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Novel physiological functions of oligosaccharides*

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Abstract: This paper reviews the evidence for novel physiological functions of oligosaccharides. A history of the research and development and the types of health benefits are discussed. A key property of the physiological functions lies in the indigestibility of oligosaccharides, which gives rise to fermentation in the large intestine, followed by an increase of *Bifidobacteria* and short-chain fatty acid (SCFA) production. This property leads to several kinds of physiological functions, classified into three types of health benefits. The first is the improvement of gastrointestinal conditions, including a normal stool frequency, less constipation, and healthy intestinal microflora. The second is the promotion of mineral absorption, including an increase of bone density and relief of anemia. The third is an immunomodulation effect, such as allergy and cancer prevention.

INTRODUCTION

During the past two decades, many types of oligosaccharides, such as fructo-, galacto-, and xylo-oligosaccharides, were actively developed. Research on their characteristics found that these oligosaccharides had excellent physiological properties that are both scientifically interesting and beneficial to human health. In this paper, I introduce the novel physiological functions and the practical applications of oligosaccharides.

Enzymatic conversion of natural resources is one of the important methods for producing useful food materials. In Japan, this method has been successfully used for the production of inverted sugar, starch hydrolysates, and sugar alcohols. Likewise, many types of oligosaccharides are also produced by enzymatic conversion of natural resources. Table 1 shows chronologically the introduction of typical oligosaccharides into commercial use and the corresponding function of each. In 1979 and 1982, two pioneering oligosaccharides, Coupling sugar® and fructo-oligosaccharides (FOS), were applied in commercial use. Coupling sugar was the first oligosaccharide to claim a beneficial function. It is a low-cariogenic sweetener. FOS were the first indigestible oligosaccharides to verify physiological functions [1,2]. Since then, structurally different oligosaccharides such as galacto- and xylo-oligosaccharides, and lactosucrose were developed. In fact, beneficial functions of indigestible oligosaccharides have been applied for Foods for Specified Health Use, known as FOSHU of the Japanese government approval system. FOSHU has already approved two health claims of indigestible oligosaccharides. They improve gastrointestinal conditions and promote mineral absorption.

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Table 1 Commercial use and their functions of representative oligosaccharides.

Year	Oligosaccharides		Other saccharides	Functions or properties
	Indigestible	Digestible		
1967			Inverted sugar	Economical sweetner
1970			Maltitol	Low caloric,
1979		Maltooligosylsucrose (Coupling sugar®)		Low cariogenic
1982	Fructooligo- (Meioligo®)			Bifidogenic
1984		Maltotetraose (Fujioligo®)		Moderate sweetness
		Isomaltooligo- (Isomalto®)		Bifidogenic
			Palatinose	Low cariogenic
1989	Galactooligo- (Oligomate®)			Bifidogenic
			Erythritol	Noncaloric
1990	Xylooligo- (Xylooligo®)			Bifidogenic
	Gal-sucrose (Lactosucrose®)			Bifidogenic
1993	FOSHU Approval			Improvement of GI Condition
2000	FOSHU Approval			Promotion of Mineral Absorption

STRUCTURE AND PRODUCTION OF FOS

There are two general procedures of oligosaccharide production. One is by transglycosylation between acceptors and donors, and this procedure is applied for fructo- and galacto- oligosaccharide production. The other is by hydrolysis of polysaccharides, and this procedure is used for xylo- and malto-oligosaccharide production. Figure 1 shows the chemical structure of FOS and their enzymatic preparation scheme from sucrose. FOS are oligomers with insulin-type β-(2,1)-fructosyl linkages and exist in natural plants, such as vegetables, fruits, and crops [3]. Commercially available FOS are mixtures of

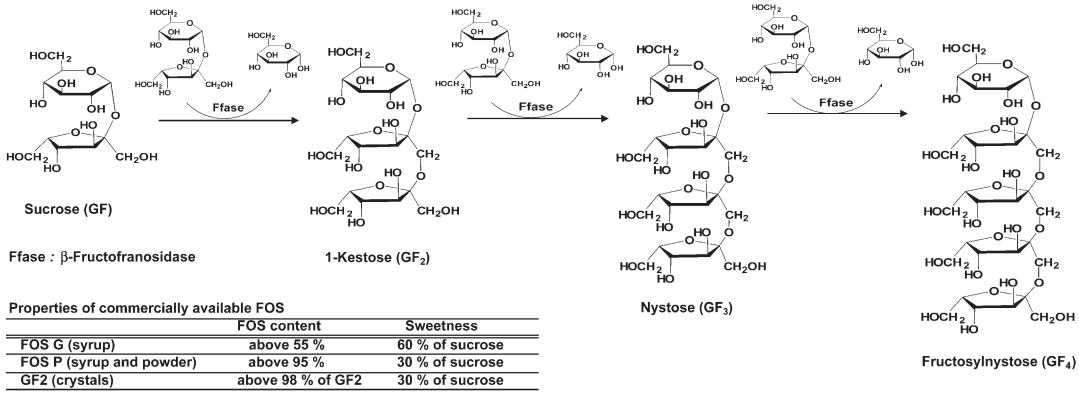

Fig. 1 Chemical structure of FOS and its enzymatic preparation from sucrose.

kestose (GF_2), nystose (GF_3), and fructosylnystose (GF_4) produced from sucrose by the transfructosylating activity of β-fructofranosidase, which can transfer a fructosyl group of sucrose to the terminal fructose of acceptors [4]. Two types of FOS, G and P, are commercially available, and their FOS contents are shown above 55 and 95 %, respectively. Both have good sweetness properties similar to sucrose and have been used for foodstuffs.

INDIGESTIBILITY OF OLIGOSACCHARIDES AS A KEY PROPERTY OF PHYSIOLOGICAL FUNCTIONS

Figure 2 shows the difference in the metabolic pathway between digestible and indigestible saccharides. This difference is an essential property to understand the physiological functions of oligosaccharides [5,6]. The left column in Fig. 2 shows the digestive tract, and the right rectangle shows the inside of the body. Food moves from the mouth to the stomach, then to the small intestine, and then to the large intestine. In the small intestine, digestible saccharides, such as sucrose and starch, undergo the digestion and absorption process. Digestible enzymes convert the saccharides to monosaccharides, which are eventually metabolized, and exhaled as breath carbon dioxide or excreted in urine. On the other hand, indigestible oligosaccharides pass through the small intestine tract. In the large intestine, they enter a fermentation and absorption process. In this process, intestinal microbes transform the oligosaccharides into short-chain fatty acids (SCFAs). Acetate, propionate, and butyrate are the most common components. Subsequently, the SCFAs are absorbed and metabolized into carbon dioxide.

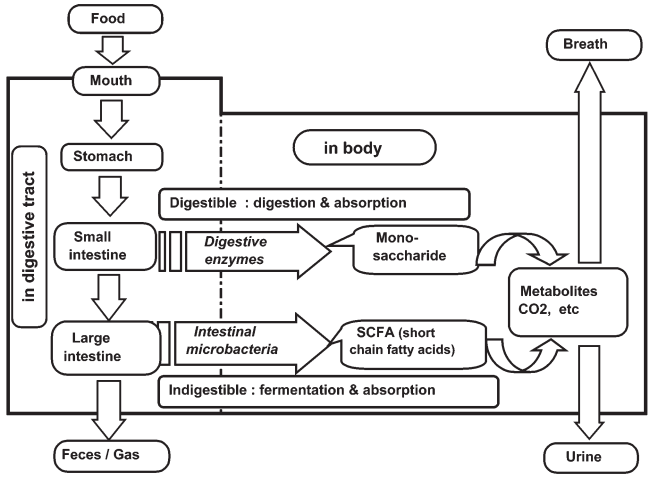

Fig. 2 Difference in the metabolic pathway between digestible and indigestible saccharides.

Figure 3 shows an overview of physiological functions and their key properties of oligosaccharides. Indigestibility gives rise to fermentation in the large intestine, followed by an increase of *Bifidobacteria* and SCFA production. The indigestibility leads to several kinds of physiological functions, and the functions are classified into three types as shown in Fig. 3. The primary function encourages a good gastrointestinal condition, including a normal stool frequency, less constipation, and healthy intestinal microflora. FOSHU approved this health claim in 1993. The second is related to better mineral absorption, including an increase in bone density and relief of anemia. This function was also approved in 2000. The third function is immunomodulation, such as allergy and cancer prevention. Studies on these functions are in progress, and FOSHU should approve this health claim in the near future.

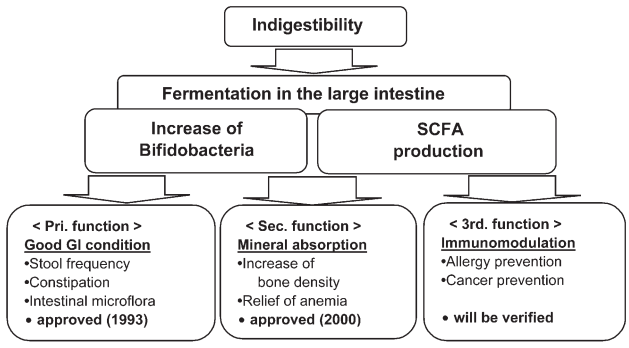

Fig. 3 An overview of physiological functions of FOS and their key properties.

BASIC STUDIES RELATED TO THE PHYSIOLOGICAL FUNCTIONS

Basic studies related to the physiological functions of FOS were done from 1979 to 1989, and major objectives focused on indigestibility, bifidogenesity, SCFA production, and fermentation and absorption.

The indigestibility of FOS was supported by a comparative monitoring study of three blood markers (plasma glucose, fructose, and insulin) between FOS and digestible sucrose after oral administration to men [7]. In the administration of sucrose, all three markers showed a clear increase and reached a maximum 30 min after administration. On the contrary, FOS administration showed no change in each of the three blood markers, indicating that FOS were not absorbed into blood.

It is well known that bacterial growth depends on the carbon resources. As shown in Table 2, glucose is utilized by almost all intestinal bacteria, including the harmful *Clostridium* and *E. coli* species. However, FOS are not utilized by all of them, but selectively utilized by beneficial bacteria of the *Bifidobacterium* species [8]. The proliferation of *Bifidobacteria* by this selective utilization is named "bifidogenesity" and is also recognized as a key property for physiological functions. The SCFA feature of intestinal bacterial fermentation was studied in anaerobic fecal incubation of labeled FOS, showing that the amount of FOS rapidly reduced with an increase in the fermentation products [6]. The major components of the SCFAs produced were acetate, propionate, and butyrate.

Figure 4 shows a study confirming the difference in the metabolic pathway between FOS and sucrose [5]. In an earlier stage of FOS studies, we had a strange result. Indigestible FOS could not be detected in feces. We solved this problem by conducting an animal study that varied the amount of intestinal bacteria in rats. Conventional rats naturally have a large number of intestinal bacteria. An antibiotics treatment reduces that amount, and germ-free rats have none. When labeled FOS was administered to conventional rats, a radioactivity rate of about 50 % was detected in exhaled carbon dioxide 8 h after administration. Similar experiments on rats treated with antibiotics showed a significant reduction in radioactivity. Furthermore, no radioactivity was detected with the germ-free rats while the germ-free condition was maintained. In the case of sucrose administration, no difference was observed among three conditions. From this result, we were convinced that the fermentation and absorption process was related to FOS. This result was supported by a human radiorespirometric study investigating the utilization of [U-^{14}C]fructo-oligosaccharides [6]. About 49 and 55 % of administered radioactivity were detected in expired $^{14}CO_2$ after 24 and 48 h, respectively.

Table 2 Selective utilization of FOS by intestinal bacteria[a].

Bacterial species (No. of strains)		Saccharide[b] Glc	LTU	FOS	Bacterial species (No. of strains)		Saccharide[b] Glc	LTU	FOS
Bifidobacterium					*Megamonas*				
adolescentis	(4)	++	++	++	*hypermegas*	(2)	++	++	++
longum	(3)	++	++	++	*Mitsuokella*				
breve	(3)	++	++	+	*multiacidus*	(2)	++	++	v
infantis	(2)	++	++	++	*Escherichia*				
bifidum	(2)	++	++	-	*coli*	(2)	++	++	-
Lactobacillus					*Klebsiella*				
acidophilus	(3)	++	++	-	*pneumoniae*	(1)	++	-	++
fermentaum	(4)	++	++	-	*Enterococcus*				
salivarius	(2)	++	++	+	*faecalis*	(1)	++	+	+
casei	(1)	++	++	-	*faecium*	(1)	++	++	+
plantarum	(1)	++	++	+	*Streptococcus*				
Eubacterium					*intermedius*	(2)	++	++	++
aerofaciens	(1)	++	++	+	*Peptostreptpcoccus*				
limonus	(1)	++	-	-	*prevotii*	(1)	++	-	-
lentum	(1)	-	-	-	*pervulus*	(1)	++	-	++
Propionibacterium					*Clastridium*				
acnes	(1)	++	-	-	*perfringens*	(4)	++	++	-
Bacteroides					*difficile*	(2)	++	-	-
fragilis	(4)	++	++	++	*paraputrificum*	(2)	++	+	-
thetaiotaomicron	(3)	++	++	++	*clostridiiforme*	(2)	++	++	+
vulgatus	(2)	++	++	++	*ramosum*	(2)	++	++	+
dislasonis	(1)	++	++	++	*butyricum*	(1)	++	++	++
ovatus	(1)	++	++	++	*Veilonella*				
melaninogenicu	(1)	++	-	++	*dispar*	(2)	-	-	-
Fusobacteriun					*Megasphaera*				
varium	(2)	++	-	-	*elsdenii*	(1)	-	-	-

a) Judgement of bacterial growth: ++, same level of growth compared with glucose; +, weaker growth compared with glucose; -, no growth; v, variable (strains may be either + or -); supersucripts indicate the result of occasional strains in the species.

b) Glc, glucose; LTU, lactulose, and FOS, fructooligosaccharides.

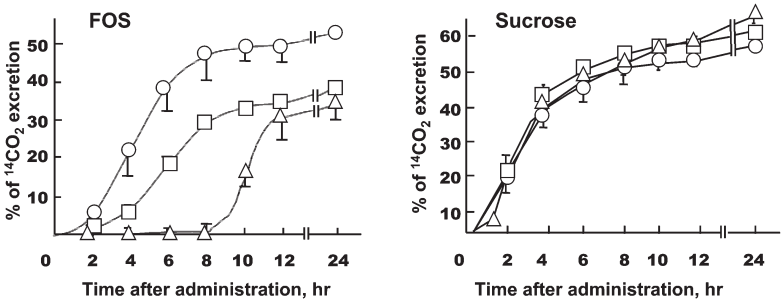

Fig. 4 Difference of metabolic pathway between indigestible FOS and digestible sucrose. Symbols: ○, conventional rats; □, antibiotics treatment rats; △, germ-free rats.

PRACTICAL BENEFITS OF THE PRIMARY FUNCTION

There are four benefits in the primary function: bifidogenesity, reduction in constipation, suppression of putrefactive substances, and reduction of fecal odor. I introduce the practical benefits in human studies.

Figure 5 shows the increase of *Bifidobacteria* during FOS intake, now known as the bifidogenic effect. When 21 human subjects took 6 g daily of FOS, numbers of *Bifidobacteria* clearly increased within a week [9]. However, after stopping intake, the numbers reduced gradually. Figure 6 shows a reduction in constipation due to FOS intake. When the patients took 6 or 12 g of FOS daily, this average number of days between stools gradually reduced to a 1-day interval of normal value [10]. FOS intake shows the suppressive effect on putrefactive substances in feces [11]. In a case study of three subjects, where each took 8 g FOS daily for a month, a decrease in putrefactive substances of *p*-cresol, skatole, and indole was confirmed, along with increases in both *Bifidobacterial* ratio and SCFA amount. As a practical application, FOS have been added to the feed for livestock and pet animals for the decrease of animal fecal odor.

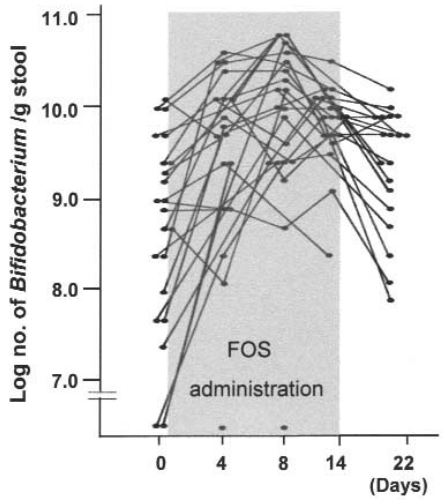

Fig. 5 Increase of *Bifidobacteria* during FOS intake (bifidogenic effect).

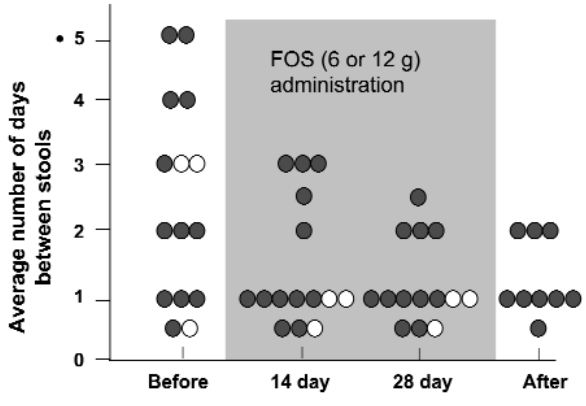

Fig. 6 Reduction in functional constipation due to FOS intake. Symbols: ●, without laxative; ○, with laxative.

PRACTICAL BENEFITS OF THE SECONDARY FUNCTION

The secondary function contains the promotion of calcium, magnesium, and iron absorption.

Figure 7 shows that dietary FOS increased apparent calcium and magnesium absorption in rats [12]. Each rat was fed different test diets containing an appropriate amount of FOS, lactose, or sucrose as a control. As a result, the absorption effect increased with an increase of FOS dosage, and the 15 % FOS diet showed the highest absorption rate. This finding was the start of subsequent mineral absorption studies. In order to examine a possible relationship between the absorption and bacterial fermentation, we carried out a similar study [13] using rats with a cecum and other rats without a cecum. After comparing the FOS and control diet in rats with a cecum, in the FOS diet promotion of both calcium and magnesium absorption was clearly observed, but the increase was lost in both FOS and control diets in rats without a cecum. This result showed that mineral absorption results from fermentation in the cecum. Photos in Fig. 8 show that a FOS diet prevented reduction of cortical and trabecular bone in rats [14]. Comparing the calcification condition of femur among test rats, gastrectomized rats fed a control diet (abbreviated to GX) were shown to be markedly osteoporotic compared to sham-operated rats

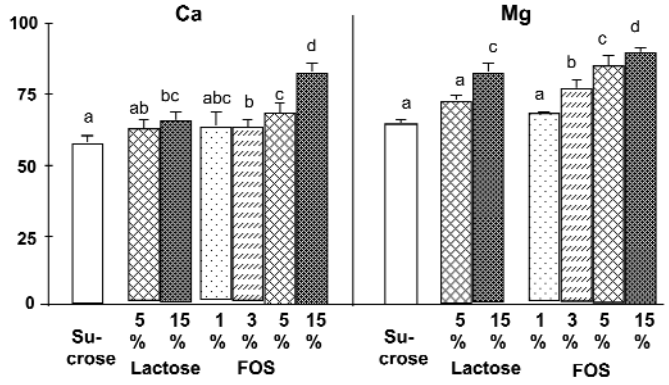

Fig. 7 Dietary FOS increase apparent Ca and Mg absorption in rats. Values are means ±SD, n = 7. Means not sharing a common superscript letter are significantly different (Tukey's test, $P < 0.05$). Apparent absorption = {(intake - fecal output) / intake} × 100.

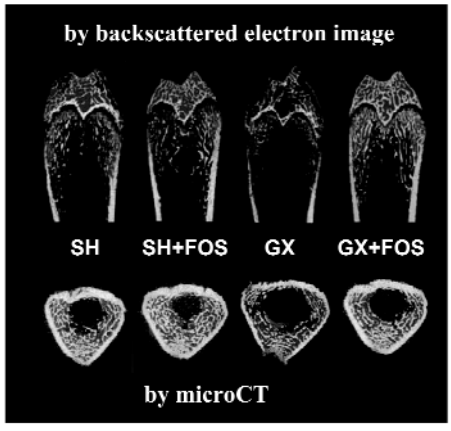

Fig. 8 FOS prevent reduction of cortical and trabecular bone of gastrectomized rats. Abbreviations: SH, sham-operated rats fed by control diet; GX, gastrectomized rats fed by control diet; SH + FOS, sham-operated rats fed by FOS diet; GX + FOS, gastrectomized rats fed by FOS diet.

(abbreviated to SX). However, after an FOS diet (abbreviated to GX + FOS), reduction of the bone was nearly identical to sham-operated rats. These results can be explained well by the following interpretation. Gastrectomy causes calcium malabsorption in the small intestine by the decrease of soluble calcium, and an FOS diet promotes calcium absorption in the large intestine by the fermentation of FOS.

FOS intake also improves iron absorption [15]. Figure 9 shows the promoting effect on recovery from post-gastrectomy anemia in rats. Comparing gastrectomized rats fed by two kinds of diets in four experimental groups, the control diet resulted in a significant decrease of both hemoglobin and hematocrit level, but the FOS diet resulted in no decrease. This result supports the function that an FOS diet can relieve anemia caused by iron malabsorption.

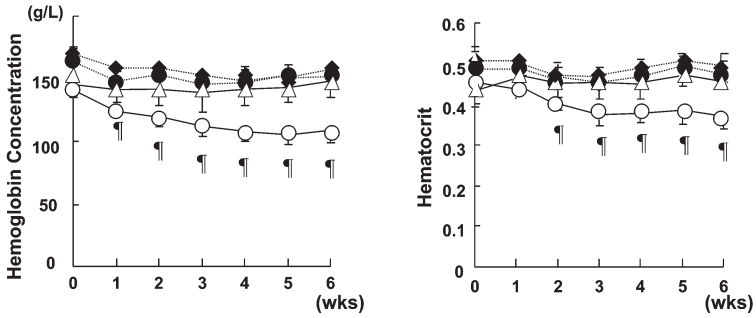

Fig. 9 Promoting effect of FOS on recovery from post-gastrectomy anemia in rats. Symbols: —●—, sham-operated rats fed by control diet; —◆—, sham-operated rats fed by FOS diet; —○—, gastrectomized rats fed by control diet; —△—, gastrectomized rats fed by FOS diet; ¶, significantly less than in other three groups ($P < 0.05$). Each point represents the mean ±SEM of n =7.

EXPECTING BENEFITS OF THE THIRD FUNCTION

The third function is related to immunomodulation effects, such as allergy and cancer prevention. These effects aren't approved as FOSHU claims yet, but their approval is expected in the future.

Raffinose is reported to have a preventing effect on atopic dermatitis, carried out as a clinical test [16]. Raffinose was administered to 50 patients, in age-appropriate dosage, for 2 weeks. Very good results were obtained as follows. Prominent efficiency, meaning a complete disappearance of dermatitis, was seen in nearly half the patients. A lower dermatitis result, called efficiency, was seen in 35 % of the patients, and no efficiency was seen in only 17 %. The preventive effect of indigestible saccharides on colon cancer was studied using min-mice, known as model mice, for colon cancer [17]. The number of colon tumors and intestinal lymphoid nodules were compared among four kinds of test diets including FOS. Figure 10 shows that the FOS diet had the best result for preventing the number of colon tumors or increasing the number of intestinal lymphoid nodules. This is still a result of animal study, but now several human studies on preventing cancer by dietary fibers have been proceeded in the world.

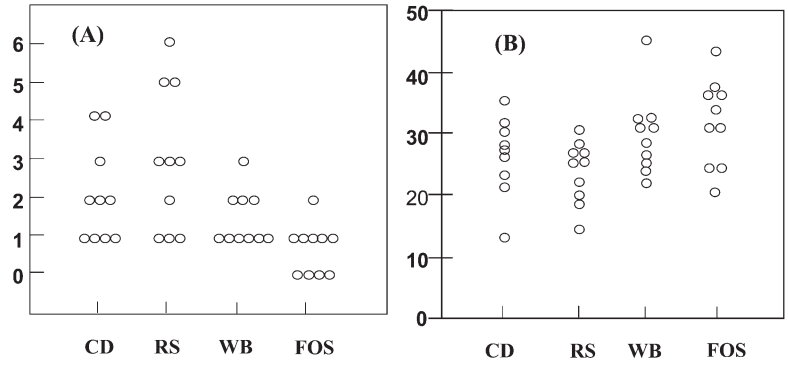

Fig. 10 Preventing effect of dietary fibers and FOS on colon tumors in min mice. (A) Number of colon tumors per mouse. (B) number of intestinal lymphoid nodules per mouse. Abbreviations: CD, control diet (starch); RS, resistant starch; WB, wheat bran; FOS, fructo-oligosaccharides.

CONCLUSION

During the 20 years after FOS development, there has been an impressive advance in the knowledge of physiological functions associated with oligosaccharides. Indigestible saccharides cause several physiological functions, but all originate with fermentation in the large intestine followed by increase of *Bifidobacteria* and SCFA production. I hope that even more physiological functions of oligosaccharides are discovered and that the health benefits are verified in the future.

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