intestinal microbiota (called intestinal dysbiosis) affects various GI functions including intestinal motility, colitis, diarrhea, IBS, IBDs and constipation (1–3). The imbalance may affect the intestinal motility by modifying the metabolic environment of the intestine due to the increase of the pH generated by undesirable bacteria, which causes a consequent reduction in the production of physiologically active beneficial compound (42). In addition, some studies indicated that there is a relationship between the brain and the intestine that influences the behavior of individuals with intestinal dysbiosis (43). Moreover, there is a decrease in the proportion of SCFAs in dysbiosis situations (44).

Since it is known that the intestinal microbiota plays an important role in human health and diseases, to rebalance these microorganisms, it is recommended to intake probiotics, prebiotics and symbiotic (the combination of probiotics and prebiotics), which works in the modulation of a healthy intestinal microbiota and favors the production of SCFAs that have a positive effect on intestinal motility (4, 5).

2.2 Probiotics

Probiotics are live microorganisms that have beneficial effects on health and well-being of the host, when ingested in adequate amount. These effects are predominantly related to support the intestinal mucosal barrier against pathogens, stimulate immune responses, activate anticarcinogenic and antimutagenic activities, synthesize some vitamins, produce metabolic products and reduce cholesterol in the plasma (45).

Probiotics can be packaged in many formulations containing just one organism or a mixture. Currently, the most commonly used probiotics are bacterial members of the genus *Bifidobacterium* and *Lactobacillus*, which are also represented important components of human intestinal microbiota (46). Compared with other anaerobic bacteria in the GI tract, lactobacilli and bifidobacteria decrease the activity of some enzymes, such as β-glucosidase, β-glucuronidase, urease, azoreductase and nitrate reductase, which are involved in the formation of mutagens and carcinogens (47). In addition to these genus, other nonpathogenic microorganisms, such as *Streptococcus*, *Enterococcus* and *Saccharomyces*, have been used in probiotic preparations (48).

Clinical studies support the use of probiotic bacteria in the treatment of GI disorders such as infectious diarrhea, antibiotic diarrhea, traveler's diarrhea, IBS and colitis (49–53). The possible mechanisms of probiotics are alterations in the composition of the microbiome, changes in enteric neuron functions, and modulation of motility with reductions in either diarrhea or constipation (54–56).

Recent experimental studies also showed the data evaluating the effects of probiotic bacteria on the intestinal motility. Wu and co-workers (2013) (57) showed the strain of probiotic bacteria and region of intestine specific effects on intestinal motility. *Lactobacillus rhamnosus* decreased jejunal and colonic frequencies, and colonic velocity, but increased jejunal velocity. *Lactobacillus reuteri* increased jejunal and colonic frequencies, and colonic velocity, but decreased jejunal velocity. Both of them could decrease jejunal and colonic intraluminal peak pressure. In *ex vivo* studies of Kunze et al., 2009 (55) and Wang et al., 2010 (58) showed that both oral administration for 9 days and incubation for 15 minutes in the tissue chamber *of Lactobacillus rhamnosus* decreased the amplitudes of rat intestinal migrating motor complex.

2.3 Prebiotics

Prebiotics are non-digestible food ingredients that selectively stimulate the growth and/or activities of specific bacteria in the GI tract, usually bifidobacteria and lactobacilli, or inhibit the growth and/ or activities of undesirable microbiota, such as *Salmonella* sp. and *Escherichia coli* (59). Compared with probiotics, which introduce exogenous bacteria into the human colon, prebiotics stimulate beneficial endogenous bacteria (Table 2.1). The criteria of prebiotics was identified by Gibson and co-workers (2004) (11), i.e., resistance to gastric juice in the stomach and enzymes in the small intestine, cannot absorbed in the GI tract, fermentation by intestinal microbiota, and selectively stimulation of the growth and/or activity of beneficial bacteria.

Prebiotics enter the large intestine without alteration by the digestive and absorptive processes, serving as energy and growth source for the beneficial bacteria that live in the large intestine. The major fermentation products of prebiotic metabolism in the colon are SCFAs, which had different effects on colonic morphology and functions such as supply of energy to the intestinal mucosa, lowering of the pH and stimulation of electrolyte transport, e.g., Na^+ and water absorption, cell differentiation and growth, and motility of proximal and distal colon (13, 14, 60).

Butyrate, which is the one important of SCFAs, is almost completely consumed by the epithelium of the colon and is an important energy source for colonocytes. Butyrate is also associated with many beneficial biological functions in the colon such as the effects on the DNA methylation, enhancing cell proliferation of normal cells, but suppressing cell proliferation of transformed cells (47, 61). Aside from butyrate, acetate and propionate are found in the byproduct of prebiotics. After intestinal absorption, these SCFAs are circulated in the blood stream and are metabolized by the liver or in the peripheral tissues, particularly on the muscular tissue. These SCFAs can act as modulators of glucose metabolism and improving insulin sensitivity (62).

Table 2.1: A comparison of the terms probiotics and prebiotics

	Probiotics	**Prebiotics**
Definition	A live microbial food supplement which beneficially affects the host by improving its intestinal microbial balance	A non-digestible food ingredient which beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon
Examples	- Bifidobacteria - Lactobacilli - Streptococci - Enterococci	- Fructo-oligosaccharides (FOS) - Galacto-oligosaccharides (GOS) **- Dragon fruit-oligosaccharides (DFO)** - Inulin
The Impact on gut health	Support the treatment of diarrhea, constipation, irritable bowel syndrome (IBS) and certain intestinal infections	
Limitations of probiotics	- The powder form of prebiotics can survive heat, cold, acid and even time - Probiotics are more fragile, vulnerable to heat and stomach acid, may also be killed overtime - Bacteremia, probiotic sepsis, endocarditis are the possible complication related to probiotic administration in the patients who had intestinal surgery or chronic illnesses, such as patients with acute pancreatitis or valvular heart disease	

Typically, the prebiotics consist of dietary fibers, polysaccharides and oligosaccharides. FOS, GOS, mannan-oligosaccharides (MOS), chito-oligosaccharide (COS), lactulose, polydextose and inulin have been demonstrated to have prebiotic properties (15–18). They are not digested by human enzymes in the stomach and small intestine, but are fermented in the large intestine by microbiota to give SCFAs which can be absorbed and metabolized by the host (63). In addition, these prebiotics can enhance the number of bifidobacteria and lactobacilli, which play a beneficial role in improving GI health (64, 65). Currently, most studies have been done on inulin, FOS and GOS, however other non-digestible oligosaccharides have also been tested for their prebiotic properties especially prebiotic-rich fruit and vegetables.

2.4 Dragon fruit oligosaccharide

Dragon fruit (pitaya or *Hylocereus*) is an interested agricultural product in Thailand. It is originally native to Mexico and widely distributed to Central America and to other parts of the world. The fruit is known under several commercial and native names, but pitahaya or pitaya prevails all around. There are two or three famous varieties of pitaya in Thailand, i.e., red pitaya with white-flesh (*Hylocereus undatus* (Haw.)), red pitaya with red-flesh (*Hylocereus polyrhizus*) and yellow pitaya (*Hylocereus megalanthus*).

Dragon fruit is found to be rich in many nutrients, such as β-carotene, lycopene, vitamin E and essential fatty acids (22, 23), and also has antioxidant activity (24). Thus, dragon fruit has potential for use as a source of functional ingredients to provide nutrients that may prevent nutrition-related diseases and improve physical and mental well-being of the consumers. Moreover, both flesh and peel of the dragon fruit have been reported as a source of glucose, fructose and non-digestible oligosaccharides which has prebiotic properties (25, 26).

The dragon fruit oligosaccharides (DFO) have prebiotic characteristic, since it is resistant to hydrolysis by artificial human gastric juice and human α-amylase, and stimulate the growth of lactobacilli and bifidobacteria in artificial colon (25, 27), thus it can improve the human GI system. Even though, prebiotic properties of DFO are quite known, there is no study the prebiotic effects of DFO on GI motility.

2.5 Intestinal motility

There are two primary intestinal motility patterns, i.e., propulsive and non-propulsive contractions. Peristalsis is a propulsive contraction which propels the luminal contents in an anal direction, while segmentation is considered to be a non-propulsive contraction which mixes the luminal contents to facilitate digestion and absorption. Intestinal motility refers to the contractions of SM layers located in the intestinal wall which consist of an outer longitudinal and an inner circular layers. The longitudinal muscle contracts in the orad and caudad direction along the intestine, whereas the circular muscle contracts to occlude the lumen. Segmentation results from the contraction of circular layer whereas peristalsis results from the coordination between two layers contractions.

The motility is initiated when the intestinal wall is stretched by the luminal contents and can be increased or decreased by autonomic nervous system (ANS). However, the motility can occur independently of extrinsic innervation. The enteric nervous system (ENS) is the intrinsic innervation of the GI tract. It contains complete reflex circuits located several plexuses in the intestinal wall. The most prominent, myenteric plexus, lies between the circular and longitudinal layers of smooth muscle. The neurons of this plexus extend projections into both muscle layers and release a multitude of neuropeptides and neurotransmitters which regulates the various motility patterns in the intestine. In addition, the neurons of submucosal plexus, which lies between the submucosa and circular muscle layer, can receive signals from epithelial cells in the mucosal layer via afferent neurons and control the motility patterns by synapsing with SM layers and neurons of myenteric plexus.

Usually, once ingested foods exit the stomach, they are broken down for further digestion and absorption in the small intestine. However, certain compounds, such as fiber and some types of oligosaccharides, are not digestible by the enzymes in the small intestine. These compounds reach the ileum and are propelled into the cecum through the ileocecal valve. At this point, luminal contents are condensed into feces and are fermented by microbiota that resides in the colon. The byproducts of these compounds fermentation or the compounds themselves may modulate the colonic motility via stimulating chemoreceptors and sending the signal to the ENS. (Figure 2.1)

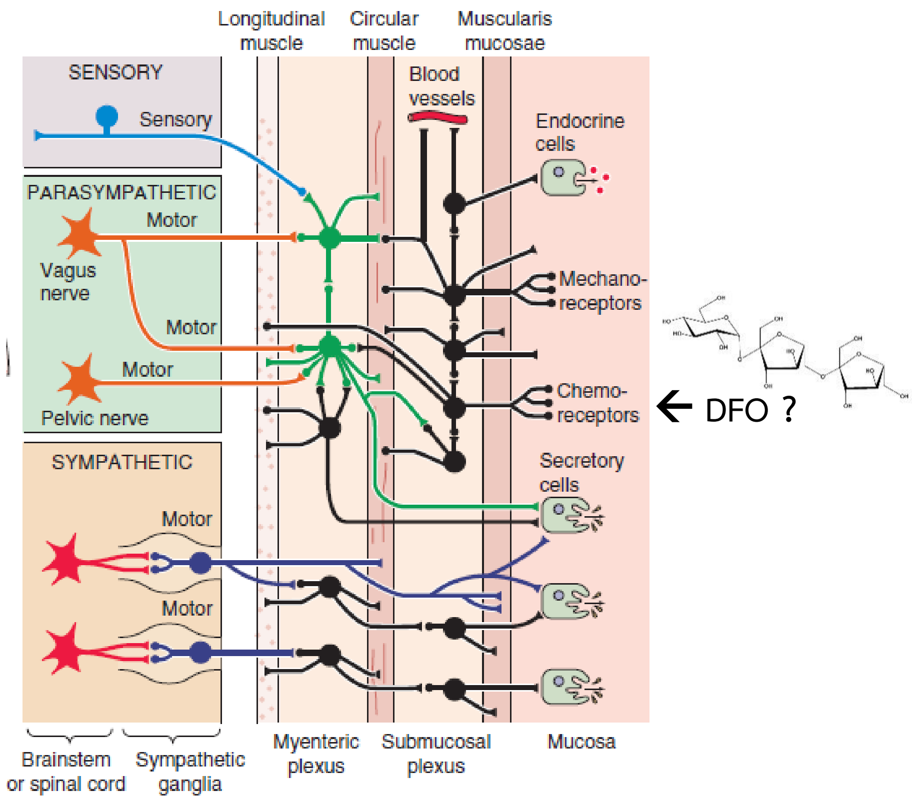


Figure 2.1: Diagram of the gastrointestinal (GI) wall. The layers of the GI tract move out from the lumen are the mucosa, submucosa, submucosal plexus, circular muscle layer, myenteric plexus, longitudinal muscle layer and serosa. The enteric nervous system (ENS) consists of sensory neurons, interneurons and motor neurons. Some sensory signals travel centrally from the ENS. Both the parasympathetic and the sympathetic divisions of the ANS, mechanical and chemical stimulants from intestinal lumen (possibly, prebiotic DFO) can modulate the ENS. (66)

2.6 Gastrointestinal Motility Monitor (GIMM) System

The GI motility has been principally measured using intraluminal pressure or SM contraction recordings from *in vitro* isolated intestinal segments. In these methods, the concentrations of substances which are incubated in the tissue chamber can be controlled and reducing the influences of circulating hormones and ANS. However, measuring more detailed aspects of motility pattern, i.e., peristalsis and segmentation has been relatively qualitative and difficult to assay in these approaches. To solve this problem, video technology began to be utilized to record isolated tissue preparations for more detailed analysis. These analyses generate spatiotemporal maps of video, which measure the distance from one lateral edge of the tissue to the other, and thus indicate either distension or occlusion of the intestinal lumen (67, 68). In the decade since the inception of this method, it had been developed commercially and sold as a Gastrointestinal Motility Monitor (GIMM) system (69). (Figure 2.2)

GIMM system has been used to monitor propulsion of artificial or natural fecal pellets in the colon for studying the dysmotility of pellet propulsion in several animal models of various GI disorders including IBDs (70). In addition, this system has been used to analyze peristalsis and segmentation by perfusing fluid in the small and large intestine (71).

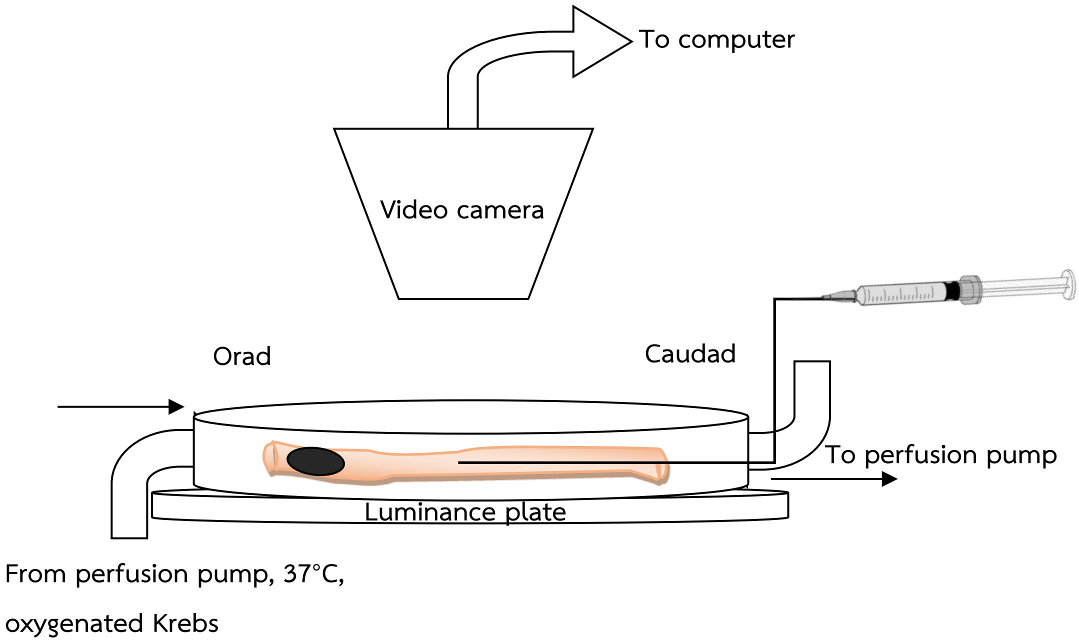


Figure 2.2: Diagram indicating setup of the Gastrointestinal Motility Monitor (GIMM). The segment of the intestine is excised and placed in an organ bath with circulating Krebs solution (pH 7.4, 37°C). In the distal colon, the orad and caudad ends are attached to the walls of the organ bath. A fine catheter (PE 10) is inserted into the anal end and advanced about half way into the colon towards the pellet when added the testers. The organ bath sits on a plate that emits light from below and causes the tissue to be silhouetted in view of the video camera situated above the preparation. This camera is attached to a computer that records and analyzes the motility patterns of the tissue over a specified timeframe.

3. Objective

The objective of this project was to investigate the *in vivo* effects of oral administration of DFO for one to two weeks on fecal output, intestinal transit time, evacuation time, patterns of colonic motility, velocity of the colonic pellet propulsion, proximal and distal colonic circular and longitudinal SM contractions and the colonic morphological changes of male ICR mice.

4. Research methodology

4.1 Animals and ethical approve

This study was approved and guided by the Animals Ethic Committee of the Prince of Songkla University, Thailand (Project license number MOE0521.11/799). Adult male ICR mice (*Mus musculus*; 5 weeks old, weighing 20–25 g) were obtained from National Laboratory Animal Center, Mahidol University and housed at Southern Laboratory Animal Facility, Prince of Songkla University. All animals were kept under standard environmental conditions (room temperature was kept in the range of 23–27°C, humidity at 50–55%, and under a 12:12 hr light/dark cycle) and were fed regular standard commercial food pellets (S.W.T., Thailand), and filtered water *ad libitum*.

4.2 Chemicals and equipment

The reference prebiotic and probiotic in these studies was FOS (Sigma-Aldrich, St. Louis, MO, USA) and *Bifidobacterium animalis* (FD-DVS nu-trish® BB-12®) (Chr. Hansen Holding A/S, Hoersholm, Danmark), respectively. The composition of the Krebs solution was as follows (in mM): 119 NaCl, 2.5 $CaCl_2$, 4.5 KCl, 2.5 $MgSO_4$, 25 $NaHCO_3$, 1.2 KH_2PO_4, and 11.1 D-glucose (72, 73) (all purchased from Merck, Co., Ltd., Darmstadt, Germany). All chemicals were kept at room temperature and the working solutions were made fresh on the day of the experiment by diluting the stock solutions in Krebs. The GIMM System which was used in an *in vitro* colonic propulsive motility study was purchased from Catamount Research and Development, St. Albans, VT.

4.3 Extraction and Purification method of DFO

DFO extraction used distilled water (DW) and pectinase and then the extract was purified by fermentation with yeast. All sugars of white flesh dragon fruit were analyzed by high performance liquid chromatography (HPLC) which consisted mostly of glucose, fructose and some oligosaccharides. The low molecular weight (MW) fraction, glucose and fructose, which were analyzed by gel permeation chromatography, were removed by biological approach using two-step yeast (*Saccharomyces cerevisiae*) cultivation. MW distribution of the mixed oligosaccharides was confirmed by mass spectrometry. The mixed DFOs consisted of four components of 716, 700, 490 and 474 Da with relative percentages of 100, 68, 45 and 21, respectively. Thus, the degree of polymerization (DP) of mixed DFOs is 3–4, which is in the same range as some FOS.

4.4 Experimental design, surgical procedure and tissue preparation

After a week acclimatization, experimental animals were randomly divided into six groups and each group were fed with 0.2 mL DW (vehicle control), 100, 500, and 1000 mg/kg DFO, 1000 mg/kg FOS, or 10^9 CFU bifidobacteria daily for a week or two weeks. Body weight (BW), food and water intakes, and fecal pellet output of all mice were recorded every day prior to start the experiments. After anesthetization by intraperitoneal injection of 70 mg/kg Thiopental sodium (Anesthal®), the abdominal cavity was dissected and rapidly removed the entire colon. The colon with contents was placed in an oxygenated (5% CO_2 and 95% O_2) ice-cold Krebs solution (pH 7.4 with an osmolality of 289–292 mmoL/kg H_2O). To study the effects of DFO on colonic propulsive motility, whole colonic segment was mounted horizontally in a 50 mL GIMM organ bath containing 37°C oxygenated Krebs solution. To study SM contractility, segments of proximal colon (3 cm distal to the cecum) and distal colon (3 cm proximal to the rectum) were separated and cut into smaller segments (1 cm in length). In the contraction of longitudinal SM study, whole thickness segments were suspended in the direction of longitudinal SM fibers, whereas in the circular SM study, the segments were opened along the mesenteric border and full-thickness muscular strip were cut in the direction of circular muscle and suspended in a thermostatically controlled (at 37ºC) 20 mL organ bath containing oxygenated Krebs solution. In the histological study, 1 cm of proximal colonic segment was fixed in 10% formalin solution.

4.5 Fecal pellet output and gut transit assay

After treatments, the number of feces was counted and weighted within six hours. For fecal water content calculation, feces was weighted and dried at 100°C for 30 minutes and the formula of fecal water content (%) is ((wet weight – dry weight)/wet weight) × 100. For the total gut transit time measurement, mice received Evan-blue marker meal (5 % Evan-blue indicator in 1.5 % methyl cellulose; 0.1 mL (i.g.)) and observe the fecal pellet every 10 minutes until first blue fecal pellet expelled. For the evacuation time, it was measured by using a bead expulsion test. A 3-mm glass bead was inserted into the colon through 1 cm proximal of anal by using the plastic tip lubricated with pure petroleum jelly. The time of bead expulsion was measured. For the upper gut transit measurement, mice received charcoal meal for 60 minutes before, and charcoal transit (%) was measured and calculated by (the distance of charcoal meal/total length of the small intestine) × 100

4.6 Measurement of *in vitro* colonic motility

The GIMM organ bath lined with Sylgard which was placed on top of luminance plates to silhouette a colonic segment was continuously perfused with fresh oxygenated Krebs solution at 10 mL/minute. To achieve 37°C in the organ bath, the perfused Krebs solution was sent through heating coils circulating water from a perfusion pump held at 50°C. The segment was pinned at both oral and caudad ends at its *in situ* length. Before recording, the segment was allowed to equilibrate in Krebs solution for 30 minutes without flushed out of natural fecal pellets. The movement of the pellets was recorded form above using a video camera connected to a computer running GIMM software. Spatiotemporal map of motility was constructed from the recording that was acquired from individual runs. The image of the colonic segment in each video frame was converted to a silhouette, the diameter at each point along the entire length was calculated and converted into a grey-scale. The small diameter (intestinal contraction) was coded as white and the large diameter (intestinal dilation) was black. The number of spontaneous contractions both propulsive and non-propulsive contractions was count per unit of time. Velocity analysis was performed using the fecal pellet tracking method in the GIMM software, in which the pellet is darkened compared to the rest of the video and tracked from the orad to caudad end. Fecal pellet velocity was calculated and displayed in mm/second.

4.7 Measurement of *in vitro* smooth muscle contractility

The distal end of each colonic segment was tied to an organ holder and the proximal end was secured with a silk thread to an isometric force transducer (Model FT03, Grass, USA), and stretched passively to an initial tension of 500 mg. Signal output of the mechanical activity was amplified and digitized via Bridge Amp and PowerLab® System (AD Instruments, Australia), recorded on a computer for later analysis using LabChart7 program software. After the equilibration time (30 minutes to obtain a regular spontaneous activity), spontaneous contractions in the colonic SM representing basal activity were recorded for 5 minutes. A single concentration (0.1, 1, and 10 μM) of carbachol (CCh) (Tocris Bioscience, Bristol, UK) was added to the Krebs solution in the organ bath in a cumulative fashion without washing between concentrations, and each concentration was incubated for 5 minutes. Subsequently, the amplitude, duration and frequency of spontaneous contractions of the isolated colonic segments were calculated. The mean amplitude (in mg) of contractions was calculated as the average of peak to peak differences over 5 minutes and were expressed as a percentage of the values recorded in the presence of 1 μM CCh (maximal contraction). The frequency of contractions was expressed as the number of contractions per minute (times/min) in a 5-min period. The duration of contractions was expressed as the mean of the period of contraction time (second) recorded in a 5-min period.

4.8 Histological study

After 10% formalin fixing, the colonic segments were embedded in paraffin, and sectioned at 7-μm-thick. Sections were deparaffinized with xylene and rehydrated in serial graded ethanol. Hematoxylin-eosin (HE) and Periodic acid–Schiff (PAS) staining were performed according to the standard protocols and tissues were observed under light microscope. The smooth muscle thickness was measured with image J software.

4.9 Data and statistical analysis

Results obtained from this study were expressed as mean ± SEM (standard error of mean) with n in parentheses denoting the number of animal. Data were analyzed using the statistical program GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, California, USA). Multiple group comparisons were made using one-way analysis of variance (one-way ANOVA) test, followed by Bonferroni post hoc test. The level of significance for all statistical tests was $P < 0.05$.

5. Results

5.1 Effects of DFO on body weight, food and water intakes, and fecal pellet output

This study aimed to investigate the effect of prebiotic DFO ingestion on energy balance by measuring food intake, fecal output, and BW change. There was no significant change in BW change, food intake, and water intake in mice either fed vehicle control or all doses of DFO for one and two weeks (Figure 5.1 and 5.2). Mean fecal pellet number of DFO treated group were not significantly different compared to control group (Figure 5.3A and 5.3C) whereas mean fecal pellet wet weight was significantly increased by 2.3 times with consumption of 500 and 1000 mg/kg DFO for a week and increase by 2 times with consumption of 500 mg/kg DFO for two weeks compared with the vehicle control (Figure 5.4A and 5.4C). The greater fecal mass was induced by ingestion of DFO, despite similar food intake to control. This result is consistent with the putative prebiotic effects of DFO as investigated by the previous study (25). However, fecal water content of each group was not significantly different (Figure 5.4B and 5.4D).

5.2 Effects of DFO on gut transit

To demonstrate the action of DFO on the time or distance of fecal pellet movement through the colon, gut transit time and upper gut transit measurement by using marker meals were determined. The total gut transit time of vehicle control was approximately 230 minutes and the transit time of DFO at 1000 mg/kg for a week and 500 mg/kg for two weeks was significantly reduced approximately 30% from the control (Figure 5.5A and 5.5C). The distance of charcoal meal of mice treated with DW for a week and two weeks was 56.03 ± 2.80% and 61.01 ± 4.40% of the small intestinal length, respectively. DFO-treated for a week at 500 and 1000 mg/kg, the charcoal meals were moved 68.22 ± 1.90% and 82.86 ± 1.62% in the small intestine, respectively. DFO-treated for two weeks at 500 mg/kg moved the charcoal meal to 77.11 ± 1.62%. Moreover, the upper gut transit of a charcoal meal was significantly increased in the 500 and 1000 mg/kg DFO in relation with the control (Figure 5.5B and 5.5D). Evacuation time of this study was measured from glass bead emptying via the anus. Both one and two weeks treatment of DFO showed that the evacuation time was not significantly different compare to control group (Figure 5.3B and 5.3C).

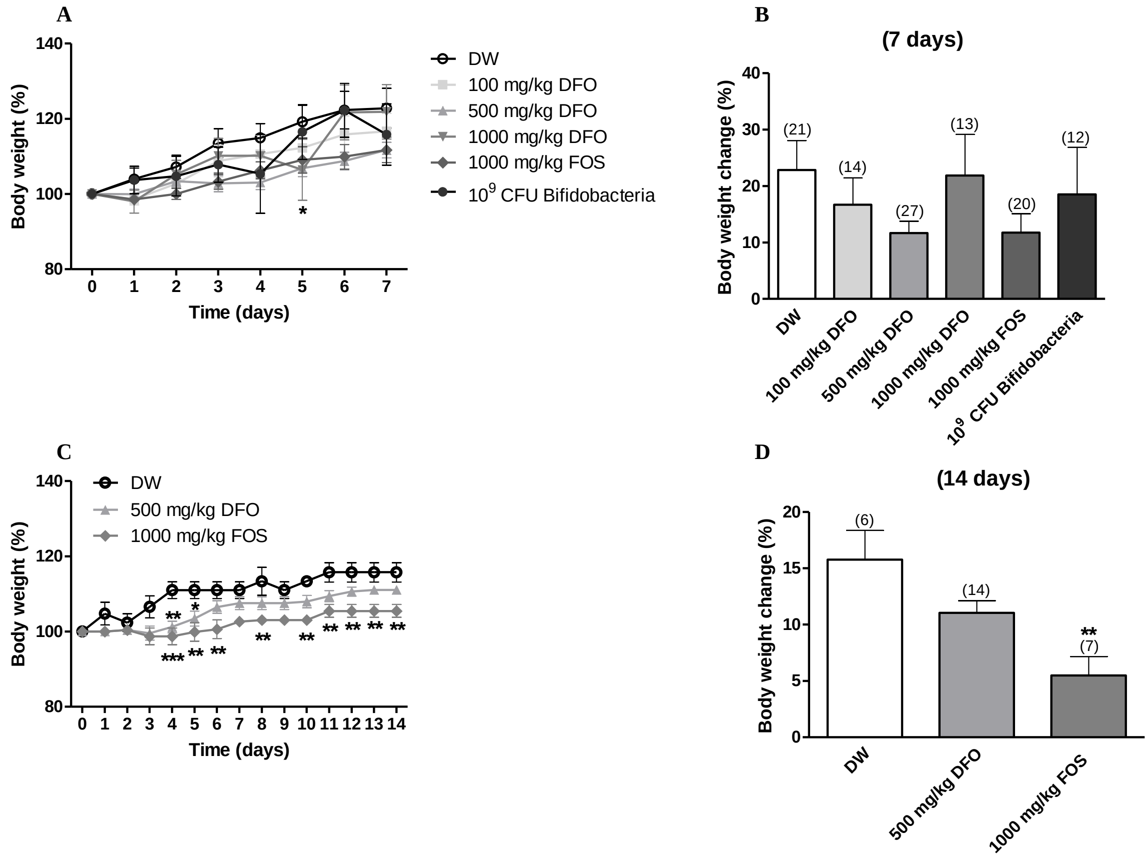


Figure 5.1: Effect of DFO ingestion for seven (A and B) and fourteen (C and D) days on body weight (%) (A and C) and body weight change (%) (B and D) in mice. Data are means ± SEM and are expressed as a percentage of day 0 of the treatment. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ compared to control group (DW), number in parentheses is the number of animals.

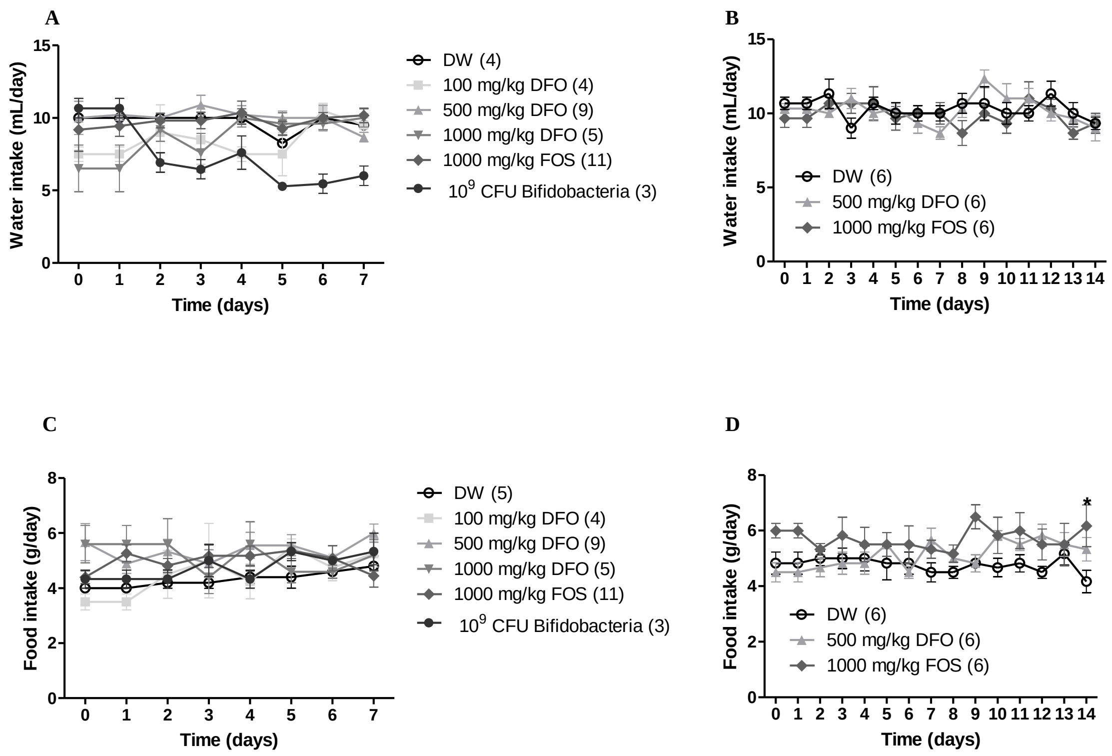


Figure 5.2: Effect of DFO ingestion for seven (A and C) and fourteen (B and C) days on food intake (C and D) and water intake (A and B) of mice. *$P < 0.05$ compared to control group (DW). Number in parentheses is the number of animals.

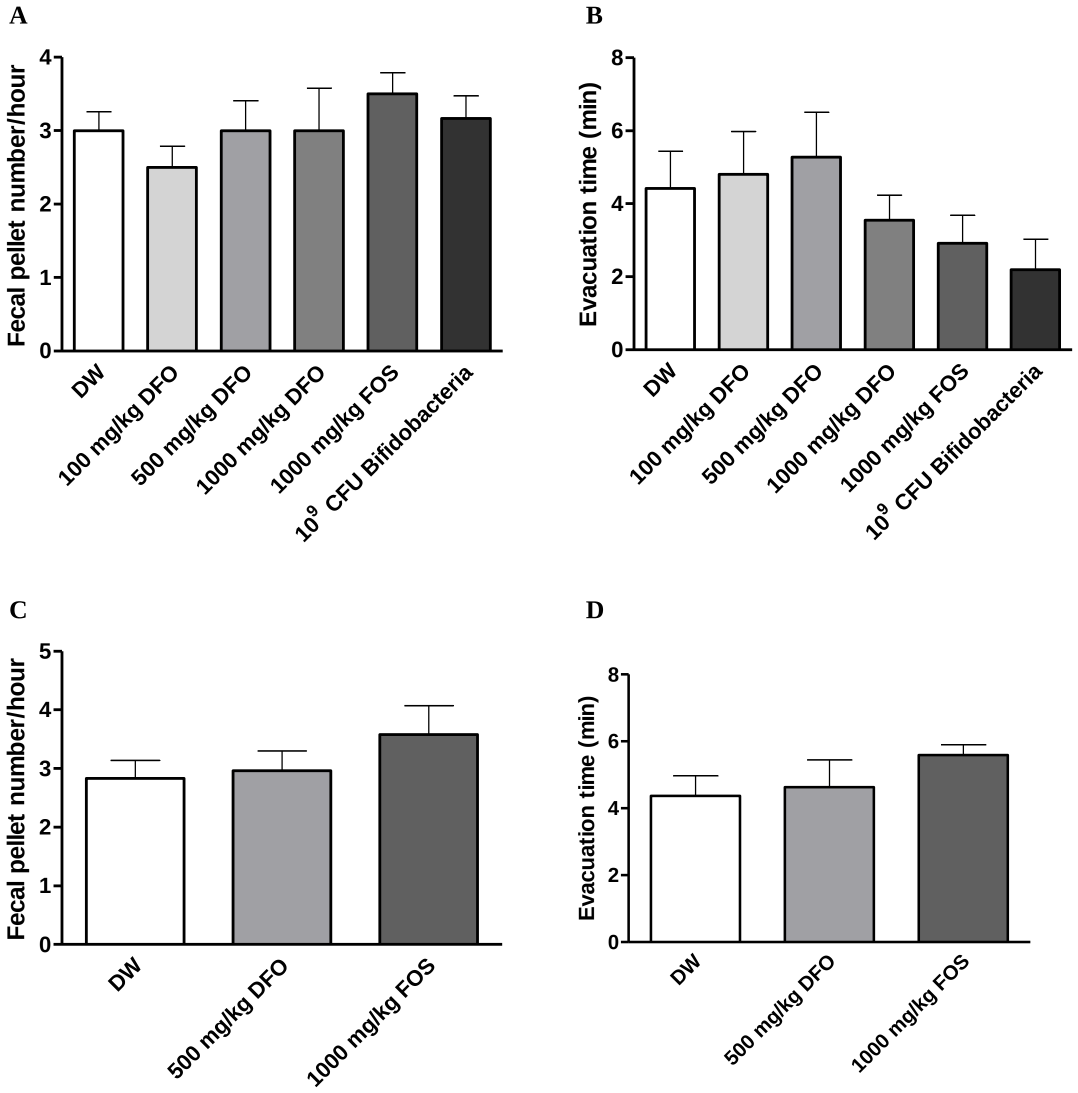


Figure 5.3: Effects of DFO ingestion for a week and two weeks on the number of fecal pellets and time of evacuation in mice. Mice were treated with 0.2 mL DW, DFO (100, 500, and 1000 mg/kg, p.o.), FOS (1000 mg/kg, p.o.), and bifidobacteria (10^9 CFU, p.o.) for a week (A and B) and treated with 0.2 mL DW, DFO (500 mg/kg, p.o.), and FOS (1000 mg/kg, p.o.), for two weeks (C and D). The fecal pellet number was counted per six hours and calculated to one hour. The evacuation time was recorded after insertion of a glass bead until the glass bead expelled. Each bar of the data represents means ± SEM (n = 4–6).

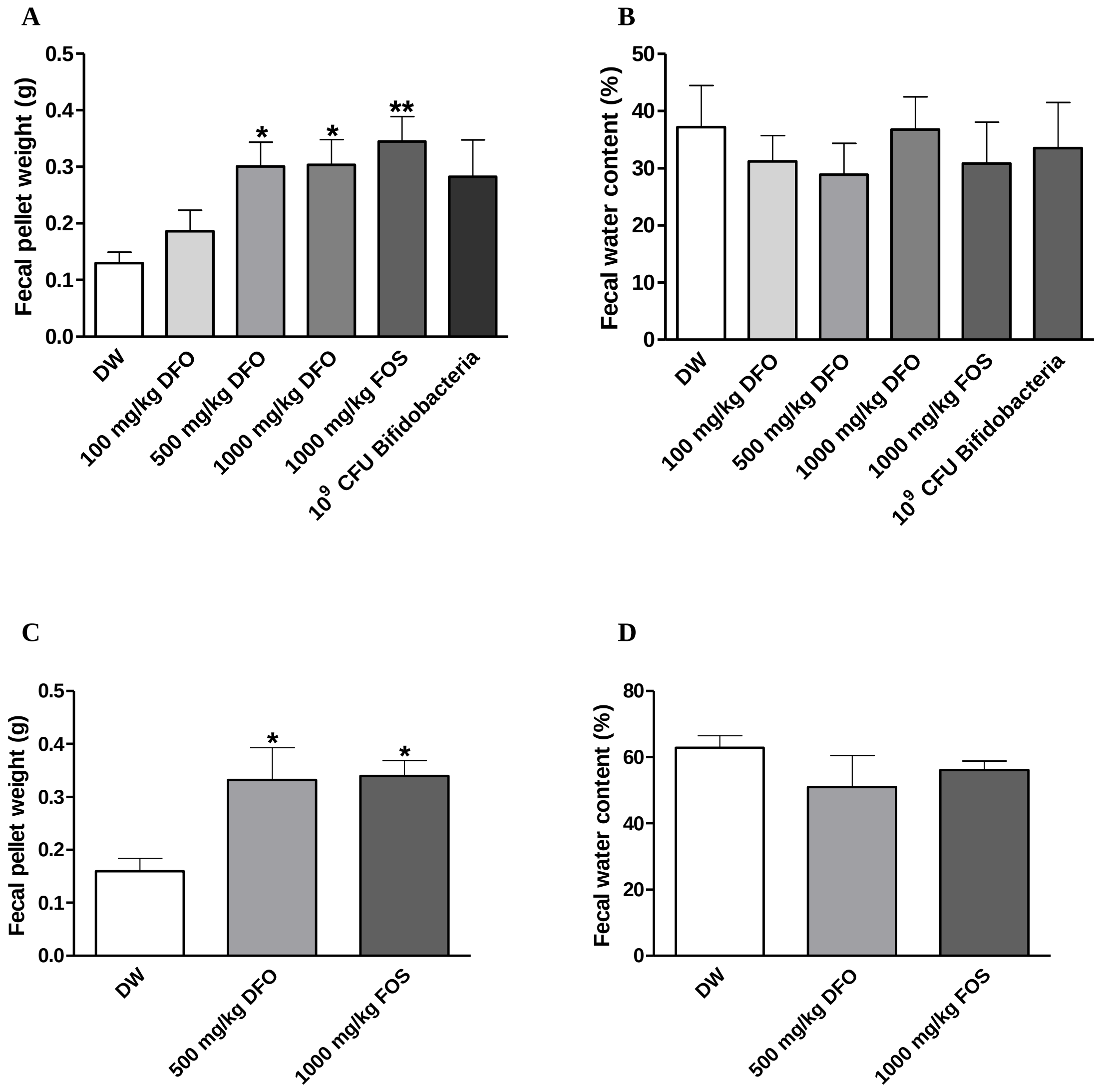


Figure 5.4: Effects of DFO ingestion for a week and two weeks on fecal pellet weight and fecal water content in mice. Mice were treated with 0.2 mL DW, DFO (100, 500, and 1000 mg/kg, p.o.), FOS (1000 mg/kg, p.o.), and bifidobacteria (10^9 CFU, p.o.) for a week (A and B) and treated with 0.2 mL DW, DFO (500 mg/kg, p.o.), and FOS (1000 mg/kg, p.o.), for two weeks (C and D). The fecal pellets were collected for six hours, weighted, and recorded in the unit of gram, after that the feces were dried and weighted again to calculate the percentage of fecal water content. Each bar of the data represents means ± SEM (n = 6–10). $^*P < 0.05$ and $^{**}P < 0.01$ compared to vehicle control group (DW).

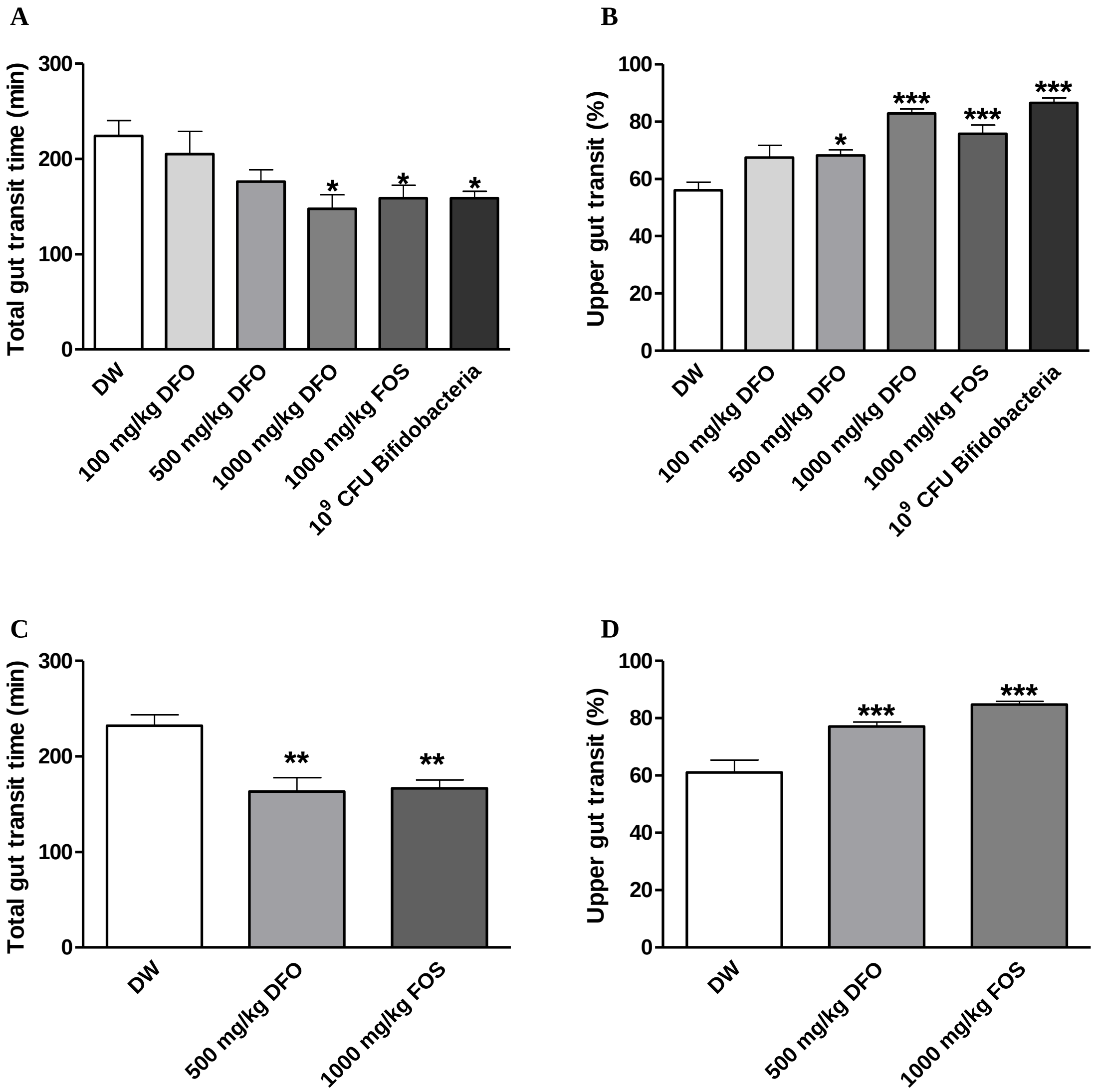


Figure 5.5: Effects of DFO ingestion for a week or two weeks on Evan-blue total gut transit time and charcoal meal upper gut transit in mice. Mice were treated with 0.2 mL DW, DFO (100, 500, and 1000 mg/kg, p.o.), FOS (1000 mg/kg, p.o.), and bifidobacteria (10^9 CFU, p.o.) for a week (A and B) and treated with 0.2 mL DW, DFO (500 mg/kg, p.o.), and FOS (1000 mg/kg, p.o.), for two weeks (C and D). The total gut transit time was recorded when first blue fecal pellet expelled and each bar represents the mean of the total gut transit time (minute) ± SEM (n = 4–6) (A and C). The whole distance of the small intestine (from pylorus to cecum) was taken as 100% and each bar represents the mean of the percentage distance of the small intestine 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).

5.3 Effects of DFO on colonic motility

Since DFO ingestion could decrease the gut transit time, it is possible that DFO could exert this effect via increased gut motility. For the spatiotemporal maps study, two patterns of motility were observed. First, the motility was induced by natural pellets. It consisted of propagation or peristaltic contractions that pushed the pellets aborally, such as, pushed the pellet at 0.6 mm/s in the 1000 mg/kg DFO (one week) treated group. The second type consisting of shallow circular muscle contractions separated by short relaxation. This type pushed the pellets forward and backward (non-propagation contraction or segmentation), thus we could not calculate the velocity in this contraction pattern. At a week treatment of 1000 mg/kg DFO, but not 100 and 500 mg/kg showed significantly increased number of total contraction, especially non-propagation pattern (Figure 5.6A and 5.6B), whereas the number of propagation contraction was not significantly different (Figure 5.6C). Similarity to a week, two weeks of 500 mg/kg DFO ingestion showed significantly increased number of total and non-propagation contractions, but not propagation pattern (Figure 5.7A, 5.7B, and 5.7C). For the fecal pellet velocity measurement, administration of 1000 mg/kg DFO for a week caused a 88.33% increase from 0.07 ± 0.02 mm/s to 0.6 ± 0.06 mm/s (Figure 5.6D) whereas administration of 500 mg/kg DFO for two weeks slightly increased the velocity but not significantly different to control (Figure 5.7D). The reference prebiotic and probiotic ingestions had the similar or even greater effects to DFO ingestion, except the treatment of 1000 mg/kg FOS for a week. FOS ingestion could not significantly enhance the velocity of fecal pellet when compare to control (Figure 5.6D).

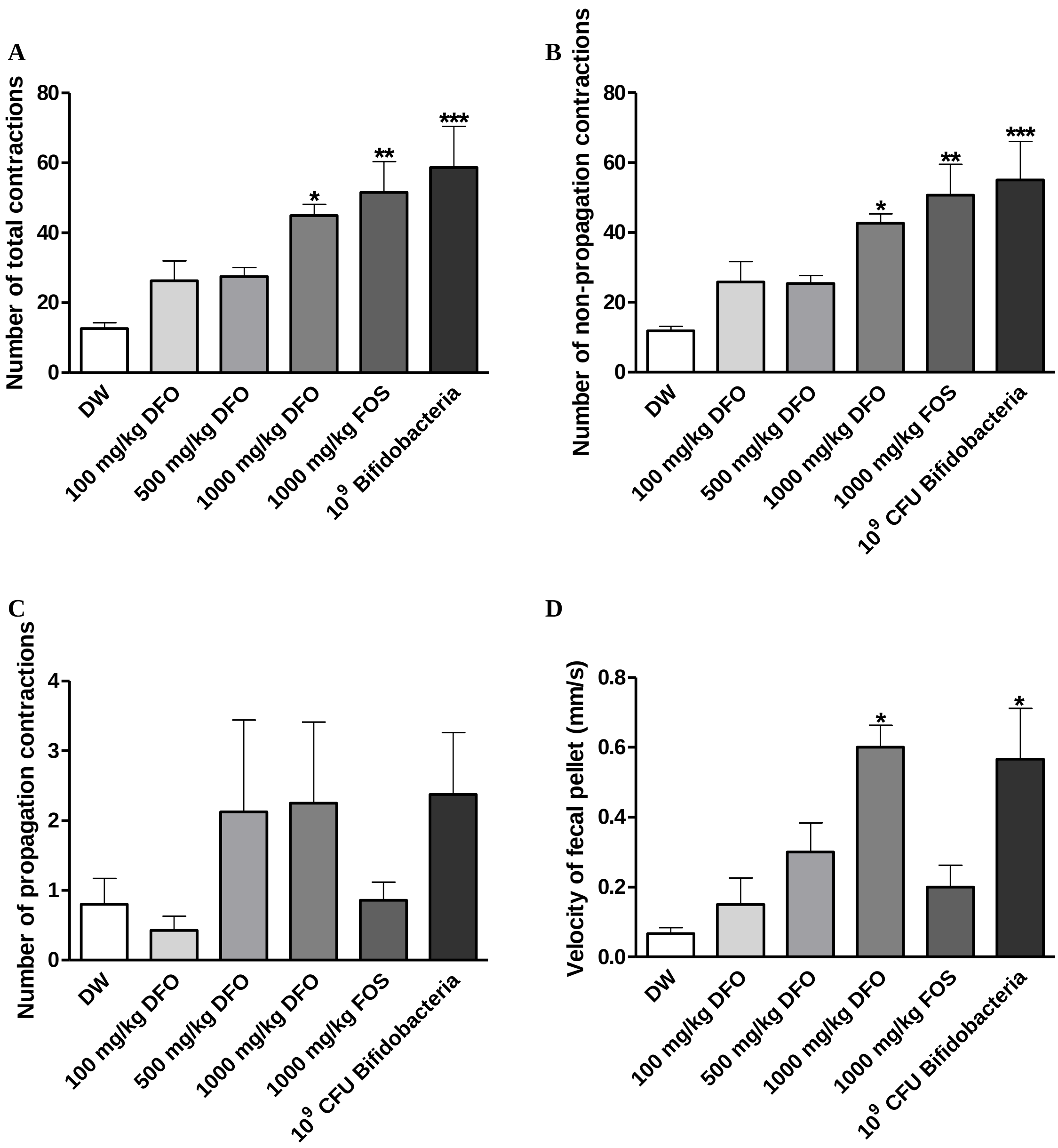


Figure 5.6: Effects of DFO ingestion for a week on the number of contractile responses for 30 minutes in the entire colon. Mice were treated with 0.2 mL DW, DFO (100, 500, and 1000 mg/kg, p.o.), FOS (1000 mg/kg, p.o.), and bifidobacteria (10^9 CFU, p.o.) for a week. Number of total contractions (A) were defined as the summation of the number of non-propagation contractions (B) and propagation contractions (C). Non-propagation contractions were defined as those contractions that failed to move the pellet go forward. Velocity of fecal pellet propulsion through whole colon (D) was determined only in the propagation contraction pattern. Data are means ± SEM (n = 5). *P < 0.05, **P < 0.01 and ***P < 0.001 compared to control group (DW).

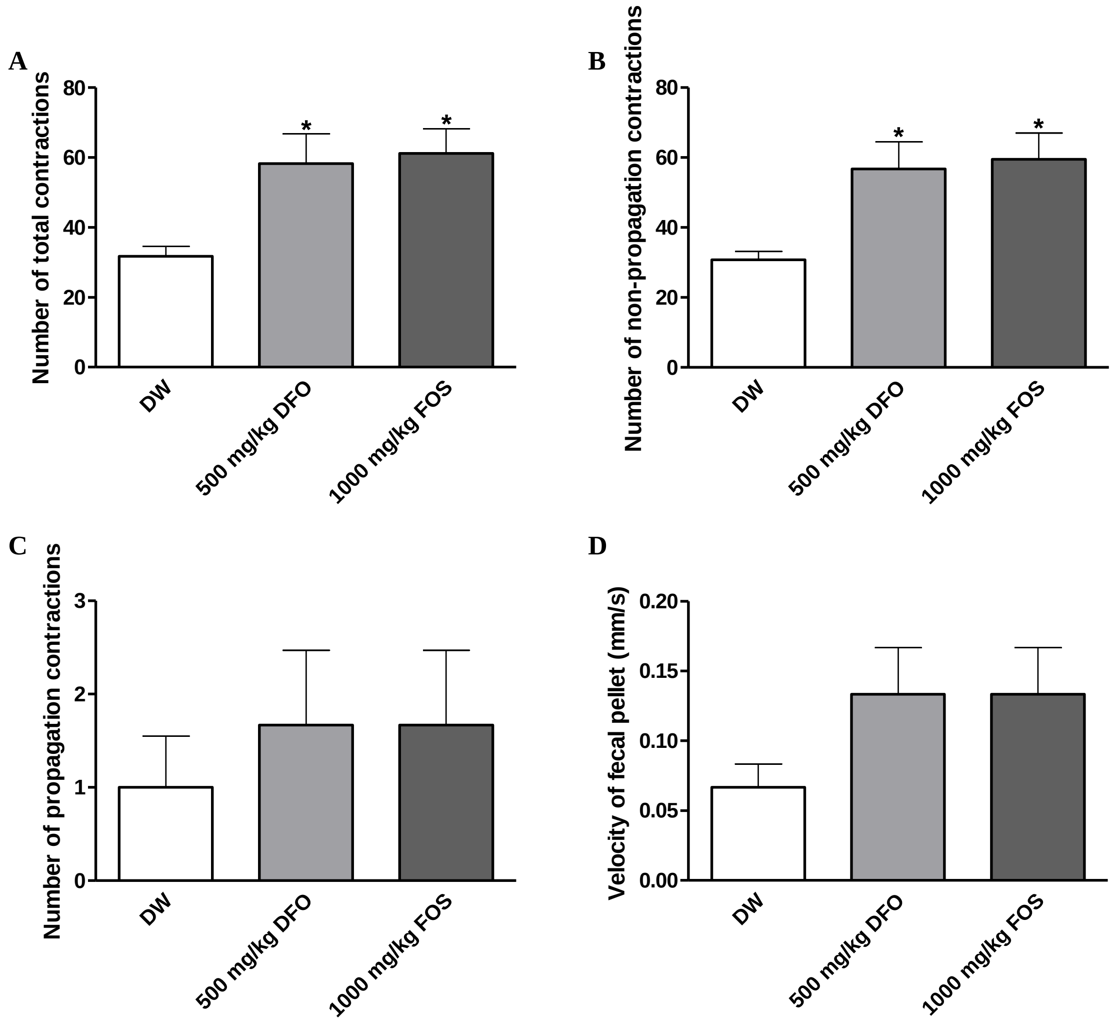


Figure 5.7: Effects of DFO ingestion for two weeks on the number of contractile responses for 30 minutes in the entire colon. Mice were treated with 0.2 mL DW, DFO (500 mg/kg, p.o.), and FOS (1000 mg/kg, p.o.) for two weeks. Number of total contractions (A) were defined as the summation of the number of non-propagation contractions (B) and propagation contractions (C). Non-propagation contractions were defined as those contractions that failed to move the pellet go forward. Velocity of fecal pellet propulsion through whole colon (D) was determined only in the propagation contraction pattern. Data are means ± SEM (n = 5–6). *$P < 0.05$ compared to control group (DW).

5.4 Effects of DFO ingestion for seven days on spontaneous proximal colonic circular and longitudinal smooth muscle contractions

This study aimed to investigate the effect of different doses of DFO, FOS, and bifidobacteria ingestion for seven days on the amplitude, frequency, and duration of contraction of the proximal colonic circular and longitudinal smooth muscles by using *in vitro* organ bath set up. The contractions induced by 1 μM carbachol were not significantly different between vehicle control and all mice treated groups in isolated proximal colonic smooth muscle segments which indicated that muscle segments from all groups worked properly (data not shown). In the circular smooth muscle segments from proximal colon, the mean amplitude of the spontaneous contraction was 30.27±4.99 % in the control group, 37.00±7.67 %, 60.49±9.28 %, and 68.95±6.98 % in 100, 500, and 1000 mg/kg DFO, respectively. Thus, the contraction amplitude of 500 and 1000 mg/kg DFO was significantly higher than the control groups (Figure 5.8A). In the longitudinal smooth muscle segments from proximal colon, the mean amplitude of spontaneous contractions was 36.27±7.79 % in the control group, 49.60±6.43 %, 63.86±3.30 %, and 70.55±8.31 % in 100, 500, and 1000 mg/kg DFO, respectively. From the statistical analysis, only the amplitude of contraction of 1000 mg/kg DFO was significantly higher than control groups (Figure 5.8B). At 1000 mg/kg of FOS and 10^9 CFU bifidobacteria also increased the contraction amplitude when compare to control, except FOS could not significantly enhanced the amplitude of contraction of longitudinal smooth muscle (Figure 5.8A and 5.8B). In both circular and longitudinal smooth muscle segments from proximal colon, the mean frequency of the spontaneous contractions was not significant difference between the responses of DFO or other treatments and control groups (Figure 5.8C and 5.8D). In contrast, the duration of contraction of 1000 mg/kg DFO and bifidobacteria was significantly longer than the control group, but 1000 mg/kg FOS could significantly increase the contraction duration only in the circular smooth muscle (Figure 5.8E and 5.8F).

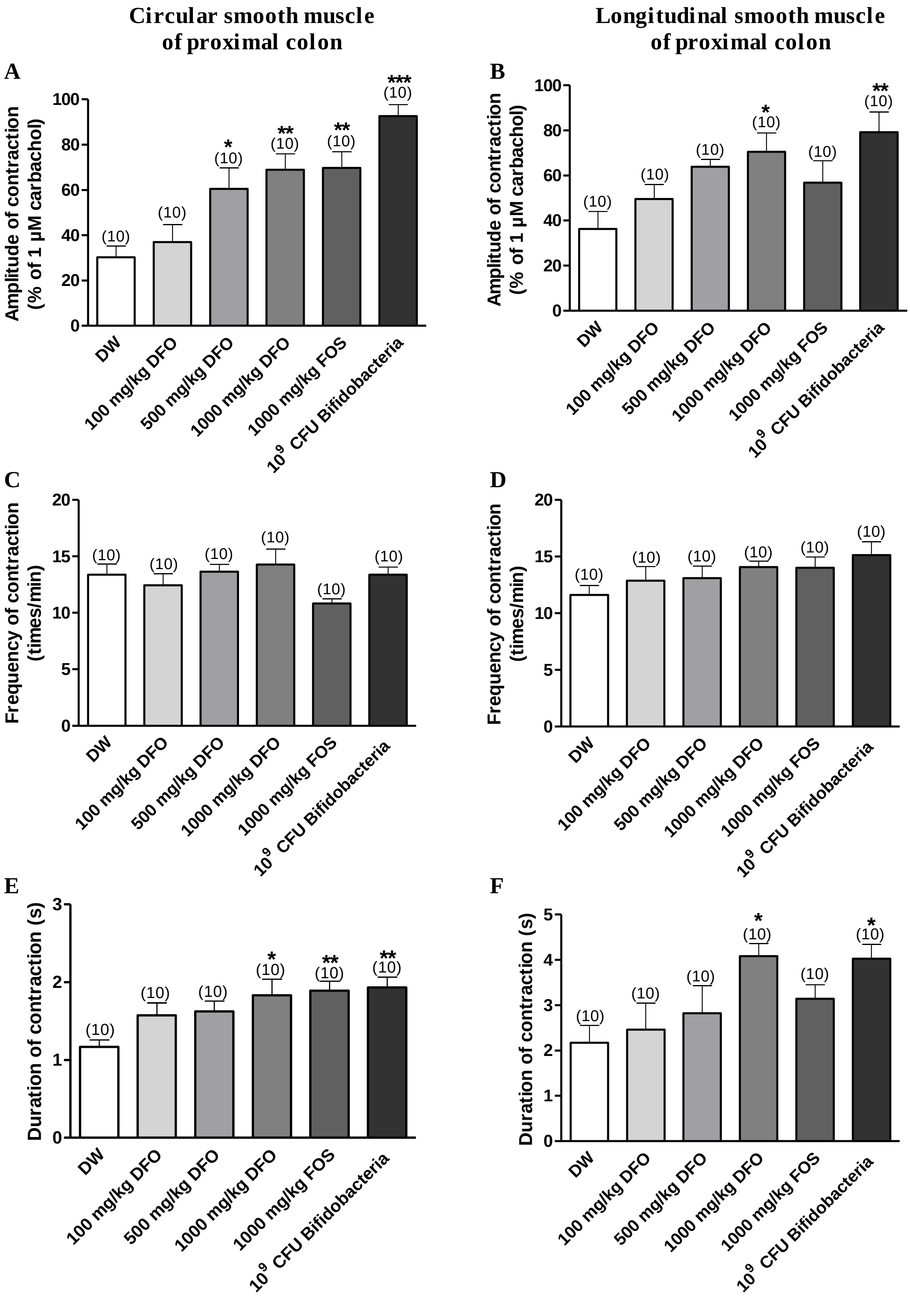
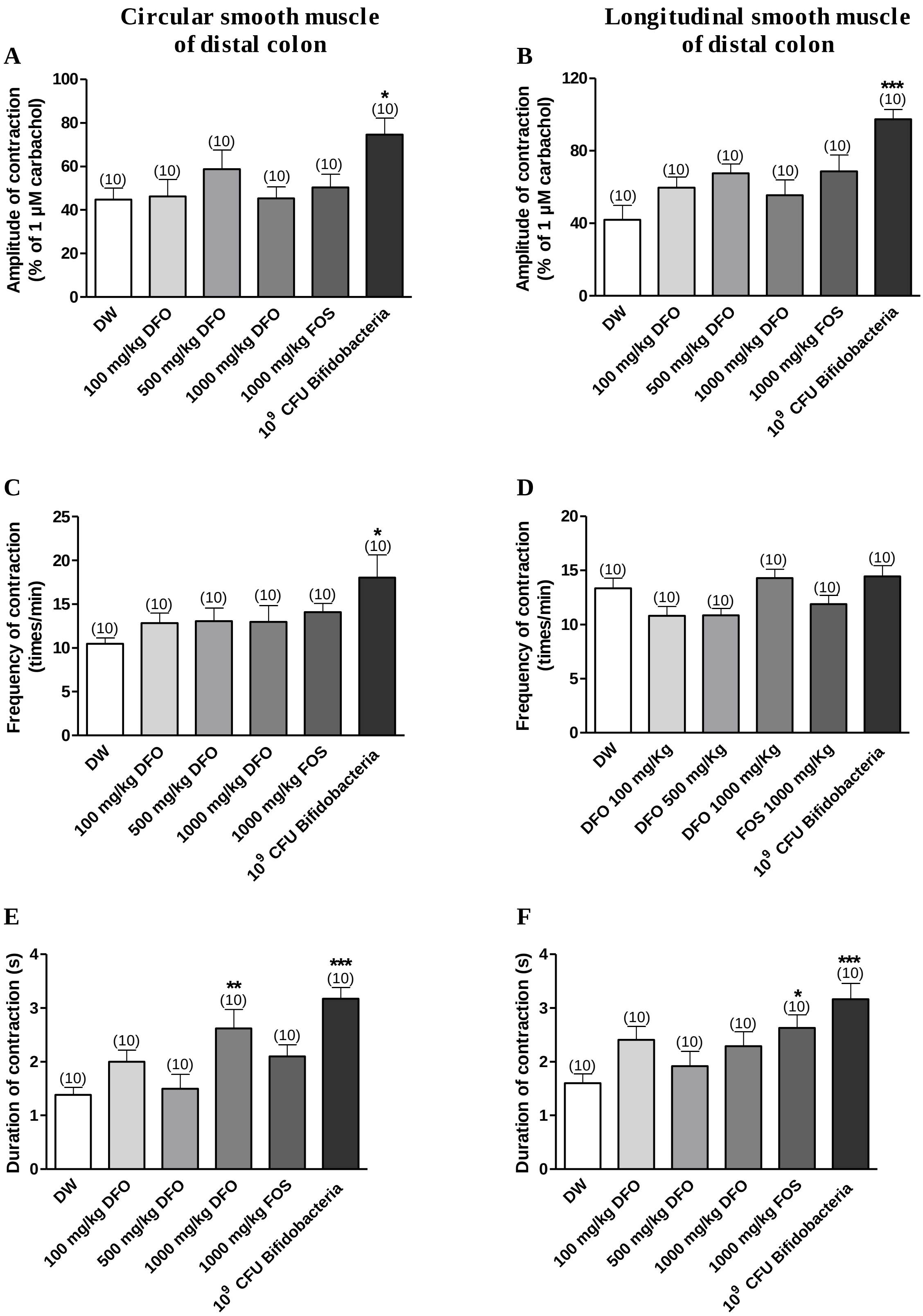

Circular smooth muscle of proximal colon
Longitudinal smooth muscle of proximal colon
A
B
C
D
E
F
Amplitude of contraction (% of 1 µM carbachol)
Frequency of contraction (times/min)
Duration of contraction (s)
DW
100 mg/kg DFO
500 mg/kg DFO
1000 mg/kg DFO
1000 mg/kg FOS
10^9 CFU Bifidobacteria
(10)
*
**
***

Figure 5.8: Effects of DFO ingestion for seven days on spontaneous proximal colonic circular (A, C, and E) and longitudinal (B, D, and F) smooth muscle contractions in mice. Mice were treated with 0.2 mL distilled water (DW), DFO (100, 500, and 1000 mg/kg, p.o.), FOS (1000 mg/kg, p.o.), and bifidobacteria (10^9 CFU, p.o.) for 7 days. Values are means + SEM (n = 10) and are expressed as a percentage of the amplitude of maximum of contraction (A and B), times/min of the frequency of contraction (C and D), and seconds of the duration of contraction (E and F). **P* < 0.05, ***P* < 0.01 and ****P* < 0.001 compared to vehicle control group (DW).

5.5 Effects of DFO ingestion for seven days on spontaneous distal colonic circular and longitudinal smooth muscle contractions

We also investigated the effect of DFO for seven days on the amplitude, frequency, and duration of contraction of the distal colonic circular and longitudinal smooth muscles. The colonic segments directly exposed to 1 μM carbachol were not significantly different between control and all mice treated groups in the contraction (data not shown). In both circular and longitudinal smooth muscle segments, the mean amplitude of the spontaneous contractions of 100, 500 and 1000 mg/kg DFO and 1000 mg/kg FOS were not significantly different when compared to control groups. Only 10^9 CFU bifidobacteria could significantly increase the contraction amplitude (Figure 5.9A and 5.9B). Similar to the amplitude, the mean frequency of the spontaneous contractions was not significant difference between the responses of DFO, FOS and control groups in both circular and longitudinal smooth muscles (Figure 5.9C and 5.9D). For the duration of contraction, 1000 mg/kg DFO and bifidobacteria significantly increased the contraction duration of circular muscle, whereas 1000 mg/kg FOS and bifidobacteria increased the contraction duration of longitudinal muscle when compared to control group (Figure 5.9E and 5.9F).



Circular smooth muscle of distal colon
Longitudinal smooth muscle of distal colon
A
B
C
D
E
F
Amplitude of contraction (% of 1 μM carbachol)
Frequency of contraction (times/min)
Duration of contraction (s)
DW
100 mg/kg DFO
500 mg/kg DFO
1000 mg/kg DFO
1000 mg/kg FOS
10^9 CFU Bifidobacteria
DFO 100 mg/Kg
DFO 500 mg/Kg
DFO 1000 mg/Kg
FOS 1000 mg/Kg
(10)
*
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Figure 5.9: Effects of DFO ingestion for seven days on spontaneous distal colonic circular (A, C, and E) and longitudinal (B, D, and F) smooth muscle contractions in mice. Mice were treated with 0.2 mL distilled water (DW), DFO (100, 500, and 1000 mg/kg, p.o.), FOS (1000 mg/kg, p.o.), and bifidobacteria (10^9 CFU, p.o.) for 7 days. Values are means + SEM (n = 10) and are expressed as a percentage of the amplitude of maximum of contraction (A and B), times/min of the frequency of contraction (C and D), and seconds of the duration of contraction (E and F). *P < 0.05, **P < 0.01 and ***P < 0.001 compared to vehicle control group (DW).

5.6 Effects of DFO ingestion for fourteen days on spontaneous proximal colonic circular and longitudinal smooth muscle contractions

To examine the effect of longer duration ingestion of DFO, DFO and the positive control FOS were administered for fourteen days. In the proximal colon, the amplitude of contraction of both circular and longitudinal smooth muscles of 500 mg/kg DFO and 1000 mg/kg FOS was significantly higher than the amplitude of the control groups (Figure 5.10A and 5.10B). In the frequency of contractions, DFO and FOS could enhance the frequency of circular, but not longitudinal, smooth muscle contractions when compared to control group (Figure 5.10C and 5.10D). In contrast, the duration of contraction of longitudinal, but not circular, smooth muscles of DFO and FOS was significantly longer than the control group (Figure 5.10E and 5.10F).

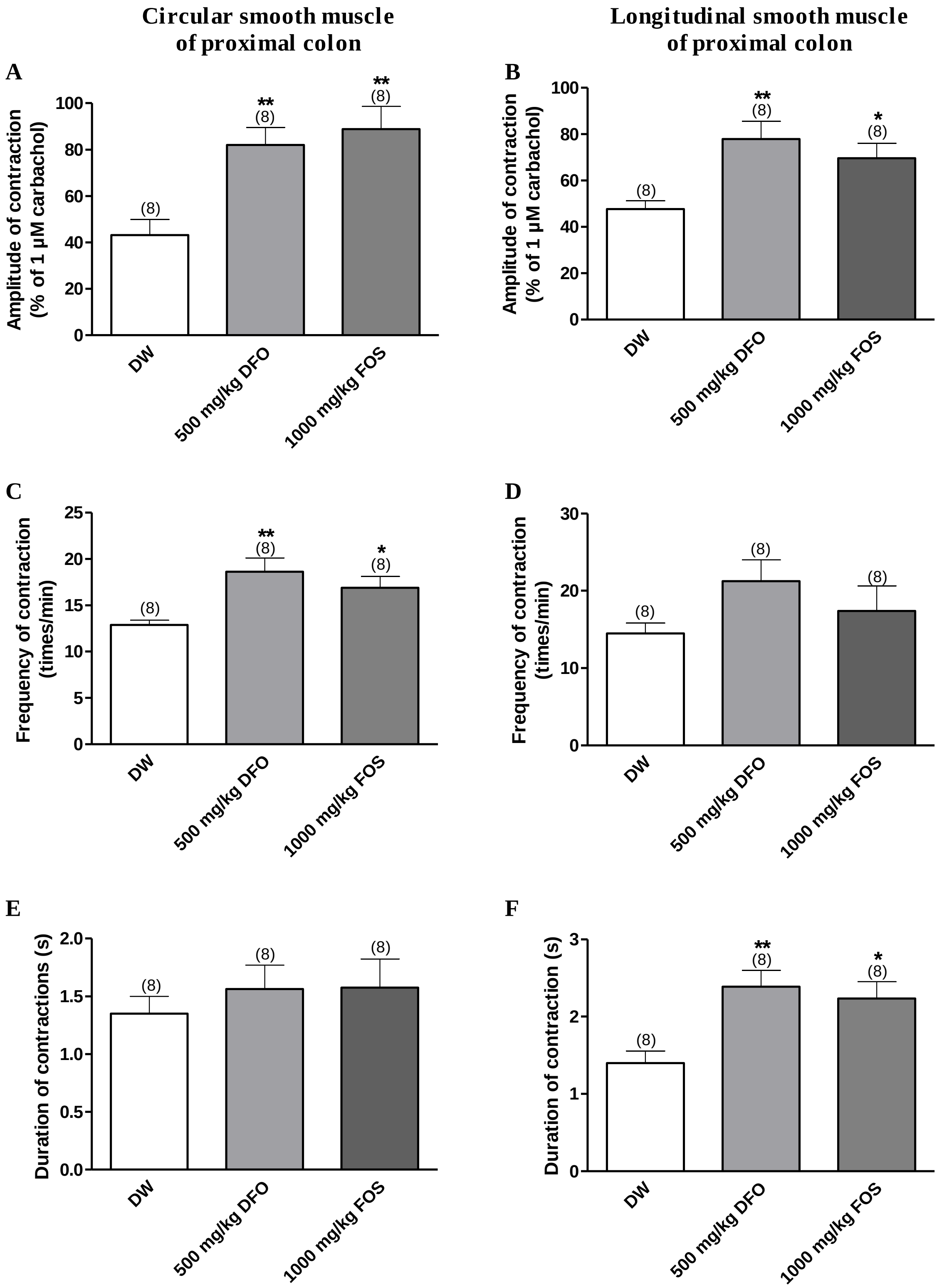
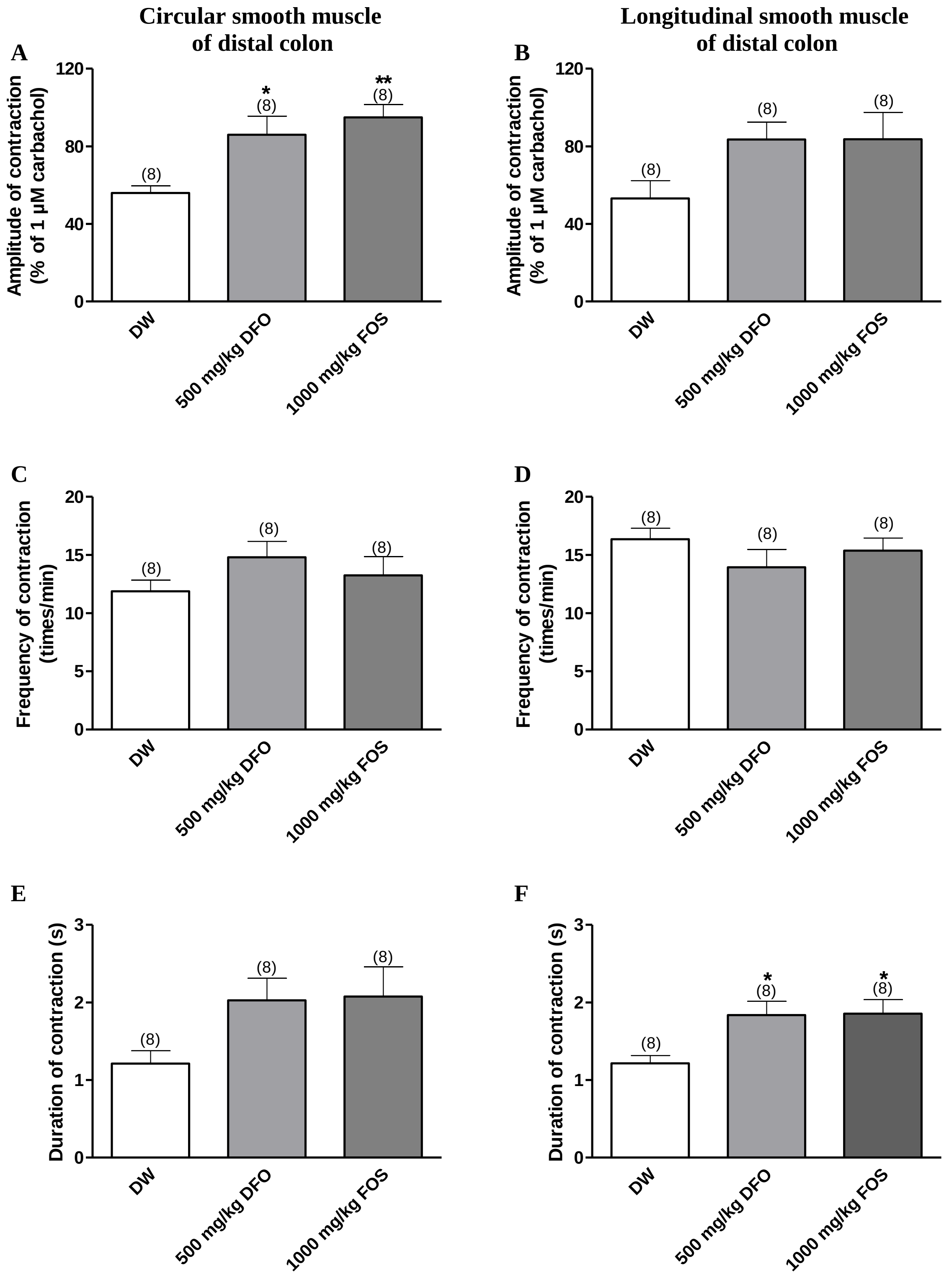

Circular smooth muscle
of proximal colon
Longitudinal smooth muscle
of proximal colon
A
Amplitude of contraction
(% of 1 μM carbachol)
B
C
Frequency of contraction
(times/min)
D
E
Duration of contractions (s)
F
Duration of contraction (s)
DW
500 mg/kg DFO
1000 mg/kg FOS
(8)
**
*

Figure 5.10 Effects of DFO ingestion for fourteen days on spontaneous proximal colonic circular (A, C, and E) and longitudinal (B, D, and F) smooth muscle contractions in mice. Mice were treated with 0.2 mL distilled water **(**DW**)**, DFO **(**500 mg/kg, p.o.**)**, and FOS **(**1000 mg/kg, p.o.**)** for 14 days**.** Values are means + SEM (n **=** 8) and are expressed as a percentage of the amplitude of maximum of contraction (A and B), times/min of the frequency of contraction (C and D), and seconds of the duration of contraction (E and F). $^*P < 0.05$ and $^{**}P < 0.01$ compared to vehicle control group (DW).

5.7 Effects of DFO ingestion for fourteen days on spontaneous distal colonic circular and longitudinal smooth muscle contractions

In the distal colon, the amplitude of contraction of circular smooth muscles of 500 mg/kg DFO and 1000 mg/kg FOS was significantly higher than the amplitude of the control groups (Figure 5.11A), while the contraction amplitude of longitudinal smooth muscles showed no change (Figure 5.11B). In contrast to the amplitude, there was no significant difference in the frequency of contraction after administration of DFO and FOS (Figure 5.11C and 5.11D). For the duration of contraction, the longitudinal, but not circular, smooth muscles of DFO and FOS treated mice was significantly longer in the duration than the control group (Figure 5.11E and 5.11F).



Circular smooth muscle
of distal colon
Longitudinal smooth muscle
of distal colon
A
B
C
D
E
F
Amplitude of contraction
(% of 1 µM carbachol)
Frequency of contraction
(times/min)
Duration of contraction (s)
DW
500 mg/kg DFO
1000 mg/kg FOS
(8)
*
**

Figure 5.11 Effects of DFO ingestion for fourteen days on spontaneous distal colonic circular (A, C, and E) and longitudinal (B, D, and F) smooth muscle contractions in mice. Mice were treated with 0.2 mL distilled water (DW), DFO (500 mg/kg, p.o.), and FOS (1000 mg/kg, p.o.) for 14 days. Values are means + SEM (n = 8) and are expressed as a percentage of the amplitude of maximum of contraction (A and B), times/min of the frequency of contraction (C and D), and seconds of the duration of contraction (E and F). *P < 0.05 and **P < 0.01 compared to vehicle control group (DW).

5.8 Effects of DFO on colonic smooth muscle histology

To determine whether DFO increased the colonic motility by increasing the thickness of the colonic smooth muscle, the histological changes of the colonic wall were determine. Continuous consumption of 500 mg/kg DFO for two weeks showed no irritating effect on colonic mucosa. It displayed normal morphology of simple squamous epithelium as same as 1000 mg/kg FOS and DW-fed groups. The number of goblet cells presence in the mucosa layer were comparable to FOS and DW-fed groups. Regarding the muscular layer, there were not significantly difference in the thickness of smooth muscle layer in DFO-fed group compared to both FOS- and DW-fed groups (Figure 2.10).

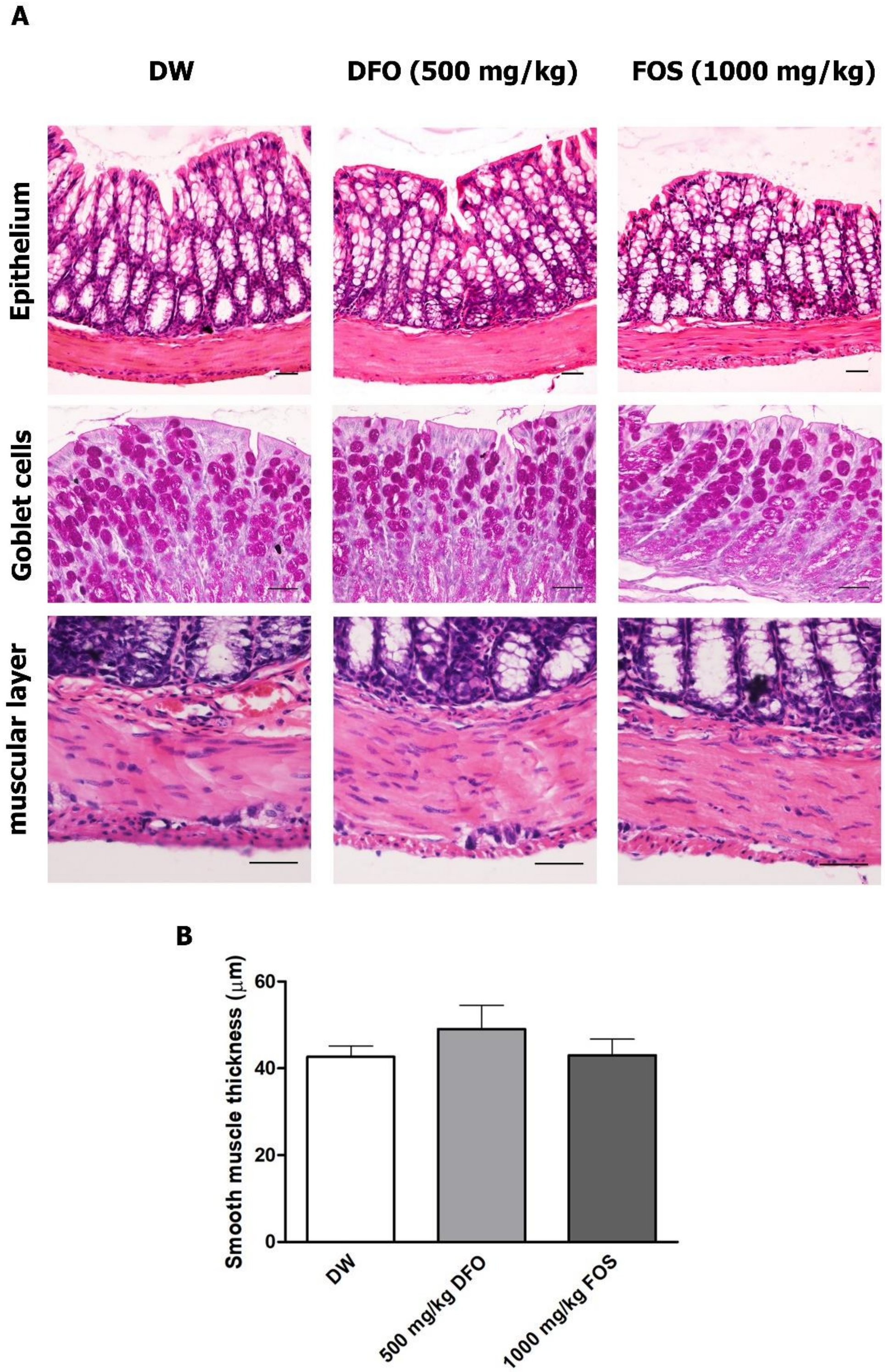


Figure 5.12: Histological cross section images (Conventional H&E and PAS staining) of mouse colon paraffin sections (A) and colonic SM thickness (B) of DFO treated mice. Mice orally administered with 0.2 mL DW, DFO (500 mg/kg, p.o.), and FOS (1000 mg/kg, p.o.) for two weeks. The figure showed normal epithelium, number of goblet cells, and thickness of muscular layer in both DFO and FOS groups compare to control group (DW). Scale bar = 20 µm

6. Conclusion and Discussion

In the present study, we found that ingestion of DFO increased the upper gut transit which reduced the time of the content to the large intestine and also reduced the total gut transit time. There were some studies reported that rapid intestinal transit was seen in malabsorptive states and diarrhea symptoms (74). In this study, however, the BW of DFO-treated group was not changed when compared to control group, and there were not shown the diarrheal feces characteristics. DFO are non-digestible, fermentable, and soluble short-chain carbohydrate fiber. When DFOs were consumed, 50% of them was estimated to reach the colon since some of them were hydrolyzed by salivary and pancreatic α-amylases (16%), by gastric juice (2.5%) and by brush-border enzymes in the small intestine (30%) (25). In the intestine, this soluble fiber had appreciable water holding capacity, however it was known to be less than that of other fibers with strong water holding capacity, i.e., wheat fiber, resulting in increase in fecal mass (75). Fecal pellet wet weight or fecal output in DFO- and FOS-treated groups in this study significantly increased when compared to control group. However, the percentages of fecal water content were not significant different in all group since this type of short-chain carbohydrates may good as stool bulking forming, but not water holding, or there was no fluid secreting effects in the colonic epithelial cells. The increased intestinal content, especially in the colon, can stimulate peristaltic contraction, and also accelerates intestinal transit (76).

The peristaltic or propagation contractions reduced the diameter of the colon, without occluding the lumen. Thus they did not empty the proximal colon in a single sweep, but rather slowly pushed small amounts of content into the distal colon. In this study also shown the effect of DFO ingestion on the colonic motility pattern. Even though the propagation pattern of the DFO-treated group was showed increasing trend, but not significant different when compared to control group. The velocity of fecal pellet movement through the entire colon significantly increased in one week of 1000 mg/kg DFO treatment when compared to control group. Distention by natural fecal pellets is a major trigger for neutrally mediated propulsion in the colon (77, 78). Thus it suggests that peristaltic contractions in the colon result from a distension-triggered motor pattern generator mediated by enteric nervous system (79).

In addition to distend the colonic wall by the colonic content, DFO might alter bowel motility through a change in the colonic environment. *In vitro* studies had shown that the bacterial fermentation of oligosaccharides increased the production of SCFAs (80, 81) leading to lower colonic pH (82). The lower pH in the colon stimulated the growth of lactobacilli and bifidobacteria and suppressed the growth of harmful bacteria (81). Increasing fermentation by-products, such as gas and SCFAs, could increase stool bulk and also stimulate the gut motility (83). The motor patterns in the colon of different species vary significantly depending on the diet. There were many conflicting studies about the effects of prebiotics and probiotics on GI motility. Some of these studies suggested that these supplements increased intestinal motility, while the others showed opposite results (84–86). *Lactobacillus reuteri* ingestion could reduce the amplitudes of colonic contractions at constant luminal pressure and increased the threshold luminal pressure which required to induce phasic contractions in rats (58). On the other hand, the administration of fermented milk prepared with *Lactobacillus casei* enhanced colonic propulsive contraction and defecation rate in pigs (85). In a study in healthy newborns fed with breast milk had lower stool consistency and higher stool frequency than newborns fed with bovine milk. In addition, supplementation of a mixture of GOS and FOS resulted in a reduction in stool consistency and an increase in stool frequency (86). Our study was consistent with the previous studies. We found that DFO increased the gut transit by increasing intestinal motility, increased fecal pellet velocity and also the number of whole colonic contraction, especially non-propagation pattern.

Non- propagation or segmenting contractions cause mixing and local circulation of contents. This contraction pattern may slow the gut transit to abolish the high effect of DFO on peristaltic contraction, finally, there were no adverse effects of the DFO supplement, e.g., diarrhea and malabsorption in this study. Normally the anal canal of the GI tract is closed because of internal anal sphincter contraction. When the rectum is distended by fecal material, which we used glass bead in this experiment, the internal sphincter relaxes as part of the rectosphincteric reflex. Rectal distention also elicits a sensation that signals the urge for defecation which is prevented by the external anal sphincter. The external anal sphincter contraction is maintained by reflex activation through dorsal roots in the sacral spinal cords. In this study, there were no significant different of evacuation time which means that the prebiotic DFO may not affect the neural control of the last process of the defecation.

Delayed transit of contents through the colon or decreased colonic motility is common which leads to constipation, however, this is dietary in origin. There is a direct correlation among increased dietary fiber or prebiotics, increased colonic intraluminal bulk, and enhanced transit through the colon or increased the motility. Many studies reported that prebiotics will improve health in a way similar to probiotics, whereas at the same time being cheaper, harmless and being easier to incorporate into the diet than probiotics. However, excessive intake of short-chain carbohydrates can cause undesirable side effects, such as flatulence, bloating, rumbling, cramps, and liquid stools, caused by gas formation and osmotic effects of certain fermentation products (e.g., SCFAs). Fortunately, in our present study, 1000 mg/kg/day or less of DFO is usually well tolerated in mice.

SM colonic contraction are organized to allow for optimal absorption of water and electrolytes, net aboral movement of contents, storage and orderly evacuation of feces. The intestinal muscularis externa layers display two distinct motility patterns, 1) the propagated peristaltic contractions, involving the coordinated contractions of the longitudinal and circular SM, and 2) the non-propagated segmentation contractions, involving mainly the circular muscle layer. It has been shown in this study that DFO treatment for a week increased force and duration of contraction of both circular and longitudinal SM in proximal colon, but only increased the duration of circular SM contraction in the distal colon. For two weeks treatment, DFO could increase the spontaneous contraction frequency of circular SM in the proximal, but not in the distal, colon. Short-chain carbohydrates including DFO were very rapidly fermented in the terminal ileum and proximal colon to produce SCFAs, therefore the proximal colonic SM should be affected much more than the smooth muscle in the distal colon (75). However, the underlying mechanisms of DFO on colonic SM contraction are still unknown.

Recent work has found that specific SCFAs such as butyrate increased cholinergic-mediated colonic circular SM contraction in animals (87). Hurst and co-workers (14) also reported that colonic luminal butyrate, acetate and propionate have different effects on proximal and distal colonic contraction depending on chain length, and the net effect of SCFAs on the contraction would depend on the balance of SCFAs produced by gut microbiota fermentation of non-digestible carbohydrates (14). The colonic SM cell membrane has slow wave activity which is always present whether contractions are occurring or not. The contractions, however, are initiated by a second electrical event, i.e., spike potential activity which is occured when slow wave reach the electrical threshold. When slow wave is accompanied by spike potential, the colon contracts at the same frequency as the slow wave frequency. Thus slow wave frequency sets the maximum frequency of contractions. Whereas the amplitude and frequency of spike potential on the crest of the slow wave are directly related to the amplitude or force and duration of muscle contractions. The occurrence of spike potential and contractions depend heavily on neuronal and hormonal activities, and locally chemical agents whereas slow waves are extremely regular and are only minimally influenced by neural or hormonal activities (88). Supporting to our results, DFO effect might regulate the amount of spiking, but less affected on the threshold of the slow wave, therefore it affected mostly on strength and duration of contractions instead of the frequency.

Coordinated contraction of intestinal circular and longitudinal SM results in caudal propulsion of luminal contents via peristalsis. Nerves in the enteric plexuses receive input from the receptors within the GI tract. An excitatory factors, i.e., SCFAs from the bifidogenic effect or distention from the osmotic effect may act on the free fatty acid or stretch receptors, respectively, at the intestinal epithelial cell which stimulate serotonin or 5-hydroxytryptamine (5-HT)[9] secretion. The 5-HT activates CGRP-containing neurons, and also a series of interneurons which send the signal to motor neurons for releasing acetylcholine, tachykinin, or substance P orad to the luminal stimulus. These neurotransmitters cause contraction of the circular SM and relaxation of the longitudinal SM. Vasoactive intestinal peptide and nitric oxide are released caudad to the luminal stimulus and stimulate circular SM relaxation and longitudinal SM contraction (88).

In addition to the contractions of SM, there were some studies reported the effect of prebiotic supplementation could change the colonic structure by the effect of gut microbiota and SCFAs. Butyrate was used directly by the colonic cells, exerting a trophic effect on these cells. However, in the present study, DFO did not have the trophic effect on the gut wall. As mucin plays a cytoprotective functions in colonic mucosa against a variety of luminal hazards. Alteration in goblet cell number was observed in intestinal infections (89). Previous studied reported that high fiber diet increased both secretory activity and numbers of mucin-secreting goblet cells in rat colon (90). Conversely, our study showed no effect of DFO on both epithelium and goblet cell numbers. Moreover, DFO did not affect the thickness of SM. Based on general criteria in histomorphological scores for intestinal inflammation (91), consumption of DFO did not follow either epithelial or mucosa architecture changes. These may confirm the safety of this product as a supplement.

Taken together, these data suggested that in addition to be a prebiotic as reported previously (25), DFO also acts as a bulk laxative, which absorb water from the intestinal lumen to increase fecal mass (osmotic effect), as well as being a stimulant laxative that increases intestinal motility in mice. We also showed an association between ingestion of DFO and alteration in the colonic SM contractility. According to these findings it seems that DFO may be suitable for inclusion as food supplements in a wide variety of food products, e.g., prebiotic/probiotic/synbiotic products, laxative product, and may be a promising nutritional therapy for GI motility disorders, such as constipation and IBS. Nevertheless, further investigation is required to identify the underlying mechanisms responsible for diet- or gut bacteria-induced changes in GI motility.

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Output

(Acknowledge the Thailand Research Fund)

1. International Journal Publication

One international paper was submitted as followed.

Title: Prebiotic oligosaccharide from dragon fruit alters intestinal motility in mice.

Authors: Pissared Khuituan, Sakena K-da, Kanrawee Bannob, Fittree Hayeeawaema, Saranya Peerakietkhajorn, Chittipong Tipbunjong, Santad Wichienchot, and Narattaphol Charoenphandhu

Journal: The Journal of Nutrition (Quartile in JCR Category = Q1, Impact Factor 2016 = 4.145)

2. Research Utilization and Application

2.1 Basic medical science: novel information on the gastrointestinal physiological roles, especially intestinal motility, of dragon fruit oligosaccharides (DFO)

2.2 Translational medical science: the present results provided foundation for further investigation of the role of DFO in health and diseases, such as diarrhea, constipation, inflammatory bowel diseases (IBDs), and irritable bowel syndrome (IBS). This finding is a preclinical step. We found that DFO could act as a bulk and a stimulant laxative which was similar to other commercial prebiotics, such as, FOS and GOS. However, DFO is derived from a dragon fruit which is a very common fruit in Thailand. DFO also improve health in a way similar to probiotics, whereas at the same time being cheaper, harmless and being easier to incorporate into the diet than probiotics. Nonetheless, further investigation is required to identify the underlying mechanisms responsible for diet- or gut bacteria-induced changes in GI motility. In addition, this investigation should be tested or confirmed by using human model before actual treatment.

2.3 Bioactive natural product: knowing the impact of prebiotic DFO confirms the benefit of nutritional supplements from dragon fruit which is important in supporting agricultural industry in Thailand.

3. Others e.g. national journal publication, proceeding, international conference, book chapter, patent

One poster presentation will be presented at the following international conference:

The 9^{th} Federation of the Asian and Oceanian Physiological Societies Congress (FAOPS 2019) from March 28^{th} to March 31^{st}, 2019, in Kobe, Japan.

Appendix

(Submitted Manuscript)

The Journal of Nutrition

Prebiotic oligosaccharide from dragon fruit alters intestinal motility in mice

--Manuscript Draft--

Manuscript Number:	JN-2018-0422
Full Title:	Prebiotic oligosaccharide from dragon fruit alters intestinal motility in mice
Short Title:	Oligosaccharide, dragon fruit, intestinal motility
Article Type:	Original Research Article
Section/Category:	Nutrient Physiology, Metabolism, and Nutrient-Nutrient Interactions (including Nutritional Toxicity)
Keywords:	dragon fruit oligosaccharide; colonic contractility; gut transit time; colonic smooth muscle; spatiotemporal map
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Abstract:	Background: Dragon fruit oligosaccharide (DFO) has prebiotic properties which improve gut health by selectively stimulating the colonic microbiota. Altering microbiota composition may affect intestinal motility; however, there is no study on DFO effects on gut motor functions. Objectives: This research aimed to investigate DFO effects on fecal pellet propulsions and spontaneous motility patterns in the isolated mouse colon, and to examine the morphology and physiology of colonic smooth muscle (SM). Methods and Results: Administration of 1000 mg/kg prebiotic reference (fructo-oligosaccharide (FOS)) for one and two weeks to adult mice significantly increased fecal pellet weight when compared to control. Similarly to the positive control, fecal pellet weight of 500 and 1000 mg/kg DFO-treated mice were significantly increased. Moreover, mice treated with FOS and probiotic bifidobacteria significantly reduced the transit time and increased the distance of upper gut transit which was comparable to DFO. Spatiotemporal map of whole colonic wall motions which recorded with a video camera showed that DFO significantly increased the number of total colonic contractions, especially non-propagation pattern, and velocity of fecal pellet movement through the colon, consistent with the results from FOS- and bifidobacteria-treated groups. In addition, DFO increased the amplitude and duration of contractions of proximal and distal colonic circular and longitudinal SM as determined by in vitro tension measurement in an organ bath. Histological stains showed normal morphology of epithelia, crypts, goblet cells, and also the SM thickness in all groups. Conclusions: DFO-fed mice increased the colonic SM contraction without changing in the morphology, and acted as bulk-forming and stimulant laxatives which increased fecal output and intestinal motility, respectively. Regarding to these results, DFO may

	be classified as a dietary supplement for promoting gut health and recovering gastrointestinal motility disorders.
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Opposed Reviewers:	
Additional Information:	
Question	**Response**
Author Comments:	Pissared Khuituan, Ph.D. Prince of Songkla University 15 Karnjanavanich Rd., Hat Yai Songkhla 90112, Thailand pissared.k@psu.ac.th Prof. Teresa A. Davis Editor-in-Chief The Journal of Nutrition May 7, 2018 Dear Prof. Davis, We would like to submit our original research article entitled "Prebiotic oligosaccharide from dragon fruit alters gut motility in mice" by Khuituan et al., for publication in The Journal of Nutrition. Regarding the key novel findings of this manuscript, our results clearly suggested that an ingestion of 1000 mg/kg oligosaccharide purified from dragon fruit (DFO) for a week and 500 mg/kg DFO for two weeks significantly increased fecal output, the number of colonic non-propagation contractions, and velocity of fecal pellet movement in mice, as determined by in vivo transit-time and ex vivo spatiotemporal mapping techniques. Thus, besides it prebiotic-like effects, DFO also acts as a bulk laxative and a stimulant laxative. Moreover, we demonstrated the association between ingestion of DFO and alteration in the colonic smooth muscle contractility by using isolated organ bath techniques. We, therefore, conclude that DFO is suitable for inclusion as a food supplement or ingredient in a wide variety of food products, e.g., prebiotic/probiotic/synbiotic products, laxative product for promoting gut health, and may be a promising nutritional intervention for gastrointestinal motility disorders, such as constipation and irritable bowel syndrome. We warrant that our manuscript represents an original work, which is not being considered for publication in another journal. All authors have contributed significantly and approved the manuscript and this submission. There is no conflict of interest to disclose. All procedures comply with the institutional and national guide for the care and use of laboratory animals (Ethic license number MOE0521.11/799). Thank you for your consideration. We look forward to hearing from you. Sincerely yours, Pissared Khuituan, Ph.D.

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Prebiotic oligosaccharide from dragon fruit alters intestinal motility in mice[1-3]

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[3] Supplemental Figure 1 and 2 are available from the "Online Supporting Material" link in the online version of the article.

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Total Number of Words: 4,976 words (introduction through discussion)

The Number of Figures: 8 figures

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Supplementary data submitted: 1 table and 2 figures with figure legends

Running Title: Oligosaccharide, dragon fruit, and intestinal motility

[9]Abbreviations: 5-HT, 5-hydroxytryptamine; BW, body weight; CFU, colony-forming unit; DFO, dragon fruit oligosaccharide; DP, degree of polymerization; DW, distilled water; FOS, fructo-oligosaccharide; GI, gastrointestinal; GIMM, gastrointestinal Motility Monitor; GOS, galacto- oligosaccharides; HE, Hematoxylin- eosin; HPLC, high performance liquid chromatography; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; MW, molecular weight; PAS, periodic acid-Schiff; SCFAs, short chain fatty acids; SM, smooth muscle

Abstract

Background: Dragon fruit oligosaccharide (DFO)[9] has prebiotic properties which improve gut health by selectively stimulating the colonic microbiota. Altering microbiota composition may affect intestinal motility; however, there is no study on DFO effects on gut motor functions.

Objectives: This research aimed to investigate DFO effects on fecal pellet propulsions and spontaneous motility patterns in the isolated mouse colon, and to examine the morphology and physiology of colonic smooth muscle (SM)[9].

Methods and Results: Administration of 1000 mg/kg prebiotic reference (fructo-oligosaccharide (FOS)[9]) for one and two weeks to adult mice significantly increased fecal pellet weight when compared to control. Similarly to the positive control, fecal pellet weight of 500 and 1000 mg/kg DFO-treated mice were significantly increased. Moreover, mice treated with FOS and probiotic bifidobacteria significantly reduced the transit time and increased the distance of upper gut transit which was comparable to DFO. Spatiotemporal map of whole colonic wall motions which recorded with a video camera showed that DFO significantly increased the number of total colonic contractions, especially non-propagation pattern, and velocity of fecal pellet movement through the colon, consistent with the results from FOS- and bifidobacteria-treated groups. In addition, DFO increased the amplitude and duration of contractions of proximal and distal colonic circular and longitudinal SM as determined by *in vitro* tension measurement in an organ bath. Histological stains showed normal morphology of epithelia, crypts, goblet cells, and also the SM thickness in all groups.

Conclusions: DFO-fed mice increased the colonic SM contraction without changing in the morphology, and acted as bulk-forming and stimulant laxatives which increased fecal output and intestinal motility, respectively. Regarding to these results, DFO may be classified as a dietary supplement for promoting gut health and recovering gastrointestinal motility disorders.

Keywords:

prebiotic, dragon fruit oligosaccharide, colonic contractility, gut transit time, colonic smooth muscle, spatiotemporal map, organ bath

Introduction

The imbalance of adverse and beneficial enteric microbiota affects various gastrointestinal (GI)[9] functions including GI motility (1), resulting in inflammatory bowel disease (IBD)[9], irritable bowel syndrome (IBS)[9], diarrhea, or constipation (2, 3). To maintain these microbial populations in a normal state, intake of probiotics and prebiotics is extremely helpful (4, 5). Probiotics, such as bifidobacteria and lactobacilli, are live valuable microbes that are good to human and animal health, especially in the GI tract. It is well known that these bacteria have the effects on modulating intestinal motility with reductions in both diarrhea and constipation (6, 7, 8). Although ingestion of probiotics has excellent advantages, there are some limitations in critical patients, such as patients with acute pancreatitis or allergy (9, 10), and they may be destroyed easily by heat and acid and difficult to handle in some foodstuffs. The second approach to selectively modify the composition and activity of intestinal microbiota is to supply the microbiota which already present in the colon with prebiotics.

Prebiotics are non-digestible food ingredients that enter the colon without alteration by the digestion and absorption, serving as a nutrient source for the beneficial bacteria living in the colon. Prebiotics not only selectively allow specific changes in the compositions and/or activities of the GI microbiota, they also induce microbial competition and reduce the populations of undesirable microbiota (11, 12). The major products of prebiotic fermentation in the colon are short chain fatty acids (SCFAs)[9], mainly acetate, propionate and butyrate. SCFAs are the energy sources of colonic epithelial cells and play roles in electrolyte transport, cell differentiation, cell growth, and colonic motility (13, 14). The best known prebiotics is FOS, galacto-oligosaccharides

(GOS)[9] and inulin (15–18), and other non-digestible oligosaccharides have also been tested for their prebiotic properties, especially prebiotic-rich fruit and vegetables.

DFO is extracted and purified from dragon fruit or pitaya. Dragon fruit is an agricultural product which is native to Central and South America (19, 20) and has been interested in many countries such as Vietnam, Singapore, China, Philippines, Malaysia and Thailand (21). It is found to be rich in various nutrients, such as β-carotene, lycopene, vitamin E and essential fatty acids (22, 23) and also has antioxidant and anti-inflammatory activities (24). Both flesh and peel of red pitaya with white-flesh (*Hylocereus undatus* (Haw.)) and red pitaya with red-flesh (*Hylocereus polyrhizus*) have been reported as a source of DFO (25, 26). DFO is resistant to hydrolysis by artificial human gastric juice and α-amylase, and stimulates the lactobacilli and bifidobacteria growth in the artificial colon (25, 27). Even though, prebiotic properties in an *in vitro* studies of DFO are quite known, there is no study the prebiotic effects of DFO on GI functions, especially intestinal motility.

Thus, the aim of the present study was to investigate the *in vivo* effects of oral administration of DFO for one to two weeks on fecal output, intestinal transit time, evacuation time, colonic motility patterns, colonic pellet propulsion velocity, proximal and distal colonic circular and longitudinal SM contractions, and colonic morphological changes of male ICR mice. Knowing the effective doses and durations of prebiotic DFO intake on intestinal motility may very useful to improve the imbalance of intestinal microbiota ecosystem, and helping the intestinal motility disorders such as constipation and diarrhea.

Methods

Animals and ethical approve

This study was approved and guided by the Animals Ethic Committee of the Prince of Songkla University, Thailand (Project license number MOE0521.11/799). Adult male ICR mice (*Mus musculus*; 5 weeks old, weighing 20–25 g) were obtained from National Laboratory Animal Center, Mahidol University and housed at Southern Laboratory Animal Facility, Prince of Songkla University. All animals were kept under standard environmental conditions (room temperature was kept in the range of 23–27°C, humidity at 50–55%, and under a 12:12 hr light/dark cycle), and fed regular standard commercial food pellets (Perfect Companion Group Co., Ltd., Thailand) and filtered water *ad libitum*. More detailed information about the diet composition can be found in **Supplemental Table 1.**

Chemicals and equipment

The reference prebiotic and probiotic in these studies were FOS (Sigma-Aldrich, St. Louis, MO, USA) and *Bifidobacterium animalis* (FD-DVS nu-trish® BB-12®) (Chr. Hansen Holding A/S, Hoersholm, Danmark). The composition of the Krebs solution was as follows (in mM): 119 NaCl, 2.5 $CaCl_2$, 4.5 KCl, 2.5 $MgSO_4$, 25 $NaHCO_3$, 1.2 KH_2PO_4, and 11.1 D-glucose (28, 29) (all purchased from Merck, Co., Ltd., Darmstadt, Germany). All chemicals were kept at room temperature and the working solutions were made fresh on the day of the experiment by diluting the stock solutions in Krebs. The Gastrointestinal Motility Monitor (GIMM)[9] System which was used in an *ex vivo* colonic propulsive motility study was purchased from Catamount Research and Development, St. Albans, VT.

Extraction and Purification method of DFO (briefly)

Distilled water (DW)[9] and pectinase were used for DFO extraction. The extract was purified by fermentation with yeast. All sugars of white flesh dragon fruit were analyzed by high performance liquid chromatography (HPLC)[9] which consisted mostly of glucose, fructose and some oligosaccharides. The low molecular weight (MW)[9] fraction, glucose and fructose, which were analyzed by gel permeation chromatography, were removed by biological approach using two-step yeast (*Saccharomyces cerevisiae*) cultivation. MW distribution of the mixed oligosaccharides was confirmed by mass spectrometry. The mixed DFO consisted of four components of 716, 700, 490 and 474 Da with relative percentages of 100, 68, 45 and 21, respectively. Thus, the degree of polymerization (DP)[9] of mixed DFO is 3–4, which is in the same range as some FOS.

Experimental design, surgical procedure and tissue preparation

After a week acclimatization, experimental animals were fed with 0.2 mL DW (vehicle control), 100, 500, and 1000 mg/kg DFO, 1000 mg/kg FOS, or 10^9 CFU bifidobacteria daily for a week or two weeks. Body weight (BW)[9], food and water intakes, and fecal pellet output of all mice were recorded every day. After anesthetization by intraperitoneal injection of 70 mg/kg Thiopental sodium (Anesthal®), the abdominal cavity was dissected and rapidly removed the entire colon. The colon with contents was placed in an oxygenated (5% CO_2 and 95% O_2) ice-cold Krebs solution (pH 7.4 with an osmolality of 289–292 mmol/kg H_2O). To study DFO effects on colonic propulsive motility, whole colonic segment was mounted horizontally in a 50 mL GIMM organ bath containing 37°C oxygenated Krebs solution. To study SM contractility, segments of proximal colon (3 cm distal to the cecum) and distal colon (3 cm proximal to the rectum) were separated and cut into smaller segments (1 cm in length). In the longitudinal SM contraction study, whole thickness segments were suspended in the direction of longitudinal SM fibers, whereas in the circular SM study, the segments were opened along the mesenteric border and full- thickness muscular strip were cut in the direction of circular muscle and suspended in a thermostatically controlled (at 37ºC) 20 mL organ bath containing oxygenated Krebs solution.

Fecal pellet output and gut transit assay

After treatments, the fecal pellet number was counted and weighted. For fecal water content calculation, feces was weighted and dried at 100°C for 30 min and the formula of fecal water content (%) is ((wet weight – dry weight)/wet weight) × 100. For total gut transit time measurement, mice received Evan-blue marker meal (5% Evan-blue in 1.5% methyl cellulose; 0.1 mL (i.g.)) and observed the fecal pellet every 10 min until first blue pellet expelled. For the evacuation time, it was measured by using a bead expulsion test. A 3- mm glass bead was inserted into the colon through 1 cm proximal of anal by using the plastic tip lubricated with pure petroleum jelly. The bead expulsion time was measured. For upper gut transit measurement, mice received charcoal meal for 60 min, and charcoal transit (%) was measured and calculated by (the distance of charcoal meal/total length of the small intestine) × 100

Measurement of *ex vivo* colonic motility

The GIMM organ bath lined with Sylgard, which was placed on top of luminance plates to silhouette a colonic segment, was continuously perfused with Krebs solution at 10 mL/ min. The segment was pinned at both oral and caudad ends at its *in situ* length. Before recording, the segment was allowed to equilibrate in Krebs solution for 30 min without natural fecal pellets flushed out. The pellets movement was recorded from above using a video camera connected to a computer running GIMM software. Spatiotemporal map of motility was constructed from the recording that was acquired from individual runs. The colonic segment image in each video frame was converted to a silhouette. The diameter at each point along the entire length was calculated and converted into a grey-scale. The small diameter (intestinal contraction) was coded as white and the large diameter (intestinal dilation) was black. The number of spontaneous contractions both propulsive and non-propulsive contractions was count per unit of time. Velocity analysis was performed using the fecal pellet tracking method in the GIMM software, in which the pellet is darkened compared to the rest of the video and tracked from the orad to caudad end. Fecal pellet velocity was calculated and displayed in mm/second.

Measurement of *in vitro* smooth muscle contractility

The distal end of each colonic segment was tied to an organ holder and the proximal end was secured with a silk thread to an isometric force transducer (Model FT03, Grass, USA), and stretched passively to an initial tension of 500 mg. Signal output of the mechanical activity was amplified and digitized via Bridge Amp and PowerLab® System (AD Instruments, Australia), recorded on a computer for later analysis using LabChart7 program software. After the equilibration time (30 min to obtain a regular spontaneous activity), spontaneous contractions in the colonic SM representing basal activity were recorded for 5 min. A single concentration (0.1, 1, and 10 μM) of carbachol (CCh) (Tocris Bioscience, Bristol, UK) was added to the Krebs solution in the organ bath in a cumulative fashion without washing between concentrations, and each concentration was incubated for 5 min. Subsequently, the amplitude, duration and frequency of isolated colonic segments contractions were calculated. The mean amplitude (in mg) of contractions was calculated as the average of peak to peak differences over 5 min and were expressed as a percentage of the values recorded in the presence of 1 μM CCh (maximal contraction). The frequency and duration of contractions were expressed as the number of contractions per minute (times/min) and the mean of contraction time (second) recorded in a 5-min period.

Histological study

After 10% formalin fixing, the colonic segments were embedded in paraffin, and sectioned at 7-μm-thick. Sections were deparaffinized with xylene and rehydrated in serial graded ethanol. Hematoxylin-eosin (HE)[9] and Periodic acid-Schiff (PAS)[9] staining were performed according to the standard protocols and tissues were observed under light microscope. The SM thickness was measured with image J software.

Data and statistical analysis

Results obtained from this study were expressed as mean ± SEM (standard error of mean) with n in parentheses denoting the number of animal. Data were analyzed using the statistical program GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, California, USA). Multiple group comparisons were made using one-way analysis of variance (one-way ANOVA) test, followed by Bonferroni post hoc test. The level of significance for all statistical tests was $P < 0.05$.

Results

Effects of DFO on body weight, food and water intakes, and fecal pellet output

This study aimed to investigate the effect of prebiotic DFO ingestion on energy balance by measuring food intake, fecal output, and BW change. There was no significant change in BW, food intake, and water intake in mice either fed vehicle control or all doses of DFO for one and two weeks (**Supplemental Figure 1, 2**). Mean fecal pellet number of DFO-treated group were not significantly different compared to control group (**Figure 1**A, C) whereas mean fecal pellet wet weight was significantly increased by 2.3 times with consumption of 500 and 1000 mg/kg DFO for a week and increase by 2 times with consumption of 500 mg/kg DFO for two weeks compared with the vehicle control (**Figure 2**A, C). The greater fecal mass was induced by ingestion of DFO, despite similar food intake to control. This result is consistent with the putative prebiotic effects of DFO as investigated by the previous study (25). However, fecal water content of each group was not significantly different (Figure 2B, D).

Effects of DFO on gut transit

To demonstrate the DFO action on the time or distance of fecal pellet movement through the colon, gut transit time and upper gut transit measurement by using marker meals were determined. The total gut transit time of vehicle control was approximately 230 min and the transit time of DFO at 1000 mg/kg for a week and 500 mg/kg for two weeks was significantly reduced approximately 30% from the control (**Figure 3**A, C). The distance of charcoal meal of mice treated with DW for a week and two weeks was 56.03 ± 2.80% and 61.01 ± 4.40% of the small intestinal length, respectively. DFO-treated for a week at 500 and 1000 mg/kg, the charcoal meals were moved 68.22 ± 1.90% and 82.86 ± 1.62% in the small intestine, respectively. DFO-treated for two weeks at 500 mg/kg moved the charcoal meal to 77.11 ± 1.62%. Thus, the upper gut transit of a charcoal meal was significantly increased in the 500 and 1000 mg/kg DFO in relation with the control (Figure 3B, D). Nevertheless, both one and two weeks treatment of DFO showed that the evacuation time was not significantly different compare to control group (Figure 1B, C).

Effects of DFO on colonic motility

Since DFO ingestion could decrease the gut transit time, it is possible that DFO could exert this effect via increased gut motility. For the spatiotemporal maps study, two patterns of motility were observed. First, the motility was induced by natural pellets. It consisted of propagation or peristaltic contractions that pushed the pellets aborally, such as, pushed the pellet at 0.6 mm/s in the 1000 mg/kg DFO-treated group. The second type consisting of shallow circular muscle contractions separated by short relaxation. This type pushed the pellets forward and backward (non- propagation contraction or segmentation), thus we could not calculate the velocity in this contraction pattern. At a week treatment of 1000 mg/kg DFO, but not 100 and 500 mg/kg, showed significantly increased number of total contraction, especially non-propagation pattern (**Figure 4**A, B), whereas the number of propagation contraction was not significantly different (Figure 4C). Similarity to a week, two weeks of 500 mg/kg DFO ingestion showed significantly increased number of total and non-propagation contractions, but not propagation pattern (**Figure 5**A-C). For the fecal pellet velocity measurement, administration of 1000 mg/kg DFO for a week caused a 88.33% increase from 0.07 ± 0.02 to 0.6 ± 0.06 mm/s (Figure 4D) whereas administration of 500 mg/kg DFO for two weeks slightly increased the velocity but not significantly different to control (Figure 5D). The reference prebiotic and probiotic ingestions had the similar or even greater effects to DFO ingestion, except the treatment of 1000 mg/kg FOS for a week. It could not significantly enhance the velocity of fecal pellet when compare to control (Figure 4D).

Effects of DFO ingestion for a week on proximal and distal colonic circular and longitudinal smooth muscle contractions

This study aimed to investigate the effect of DFO ingestion for a week on the amplitude, frequency, and duration of contraction of the colonic SM. In the circular SM segments from proximal colon, the mean amplitude of contraction was 30.27±4.99% in the control group, 37.00±7.67%, 60.49±9.28% and 68.95±6.98% in 100, 500, and 1000 mg/kg DFO, respectively. Thus, the contraction amplitude of 500 and 1000 mg/kg DFO was significantly higher than the control group (**Figure 6**A). In the longitudinal SM segments from proximal colon, the mean amplitude of contractions was 36.27±7.79% in the control group, 49.60±6.43%, 63.86±3.30% and 70.55±8.31% in 100, 500, and 1000 mg/kg DFO, respectively. Only 1000 mg/kg DFO, the amplitude of contraction was significantly higher than control group (Figure 6A). The 1000 mg/kg of FOS and 10^9 CFU bifidobacteria also increased the contraction amplitude when compare to control, except FOS could not significantly enhance the amplitude of contraction of longitudinal SM (Figure 6A). In both circular and longitudinal SM segments from proximal colon, the mean frequency of the spontaneous contractions in all groups was not significant difference with control group (Figure 6C). In contrast, the duration of contraction in 1000 mg/kg DFO and bifidobacteria groups were significantly longer than the control group, but 1000 mg/kg FOS could significantly increase the contraction duration only in the circular SM (Figure 6E). We also investigated the effect of DFO on the contraction of the distal colonic SM. In both circular and longitudinal SM segments, the mean amplitude of contractions in 100, 500 and 1000 mg/kg DFO and 1000 mg/kg FOS were not significantly different when compared to control groups. Only bifidobacteria could significantly increase the contraction amplitude (Figure 6B). Similar to the amplitude, the mean frequency of the spontaneous contractions was not significant difference between the responses of DFO-, FOS- and control groups in both circular and longitudinal SM (Figure 6D). For the duration of contraction, 1000 mg/kg DFO and bifidobacteria significantly increased the contraction duration of circular muscle, whereas 1000 mg/kg FOS and bifidobacteria increased the contraction duration of longitudinal muscle when compared to control group (Figure 6F).

Effects of DFO ingestion for two weeks on proximal and distal colonic circular and longitudinal smooth muscle contractions

To examine the effect of longer duration ingestion of DFO, DFO was administered for two weeks. For both circular and longitudinal SM of 500 mg/kg DFO and 1000 mg/kg FOS, proximal colonic amplitude of contraction was significantly higher than the amplitude of the control group (**Figure 7**A). In the frequency of contractions, DFO and FOS could enhance the frequency of circular SM contractions when compared to control group (Figure 7C). In contrast, the duration of contraction of longitudinal SM of DFO and FOS was significantly longer than the control group (Figure 7E). In the distal colon, the amplitude of contraction of circular SM of 500 mg/kg DFO and 1000 mg/kg FOS was significantly higher than the amplitude of the control group, while the contraction amplitude of longitudinal SM showed no change (Figure 7B). In contrast to the amplitude, there was no significant difference in the frequency of contraction after administration of DFO and FOS (Figure 7D). For the duration of contraction, the longitudinal SM of DFO- and FOS-treated mice was significantly longer in the duration than the control group (Figure 7F).

Effects of DFO on colonic smooth muscle histology

To determine whether DFO increased the colonic contractility by increasing the thickness of the colonic SM, the histological changes of the colonic wall were determine. Continuous consumption of 500 mg/kg DFO for two weeks showed no irritating effect on colonic mucosa. It displayed normal morphology of simple squamous epithelium as same as 1000 mg/kg FOS and DW-fed groups. The number of goblet cells presence in the mucosa layer were comparable to FOS and DW-fed groups. Regarding the muscular layer, there were not significantly difference in the thickness of SM layer in DFO-fed group compared to both FOS- and DW-fed groups (**Figure 8**).

Discussion

In the present study, we found that ingestion of DFO increased the upper gut transit which reduced the time of the content to the large intestine and also reduced the total gut transit time. There were some studies reported that rapid intestinal transit was seen in malabsorptive states and diarrhea symptoms (30). In this study, however, the BW of DFO-treated group was not changed when compared to control group, and there were not shown the diarrheal feces characteristics. DFO is non-digestible, fermentable, and soluble short-chain carbohydrate fiber. When DFO was consumed, 50% were estimated to reach the colon since some were hydrolyzed by salivary and pancreatic α-amylases (16%), gastric juice (2.5%) and small intestinal brush-border enzymes (30%) (25). In the intestine, this soluble fiber had appreciable water holding capacity, however it was known to be less than that of other fibers with strong water holding capacity, i.e., wheat fiber, resulting in increase in fecal mass (31). Fecal pellet wet weight or fecal output in DFO- and FOS-treated groups in this study significantly increased when compared to control group. However, the percentages of fecal water content were not significant different in all groups since this type of short-chain carbohydrates may good as stool bulking forming, but not water holding, or there was no fluid secreting effects in the colonic epithelial cells. The increased intestinal content, especially in the colon, could stimulate peristaltic contraction, and also accelerated intestinal transit (32).

The peristaltic or propagation contractions reduced the diameter of the colon, without occluding the lumen. Thus, they did not empty the proximal colon in a single sweep, but rather slowly pushed small amounts of content into the distal colon. In this study also showed the effect of DFO ingestion on the colonic motility pattern. Even though the propagation pattern of the DFO-treated group was not significant different when compared to control group, the trend of increasing was showed and the velocity of fecal pellet movement through the entire colon significantly increased in one week of 1000 mg/kg DFO treatment when compared to control group. Distention by natural fecal pellets is a major trigger for neutrally mediated propulsion in the colon (33, 34). Thus, it is reasonable to suggest that peristaltic contractions in the colon result from a distension-triggered motor pattern generator mediated by enteric nervous system (35).

In addition to distend the colonic wall by the colonic content, DFO might alter bowel motility through a change in the colonic environment. *In vitro* studies had shown that the bacterial fermentation of oligosaccharides increased the production of SCFAs (36, 37) leading to lower colonic pH (38). The low pH in the colon stimulated the growth of lactobacilli and bifidobacteria and suppressed the growth of harmful bacteria (37). Increasing fermentation by-products, such as gas and SCFAs, could increase stool bulk and also stimulate the gut motility (39). The motor patterns in the colon of different species vary significantly depending on the diet. There were many conflicting studies about prebiotics and probiotics effects on GI motility. Some of these studies suggested that these supplements increased intestinal motility, while the others showed opposite results (40, 41, 42). *Lactobacillus reuteri* ingestion could reduce the amplitudes of colonic contractions at constant luminal pressure and increased the threshold luminal pressure which required to induce phasic contractions in rats (40). On the other hand, the administration of fermented milk prepared with *Lactobacillus casei* enhanced colonic propulsive contraction and defecation rate in pigs (41). In a study in healthy newborns fed with breast milk had lower stool consistency and higher stool frequency than newborns fed with bovine milk. In addition, supplementation of a mixture of GOS and FOS resulted in a reduction in stool consistency and an increase in stool frequency (42).

Our study was consistent with the previous studies. We found that DFO increased the gut transit by increasing intestinal motility, fecal pellet velocity, and also the number of whole colonic contraction, especially non-propagation pattern.

Non-propagation or segmenting contractions cause mixing and local circulation of contents. This contraction pattern may slow the gut transit to abolish the high effect of DFO on peristaltic contraction. Therefore, there were no adverse effects of the DFO supplement, e.g., diarrhea and malabsorption in this study. Normally the anal canal of the GI tract is closed because of internal anal sphincter contraction. When the rectum is distended by fecal material, which we used glass bead in this experiment, the internal sphincter relaxes as part of the rectosphincteric reflex. Rectal distention also elicits a sensation that signals the urge for defecation which is prevented by the external anal sphincter. The external anal sphincter contraction is maintained by reflex activation through dorsal roots in the sacral spinal cords. In this study, there were no significant different of evacuation time which means that the prebiotic DFO may not affect the neural control of the last process of the defecation.

Delayed transit of contents through the colon or decreased colonic motility is common which leads to constipation, however, this is dietary origin. There is a direct correlation among increased dietary fiber or prebiotics, increased colonic intraluminal bulk, and enhanced transit through the colon or increased the motility. Many studies reported that prebiotics improve health in a way similar to probiotics, whereas at the same time being cheaper, harmless and being easier to incorporate into the diet than probiotics. However, excessive intake of short-chain carbohydrates can cause undesirable side effects, such as flatulence, bloating, rumbling, cramps, and liquid stools, caused by gas formation and osmotic effects of certain fermentation products. Fortunately, in our present study, 1000 mg/kg/day or less of DFO was usually well tolerated in mice.

SM colonic contraction are organized to allow for optimal absorption of water and electrolytes, net aboral movement of contents, storage and orderly evacuation of feces. The intestinal muscularis externa layers display two distinct motility patterns, 1) the propagated peristaltic contractions, involving the coordinated contractions of the longitudinal and circular SM, and 2) the non-propagated segmentation contractions, involving mainly the circular muscle layer. It has been shown in this study that DFO treatment for a week increased force and duration of contraction of both circular and longitudinal SM in proximal colon, but only increased the duration of circular SM contraction in the distal colon. For two weeks treatment, DFO could increase the spontaneous contraction frequency of circular SM in the proximal, but not in the distal, colon. Short-chain carbohydrates including DFO were very rapidly fermented in the terminal ileum and proximal colon to produce SCFAs, therefore the proximal colonic SM should be affected much more than the SM in the distal colon (31). However, the underlying mechanisms of DFO on colonic SM contraction are still unknown.

Recent study found that specific SCFAs such as butyrate increased cholinergic-mediated colonic circular SM contraction in animals (43). Hurst and co-workers also reported that colonic luminal butyrate, acetate and propionate have different effects on proximal and distal colonic contraction depending on chain length, and the net effect of SCFAs on the contraction would depend on the balance of SCFAs produced by gut microbiota fermentation of non-digestible carbohydrates (14). The colonic SM cell membrane has slow wave activity which always present whether contractions are occurring or not. The contractions, however, are initiated by a second electrical event, i.e., spike potential activity which is occured when slow wave reach the electrical threshold. When slow wave is accompanied by spike potential, the colon contracts at the same frequency as the slow wave frequency. Thus, slow wave frequency sets the maximum frequency of contractions. Whereas the amplitude and frequency of spike potential on the crest of the slow wave are directly related to the amplitude or force and duration of muscle contractions. The occurrence of spike potential and contractions depend heavily on neuronal and hormonal activities, and locally chemical agents whereas slow waves are extremely regular and are only minimally influenced by neural or hormonal activities (44). Supporting to our results, DFO effect might regulate the amount of spiking, but less affected on the threshold of the slow wave, therefore it affected mostly on strength and duration of contractions instead of the frequency.

Coordinated contraction of intestinal circular and longitudinal SM results in caudal propulsion of luminal contents via peristalsis. Nerves in the enteric plexuses receive input from the receptors within the GI tract. An excitatory factors, i.e., SCFAs from the bifidogenic effect and distention from the osmotic effect may act on the free fatty acid and stretch receptors at the intestinal epithelial cells. They stimulate serotonin or 5-hydroxytryptamine (5-HT)[9] secretion. The 5-HT activates CGRP-containing neurons, and also a series of interneurons which send the signal to motor neurons for releasing acetylcholine, tachykinin, or substance P orad to the luminal stimulus. These neurotransmitters cause contraction of the circular SM and relaxation of the longitudinal SM. Vasoactive intestinal peptide and nitric oxide are released caudad to the luminal stimulus and stimulate circular SM relaxation and longitudinal SM contraction (44).

In addition to the contractions of SM, there were some studies reported the effect of prebiotic supplementation could change the colonic structure by the effect of gut microbiota and SCFAs. Butyrate was used directly by the colonic cells, exerting a trophic effect on these cells. However, in the present study, DFO did not have the trophic effect on the gut wall. As mucin plays a cytoprotective functions in colonic mucosa against a variety of luminal hazards. Alteration in goblet cell number was observed in intestinal infections (45). Previous studied reported that high fiber diet increased both secretory activity and numbers of mucin-secreting goblet cells in rat colon (46). Conversely, our study showed no effect of DFO on both epithelium and goblet cell numbers. Moreover, DFO did not affect the thickness of SM. Based on general criteria in histomorphological scores for intestinal inflammation (47), consumption of DFO did not follow either epithelial or mucosa architecture changes. These may confirm the safety of this product as a supplement.

Taken together, these data suggested that in addition to be a prebiotic as reported previously (25), DFO also acts as a bulk-forming laxative, which absorb water from the intestinal lumen to increase fecal mass (osmotic effect), as well as being a stimulant laxative that increases intestinal motility in mice. We also showed an association between ingestion of DFO and alteration in the colonic SM contractility. According to these findings it seems that DFO may be suitable for inclusion as food supplements in a wide variety of food products, e.g., prebiotic/probiotic/synbiotic products, laxative product, and may be a promising nutritional therapy for GI motility disorders, such as constipation and IBS. Nevertheless, further investigation is required to identify the underlying mechanisms responsible for diet- or gut bacteria-induced changes in GI motility.

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Figure Legends

FIGURE 1 Effects of DFO ingestion for a week and two weeks on the number of fecal pellets and time of evacuation 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 (10^9 CFU, p.o.) for a week and (C and D) 0.2 mL DW, DFO (500 mg/kg, p.o.) and FOS (1000 mg/kg, p.o.) for two weeks. The fecal pellet number was counted per six hours and calculated to an hour. The evacuation time was recorded after insertion of a glass bead until the glass bead expelled. Each bar of the data represents means ± SEM (n = 4–6).

FIGURE 2 Effects of DFO ingestion for a week and two weeks 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 (10^9 CFU, p.o.) for a week and (C and D) 0.2 mL DW, DFO (500 mg/kg, p.o.) and FOS (1000 mg/kg, p.o.) for two weeks. The fecal pellets were collected for six hours, weighted and recorded in the unit of gram, after that the feces were dried and weighted again to calculate the percentage of fecal water content. Each bar of the data represents means ± SEM (n = 6–10). $^*P < 0.05$ and $^{**}P < 0.01$ compared to vehicle control group (DW).

FIGURE 3 Effects of DFO ingestion for a week or two weeks on Evan-blue 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 (10^9 CFU, p.o.) for a week and (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) The total gut transit time was recorded when first blue fecal pellet expelled and each bar represents the mean of the total gut transit time (min) ± SEM (n = 4–6). The whole distance of the small intestine (from pylorus to cecum) was taken as 100% and each bar represents the mean of the percentage distance of the small intestine 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).

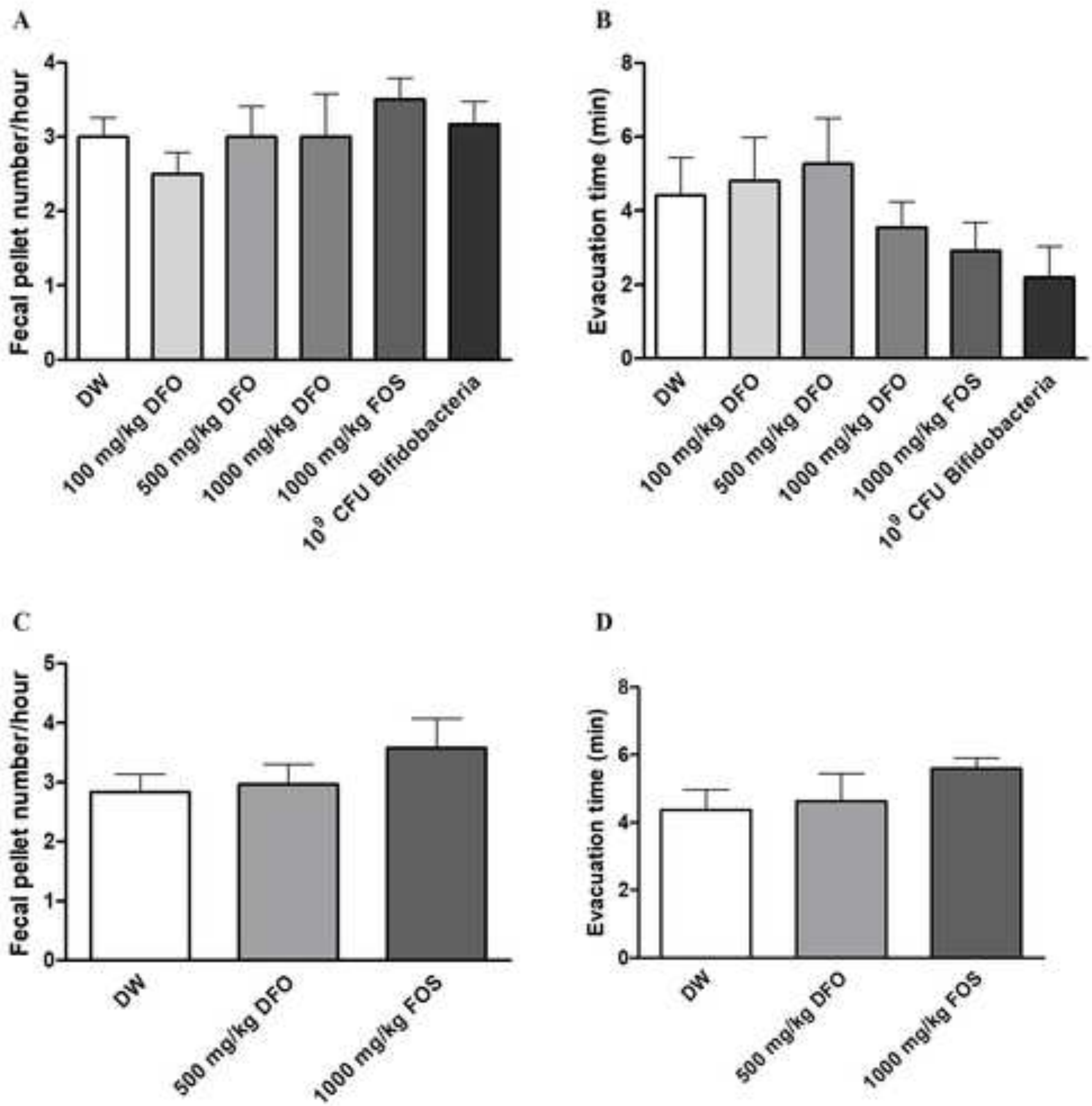
FIGURE 4 Effects of DFO ingestion for a week on the number of contractile responses for 30 min in the entire colon. Mice were treated with 0.2 mL DW, DFO (100, 500 and 1000 mg/kg, p.o.), FOS (1000 mg/kg, p.o.) and bifidobacteria (10^9 CFU, p.o.) for a week. (A) Number of total contractions were defined as the summation of (B) number of non-propagation contractions and (C) number of propagation contractions. Non-propagation contractions were defined as those contractions that failed to move the pellet go forward. (D) Velocity of fecal pellet propulsion through whole colon was determined only in the propagation contraction pattern. Data are means ± SEM (n = 5). *P < 0.05, **P < 0.01 and ***P < 0.001 compared to control group (DW).

FIGURE 5 Effects of DFO ingestion for two weeks on the number of contractile responses for 30 min in the entire colon. Mice were treated with 0.2 mL DW, DFO (500 mg/kg, p.o.) and FOS (1000 mg/kg, p.o.) for two weeks. (A) Number of total contractions were defined as the summation of (B) number of non- propagation contractions and (C) number of propagation contractions. Non-propagation contractions were defined as those contractions that failed to move the pellet go forward. (D) Velocity of fecal pellet propulsion through whole colon was determined only in the propagation contraction pattern. Data are means ± SEM (n = 5–6). *P < 0.05 compared to control group (DW).

FIGURE 6 Effects of DFO ingestion for a week 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 (10^9 CFU, p.o.) for a week. Values are means ± SEM (n = 10) and are expressed as (A and B) a percentage of the amplitude of maximum of contraction, (C and D) times/min of the frequency of contraction and (E and F) seconds of the duration of contraction. *P < 0.05, **P < 0.01 and ***P < 0.001 compared to vehicle control group (DW).

FIGURE 7 Effects of DFO ingestion for two weeks 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 (500 mg/kg, p.o.) and FOS (1000 mg/kg, p.o.) for two weeks. Values are means ± SEM (n = 8) and are expressed as (A and B) a percentage of the amplitude of maximum of contraction, (C and D) times/min of the frequency of contraction and (E and F) seconds of the duration of contraction. *P < 0.05 and **P < 0.01 compared to vehicle control group (DW).

FIGURE 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 orally administered with 0.2 mL DW, DFO (500 mg/kg, p.o.) and FOS (1000 mg/kg, p.o.) for two weeks. The figure showed normal epithelium, number of goblet cells and thickness of muscular layer in both DFO and FOS groups compare to control group (DW). Scale bar = 20 µm.



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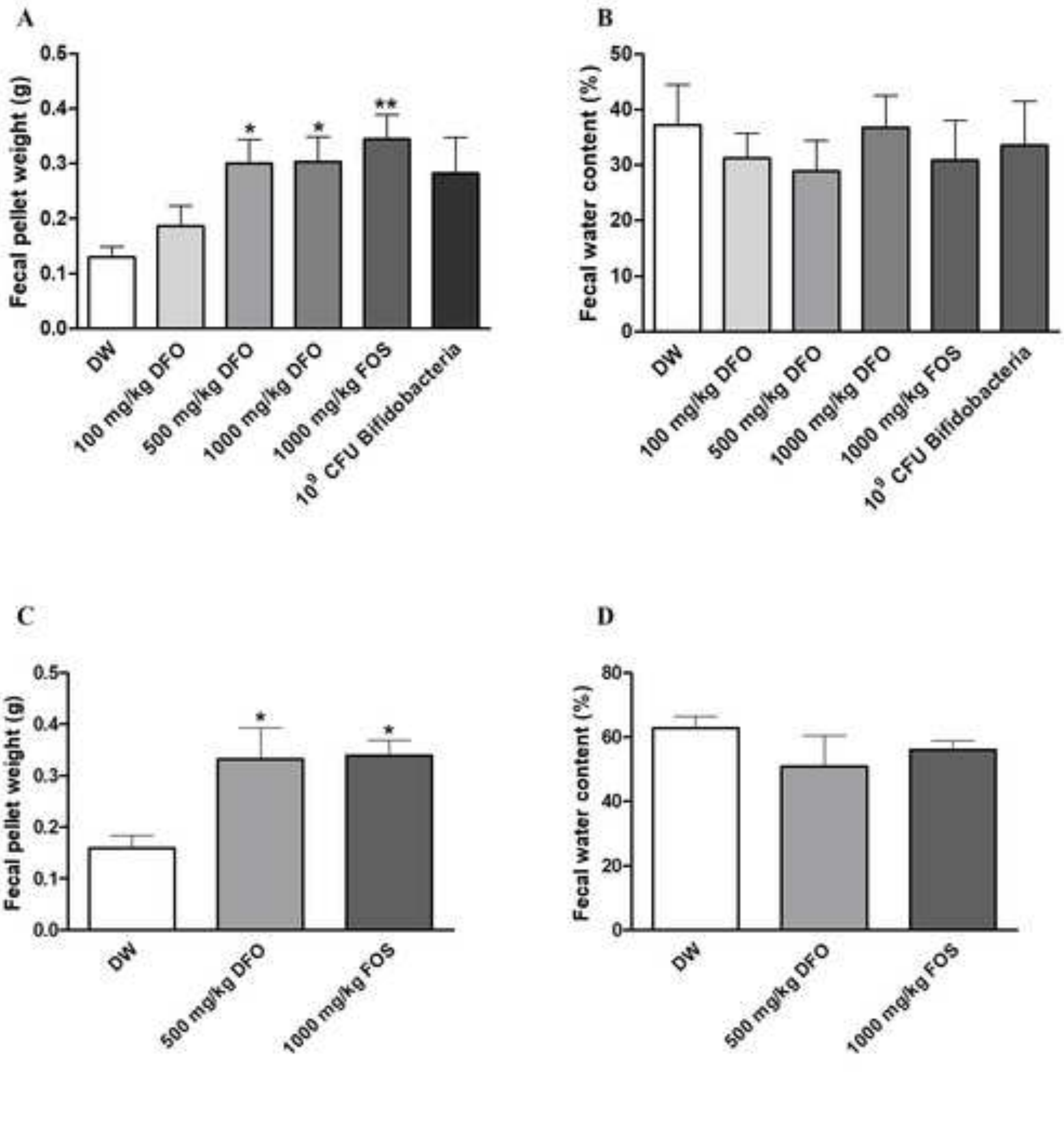

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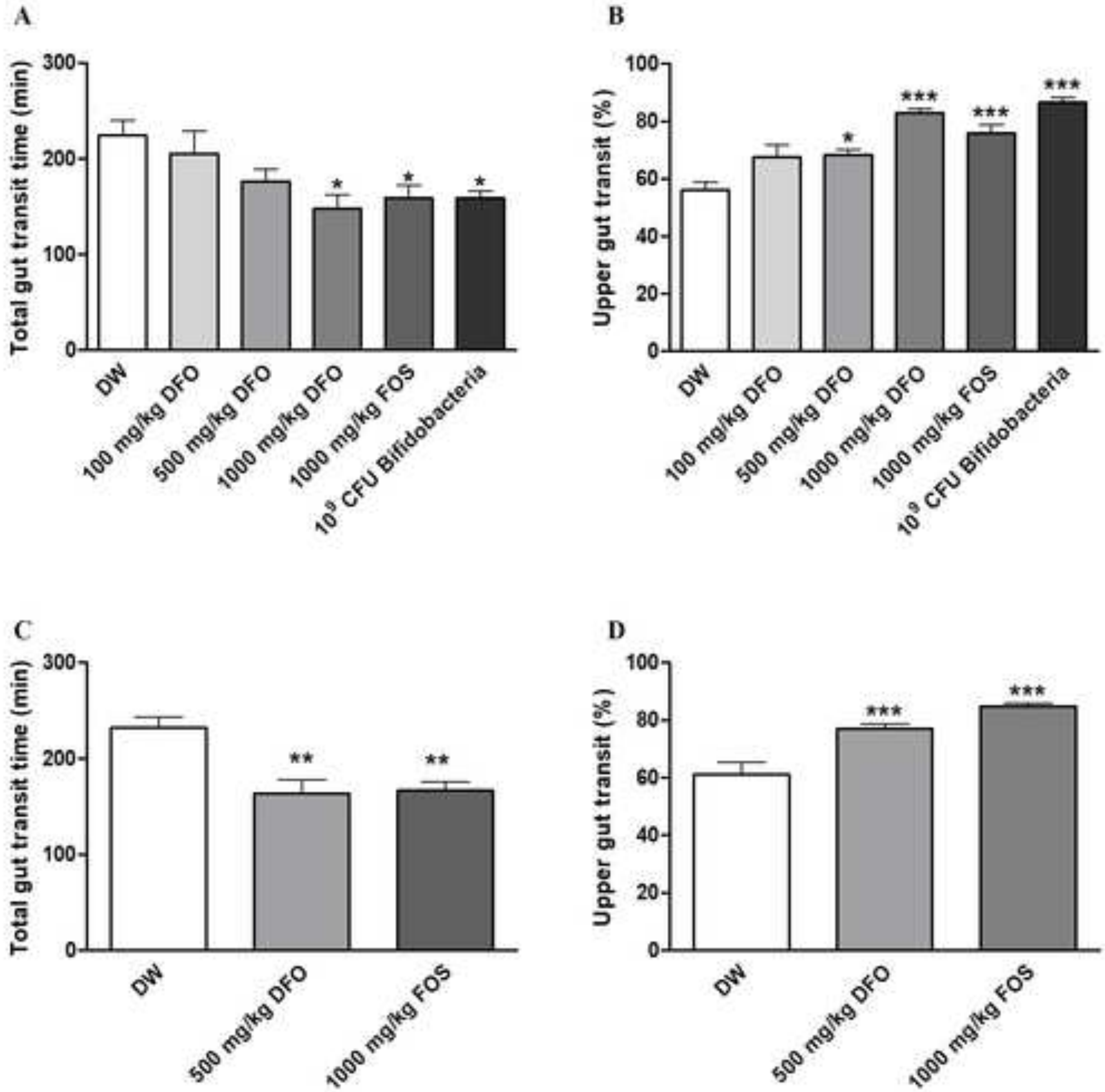

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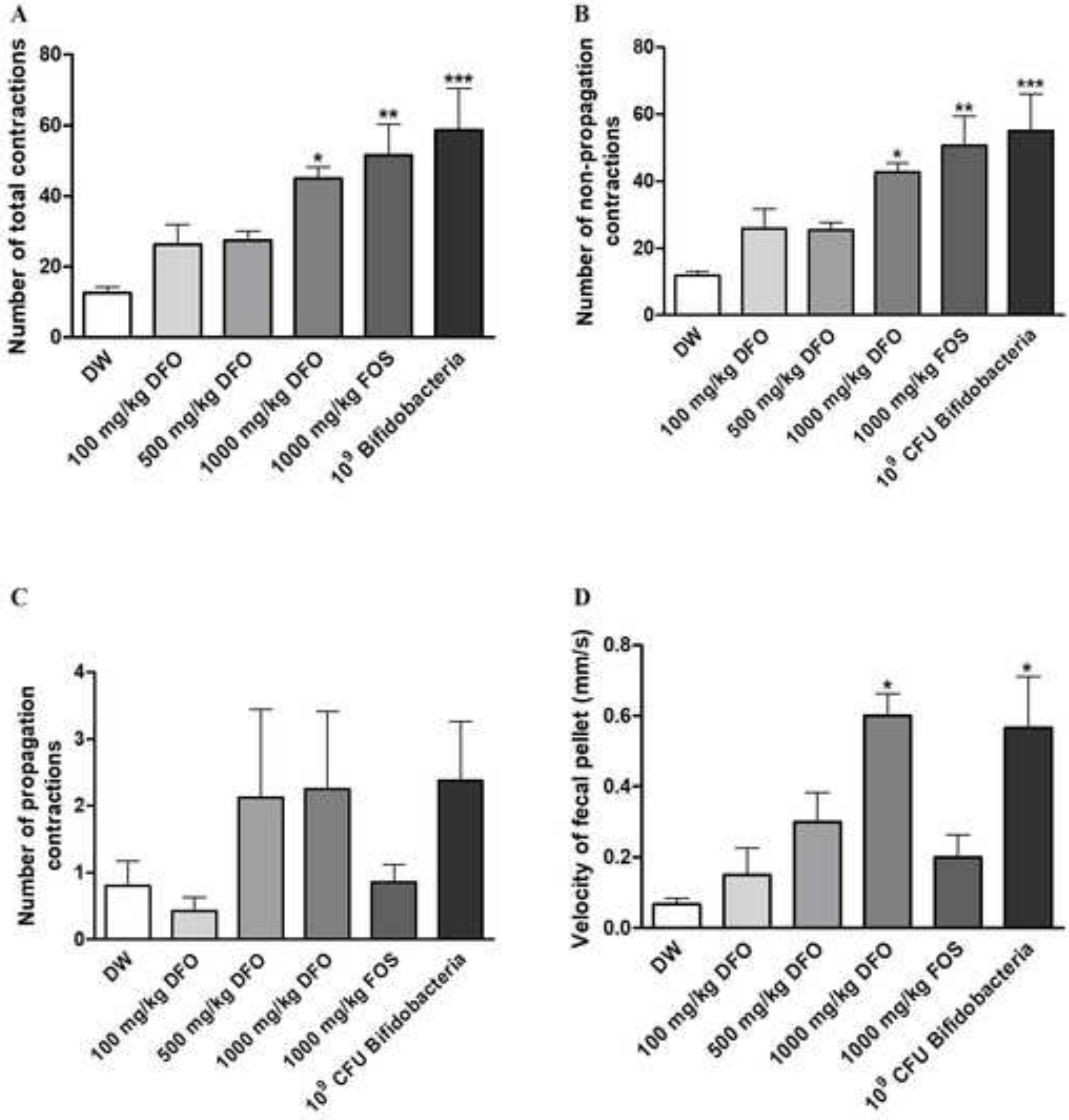

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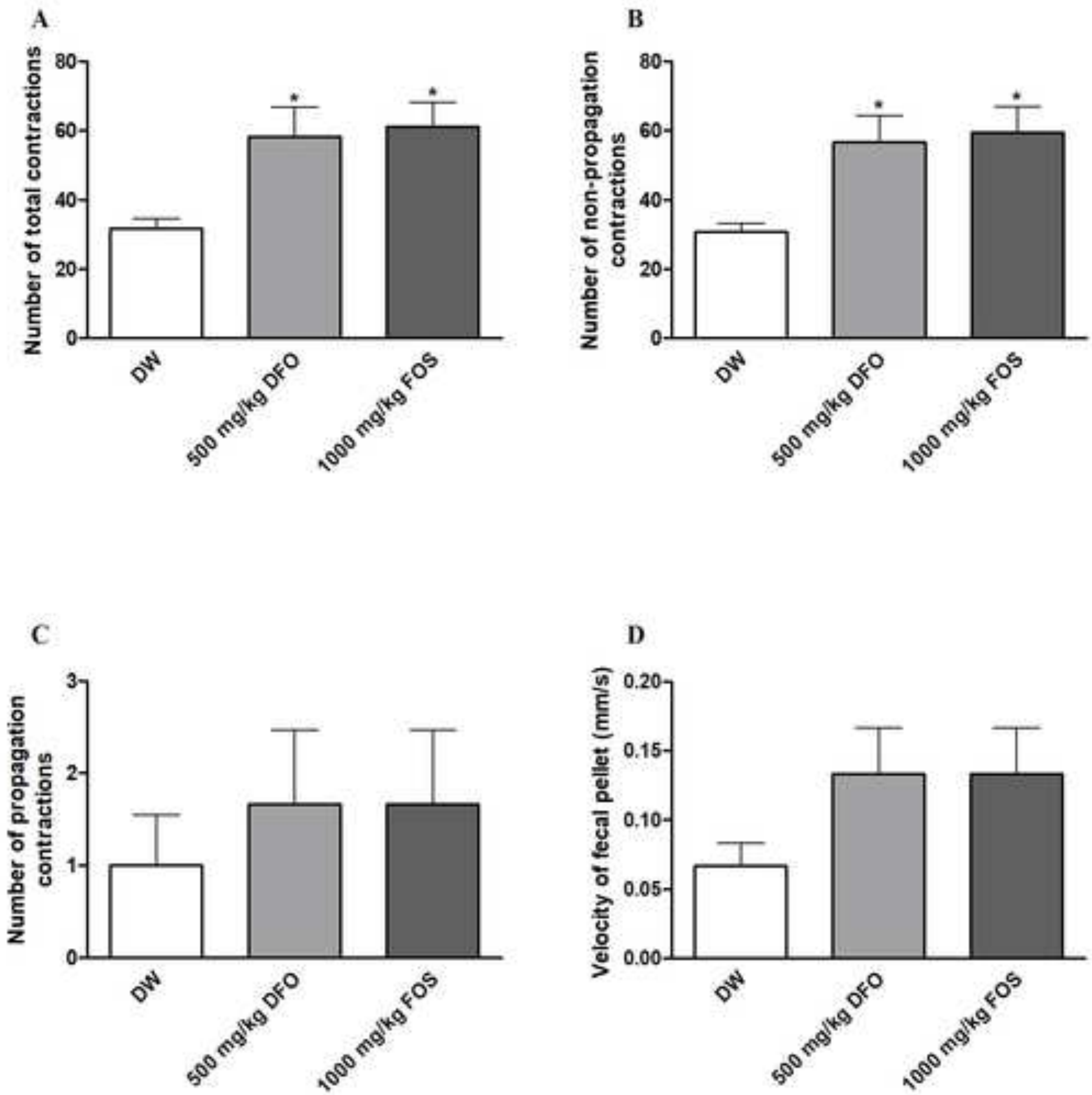

Figure 5: Khuituan et al.

Figure 6

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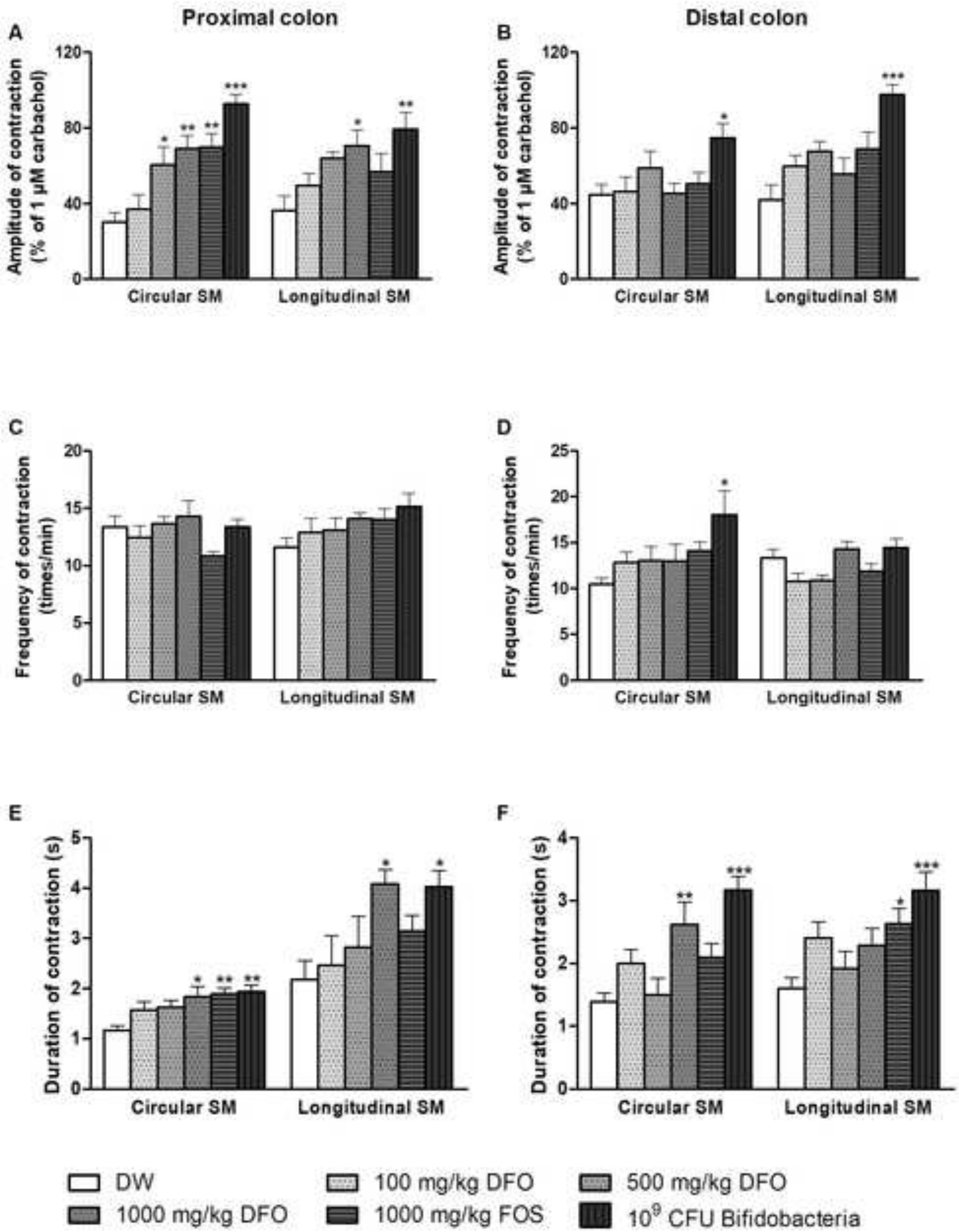

Figure 6: Khuituan et al.

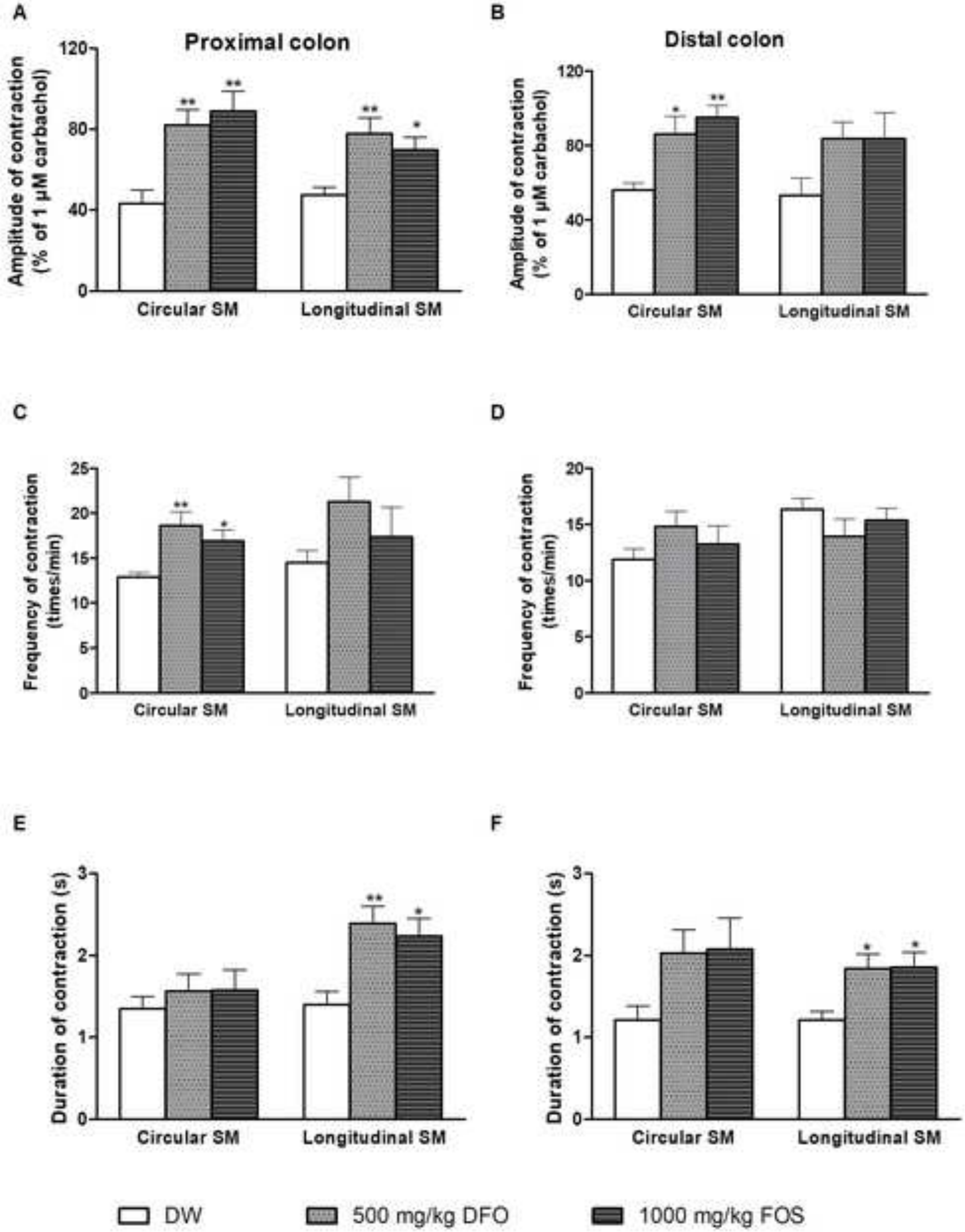

Figure 7: Khuituan et al.

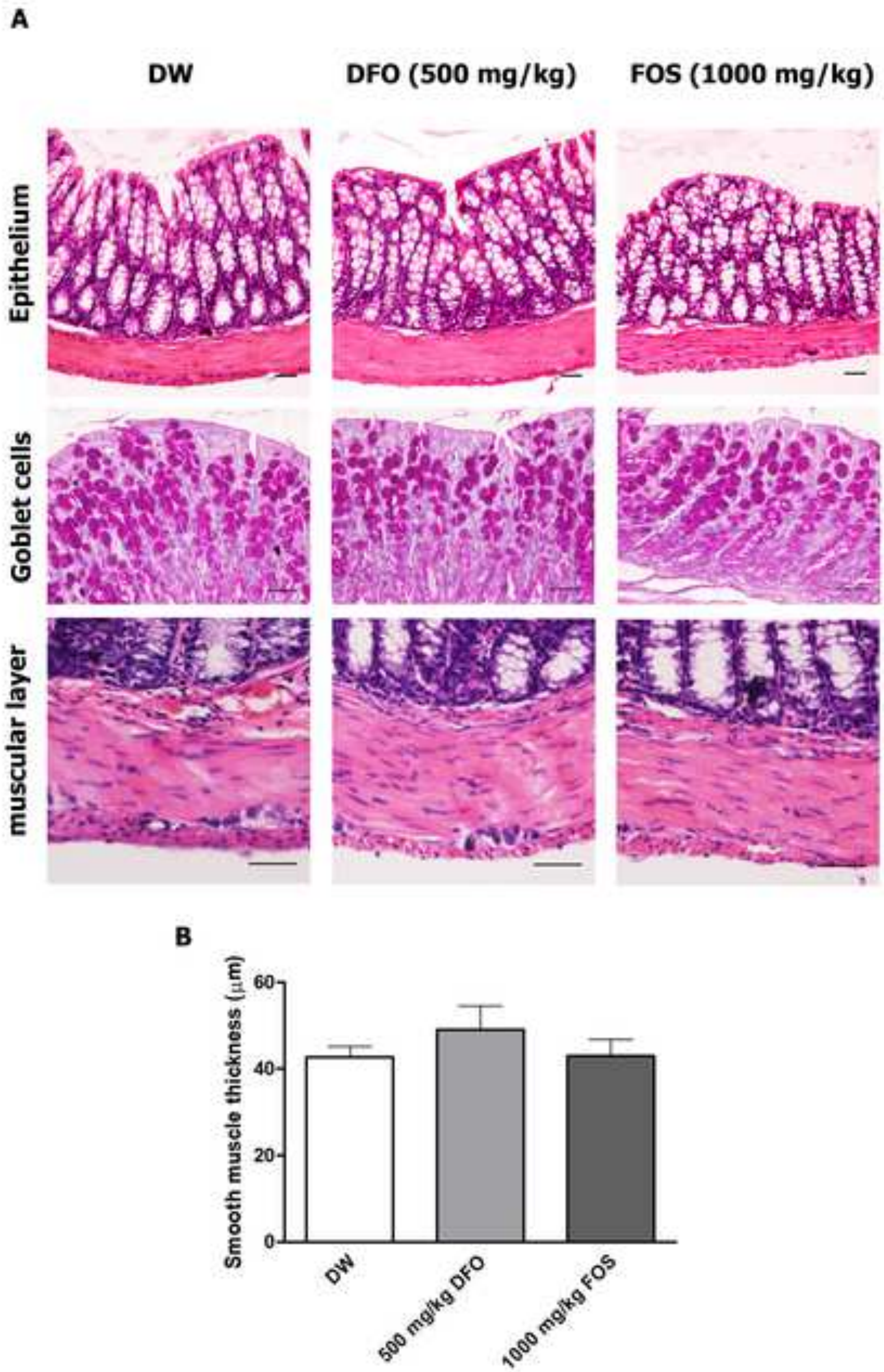

Figure 8: Khuituan et al.