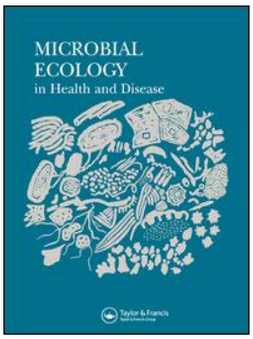

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Fermentable Fiber and the Gastrointestinal Tract Bacteria: Comparisons of Fiber Types and Mouse Strains

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The gastrointestinal tract can be considered as a small, but complex ecosystem that is responsive to inputs (e.g., diet composition, antibiotics). The present study examined the responses of the bacteria resident in different regions of the gastrointestinal tract of two strains of mice (BALB/C and C57 Black) to three types of nondigestible oligosaccharides (NDO). Replacing the poorly fermented and insoluble fiber cellulose, which was present in the diet at 10%, with three soluble NDO (oligofructose, inulin, lactosucrose) increased the densities of anaerobes, aerobes, as well as lactobacilli and streptococci, and decreased enterics and *E. coli*. The magnitude of responses was not consistent for the two strains of mice, regions of the gastrointestinal tract, and for the three NDO. Furthermore, differences were detected for the responses of two shipments of the C57 Black mice. Our findings indicate that 1) NDO can be used to manage the composition of the gastrointestinal tract bacteria, 2) there is a need to identify specific NDO or combinations that will elicit the greatest health benefits as well as the species of bacteria that are responsive, and 3) because of variability among gastrointestinal ecosystems caution is needed when extrapolating results obtained from one species, strain, individual, or one region of the gastrointestinal tract. *Key words*: nondigestible oligosaccharides, aerobes, anaerobes, lactic acid bacteria, intestine, diet, prebiotics.

INTRODUCTION

There is increasing awareness that the species composition and metabolic activities of gastrointestinal tract (GIT) bacteria have an impact on the health of the host. It is now recognized that higher proportions of lactic acid producing bacteria (LAB), which include the lactobacilli and bifidobacteria, are associated with a lower risk of cancer (1, 2) and improved resistance to enteric pathogens (3). This has been attributed to the ability of LAB to reduce the growth of pathogenic and putrefactive bacteria (4), and to possibly enhance enteric and systemic immunity.

There are two principal approaches that are used to increase the proportions of LAB. The first is the introduction of viable cultures of LAB (probiotics). The second involves supplementing the diet with compounds that are preferentially used by LAB (prebiotics). The combination of the two approaches has been called synbiotics (3). Although pre- and probiotics increase the densities and relative proportions of LAB, the responses are transient, lasting only slightly longer than the period of supplementation. A critical difference between the two approaches is that prebiotics encourage the growth of LAB already present in the GIT, and therefore already adapted to the physical, chemical, and biotic characteristics of the GIT environment.

The most commonly used prebiotics are soluble nondigestible oligosaccharides (NDO) that are readily and selectively fermented by LAB present in the GIT. Among the many possible prebiotics, most is known for oligofructose and fructooligosaccharide (FOS), which were among the first that were demonstrated to selectively increase the abundance of LAB (5). Others that have received attention include inulin, which is similar to oligofructose and differs only in having a longer chain length, lactosucrose (6), xylooligosaccharides (7), transgalactosylated oligosaccharides (8), and soy oligosaccharides (9). There are many types of NDO that are being, or could be, considered as prebiotics and virtually all studied to date are known to encourage the proliferation of LAB. A possible exception is the reported lack of increase in fecal bifidobacteria when the diet of human subjects was supplemented with transgalactoooligosaccharides (10). The responses to NDO are highly variable and this is evident when results from different animal models are compared, but even among individuals recruited for clinical studies (11).

The present study compared the responses of bacteria present in GIT to diets containing the poorly fermented fiber cellulose and three NDO that are considered as prebiotics in that they selectively encourage the proliferation of LAB (oligofructose, inulin, and lactosucrose). The

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objectives were two-fold and involved examining the influences of 1) animal strain and 2) fiber type on the assemblages of bacteria resident in the GIT. This was accomplished by enumerating six groups of bacteria (total anaerobes, total aerotolerant forms, enterics, *E. coli*, streptococci, and lactobacilli). We selected two strains of mice that are commonly used for studies of immune functions and are likely to be fed diets that can differ in composition. Because we studied samples collected from five different sites along the length of the GIT, it was also possible to determine if the responses to the different fibers were consistent along the length of the GIT. An unanticipated finding, made possible by ordering one strain of mice in two shipments, was that there is variation in the GIT bacterial assemblages of closely related individuals and how they respond to NDO.

MATERIALS AND METHODS

All phases of the research using animals were approved by the Mississippi State University Institutional Animal Care and Use Committee and were performed in facilities accredited by the American Association for the Accreditation of Laboratory Animal Care (AAALAC).

Table I

Composition of the control and experimental diets[1] fed to the mice for 6 weeks before isolating and enumerating bacteria present in the contents of the colon

Ingredient	gm
Casein, 30 mesh	200
DL Methionine	3
Corn Starch	150
Sucrose	450
Corn Oil	50
Salt Mix S10001[2]	35
Vitamin Mix V10001[3]	10
Choline Bitartrate	2
Cellulose or NDO[4]	100

[1] Diets were formulated were prepared by Research Diets, Inc (New Brunswick, NJ) and were based on the AIN 76 rodent diet.
[2] Composition of the salt mixture (amount in 35 g): calcium phosphate dibasic (Ca = 5.2 g; *P* = 4.0 g), magnesium oxide (Mg = 0.5 g), potassium citrate (K = 3.6 g); potassium sulfate (S = 0.33 g), chromium potassium sulfate (Cr = 2.0 mg), sodium chloride (Na = 1.0 g; Cl = 1.6 g), cupric carbonate (Cu = 6.0 mg), potassium iodate (I = 0.2 mg), ferric citrate (Fe = 45 mg), manganous carbonate (Mn = 59 mg), sodium selenite (Se = 0.16 mg), zinc carbonate (Zn = 29 mg), with sucrose as the remainder.
[3] Composition of the vitamin mixture (amount in 10 g): vitamin A palmitate (4000 IU), vitamin D_3 (1000 IU), vitamin E acetate (50 IU), menadione sodium bisulfite (0.5 mg menadione), biotin (0.2 mg), cyanocobalamin (10 ug), folic acid (2 mg), nicotinic acid (30 mg), calcium pantothenate (16 mg), pyridoxine-HCl (7 mg), riboflavin (6 mg), thiamin HCl (6 mg), with sucrose as the remainder.
[4] The control diet contained 100 g cellulose whereas the experimental diets had 100 g of inulin, oligofructose, or lactosucrose.

Mice and their care

Female mice of the C57 Black (C57B) and BALB/C strains were obtained at 32–35 days of age from a commercial supplier (Jackson Laboratories). The BALB/C mice were obtained as one group (40), whereas 80 C57B mice were obtained in two shipments (40 each) separated by about two months of time, but from the same production site.

After the mice arrived they were housed as groups (5 per cage) in a room maintained at 22°C with a 12:12 light:dark cycle. For the first week after arriving the mice were fed a standard rodent chow (Lab Diet, PMI Feeds, Inc., St. Louis, MO). The groups of mice were then randomly distributed to control and experimental diet groups. Throughout the acclimation and experimental periods the diets were fed to excess and water was continuously available from bottles.

Control and experimental diets

The control diet was based on the AIN 76 formulation with 10% cellulose (Table I). The three experimental diets were identical to the control, except the cellulose was completely replaced by one of three NDO [oligofructose (Raftilose P95, Orafti Belgium), inulin (Raftiline HP Oraftic, Belgium), or lactosucrose (LS 55P, Otsuka, Japan)]. All diets were prepared as pellets by Research Diets, Inc. (New Brunswick, NJ).

The control and all three experimental diets were fed to each of the two shipments of C57 Black mice (10 mice/diet for each shipment). The BALB/C mice were distributed to two groups (20 each) that were fed the control and lactosucrose diets.

Sampling

The control and experimental diets were fed for 6 weeks. The mice were then euthanitized (by carbon dioxide) and the entire postgastric alimentary canal from the pyloric sphincter to the anus was removed. The associated mesentery was severed so the alimentary canal could be straightened. Hemostats were used to isolate a section of about 5 cm long from the middle of the small intestine. In a similar manner, hemostats were used to isolate segments of proximal and distal colon. The three isolated sections were removed by cutting outside of the hemostats and were immediately transferred to an anaerobic chamber. This approach was used to minimize exposure of the internal contents to atmospheric conditions and contaminants.

Once inside the anaerobic chamber, contents of the three sections were removed and weighed. The segments of small intestine and proximal colon were then opened along their length and the mucosa was removed by gently scraping using a sterile glass microscope slide. The mucosal samples were used to enumerate populations of bacteria adhering to or associated with the mucosa. The five samples (3 of contents and 2 of mucosa) were homogenized and serially

Table II

Probability values from the PROC GLM procedure of SAS for the influences of the main effects of mouse strain (C57 Black and BALB/C), diet (cellulose and three NDO), region (five sample sites), and the interactions for the bacterial groups enumerated

Effect	Total Anaerobes	Total Aerobes	Enterics	*E. coli*	Streptococci	Lactobacilli
Strain	0.004	0.43	0.04	0.009	0.0001	0.0001
Diet	0.0001	0.0001	0.0001	0.02	0.0001	0.0001
Region	0.0001	0.0001	0.0001	0.0004	0.0001	0.0001
Strain-Diet	0.0001	0.0001	0.0001	0.008	0.0001	0.0001
Strain-Region	0.37	0.06	0.64	0.98	0.54	0.11
Diet-Region	0.31	0.27	0.45	0.98	0.10	0.01
Strain-Diet-Region	0.66	0.21	0.44	0.24	0.27	0.02

diluted in reduced yeast broth, and plated using an autoplater (Spiral Biotech, Model 4000, Bethesda, MD).

For each sample we enumerated total anaerobes on CDC anaerobe blood agar (BBL; Becton-Dickinson Co., Cockeysville, MD), total aerobes on tryptic soy agar with 5% sheep blood (BBL), enterics on MacConkey II agar (BBL), bifidobacteria on BIM-25 agar (12), lactobacilli on lactobacillus-selective agar (13), and streptococci on Columbia CNA agar(BBL), which is selective for gram positive bacteria. Aerobes were cultured in atmospheric conditions for 2–3 days and the anaerobic plates were cultured in the anaerobic chamber for 4–5 days. All plates were incubated at 37°.

Colonies were identified by gram staining, colony morphology, aerotolerance, and the Crystal System (BBL). Colonies suspected as bifidobacteria were screened by the fructose-6-phosphate phosphoketolase assay (14). When needed, the identities of suspect colonies were confirmed by gas chromatographic analysis of membrane fatty acids (Microbial Identification System, Newark, DE). Densities of each bacterial group were normalized to colony forming units per gram wet weight of sample.

Statistical analysis

The PROC GLM procedure of SAS (Statistical Analysis System, Version 6.11, Cary NC) was used to determine if the main effects of mouse strain, diet, and site influenced densities of the bacterial groups, if there were interactions among the main effects, and to detect if there were differences between the two shipments of C57B mice. Bacterial densities were log transformed for the analyses. When a significant main effect was detected, specific differences were identified using Duncan's test. $P < 0.05$ was accepted as the critical level of significance for all statistical evaluations.

RESULTS

Significant influences were detected for the main effects of site, strain, and diet on densities of the different groups of bacteria (Table II). We also detected significant interactions between the main effects for some, but not all, bacterial groups. Since the BALB/C were fed only two diets (control and lactosucrose) it was necessary to examine the main effect of mouse strain and the interactions with diet and sample site by restricting the data to the control and lactosucrose diets.

Shipment

When all diets and regions were pooled a significant effect of mouse shipment was detected for all bacterial groups, with the exception of total aerobes. The second group of C57B mice fed the cellulose diet had higher total anaerobes, aerobes, enteric, and *E. coli*, whereas streptococci were higher in the first group (Fig. 1). Only the lactobacilli did not differ between the two shipments of C57B mice fed the cellulose diet. Differences were also detected for the different groups of bacteria when mice from the two shipments were fed the three experimental diets (data not shown).

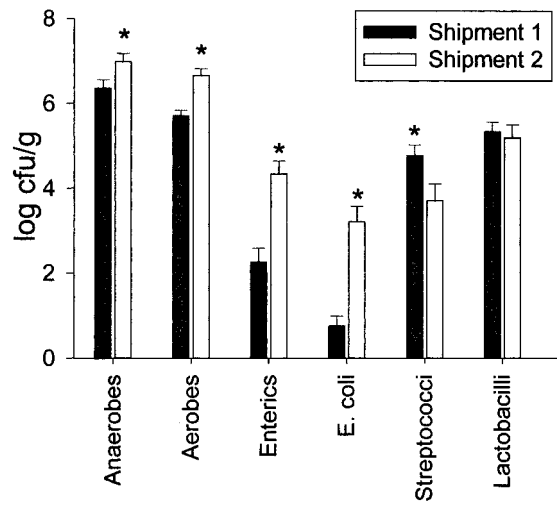

Fig. 1. Densities (colony forming units per gram) for the different bacterial groups in the colon contents of the two shipments of C57 Black mice fed the diet with 10% cellulose. Asterisks above the paired bars indicate a significant difference was detected between the two shipments.

A significant interaction was detected between the two shipments of C57B mice and the responses to the control and experimental diets. This was true for all bacterial groups, except for the streptococci ($P = 0.07$). Specifically, the magnitude of responses differed among the two shipments, however the basic patterns of responses were similar (increases or decreases). This was evident when densities for the bacterial groups enumerated in mice fed the three experimental diets were normalized to the densities in mice fed the control diet. The enterics and *E. coli* provided the most dramatic contrasts. Mice in the first group had lower densities of enterics and *E. coli* when fed the diet with cellulose (Fig. 1). Although the diets with the three NDO resulted in lower densities of enterics and *E. coli*, the responses were of greater magnitude for mice in the second shipment. As a result, feeding the NDO caused the percentage of total anaerobes represented by enterics and *E. coli* to be lower in the second group ($P < 0.05$). Because the basic patterns of response to the diets with NDO were similar for the two shipments of C57B mice, results for the two groups of C57B mice were pooled to examine the main effects of mouse strain, sample site, and diet on densities of the bacterial groups.

Total anaerobes

The distribution of total anaerobes along the alimentary canal did not differ between the two strains of mice. Densities were higher in the contents of the colon (proximal and distal), intermediate in the contents of the small intestine, and lowest in the two samples of mucosa. This pattern of distribution was not affected by diet in either strain.

Total anaerobe densities were higher when both strains of mice were fed the diet with lactosucrose compared to those fed the control diet (Fig. 2A). There was a significant interaction between strain and diet (Table II). Total anaerobes were higher in BALB/C mice when the control diet was fed, whereas densities were higher in C57B mice when the lactosucrose diet was fed. As a result, the relative increase in densities in response to the lactosucrose diet was greater for the C57B mice.

The diets with oligofructose and inulin also increased anaerobe densities of C57B mice compared to those when the control diet was fed, with the responses greater for oligofructose.

Total aerobes

Similar to the anaerobes, aerobe densities were higher in the contents of the two colon segments, intermediate for the contents of small intestine, and lowest in the two mucosal samples. The pattern of distribution did not vary among the two strains or between the control and lactosucrose diets.

Aerobe densities were lower in mice fed the control diet, and more so for C57B mice compared to the BALB/C strain (Fig. 2B). Feeding the lactosucrose diet resulted in higher densities for both strains, but more so for the C57B mice, causing a proportionally greater response.

Aerobe densities of C57B mice were lower when fed the diet with inulin compared to the diets with oligofructose and lactosucrose, which were similar.

Enterics

The lowest densities of enterics were associated with the small intestine (contents and mucosa), with comparable densities for the contents and mucosa of the colon.

There was a highly significant interaction between strain of mouse and diet ($P < 0.001$). When the control and lactosucrose diets were compared, densities of enterics for BALB/C mice did not differ, but were more than 100-fold higher in C57B mice fed the control diet (Fig. 2C).

Densities of enterics in C57B mice fed the diet with inulin were lower than those recorded from mice fed the control diet, but were higher than those fed the diets with oligofructose and lactosucrose.

E. coli

Densities of *E. coli* represented about 14% and 18% of the enterics in BALB/C and C57B mice, respectively. The regional distribution, based on densities, paralleled that for the enterics.

Densities of *E. coli* did not differ between BALB/C mice fed the control and lactosucrose diets. When C57B mice were compared to BALB/C mice, densities of *E. coli* were similar when both strains were fed the control, but were lower in C57B mice when the diet with lactosucrose was fed (Fig. 2D).

The three experimental diets resulted in comparable densities of *E. coli* in the C57B mice, despite the differences seen for the enterics.

Streptococci

The two mucosal samples supported the lowest densities of streptococci, with highest densities enumerated from the two samples of colon contents.

There were profound differences between the two strains for streptococci densities (Fig. 2E). Densities were lower in BALB/C mice fed the control diet, and did not differ among contents from the small intestine and the two colon sites. Densities averaged four log units higher in C57B mice when both strains were fed the control diet, and there was a significant increasing gradient of densities from the small intestine to the distal colon, with differences detected between contents from the three sites. Differences were also detected between strains when the diet with lactosucrose was fed, but were of much lower magnitude due to the over million-fold increase in streptococci for BALB/C mice, whereas they increased 1000-fold for C57B mice.

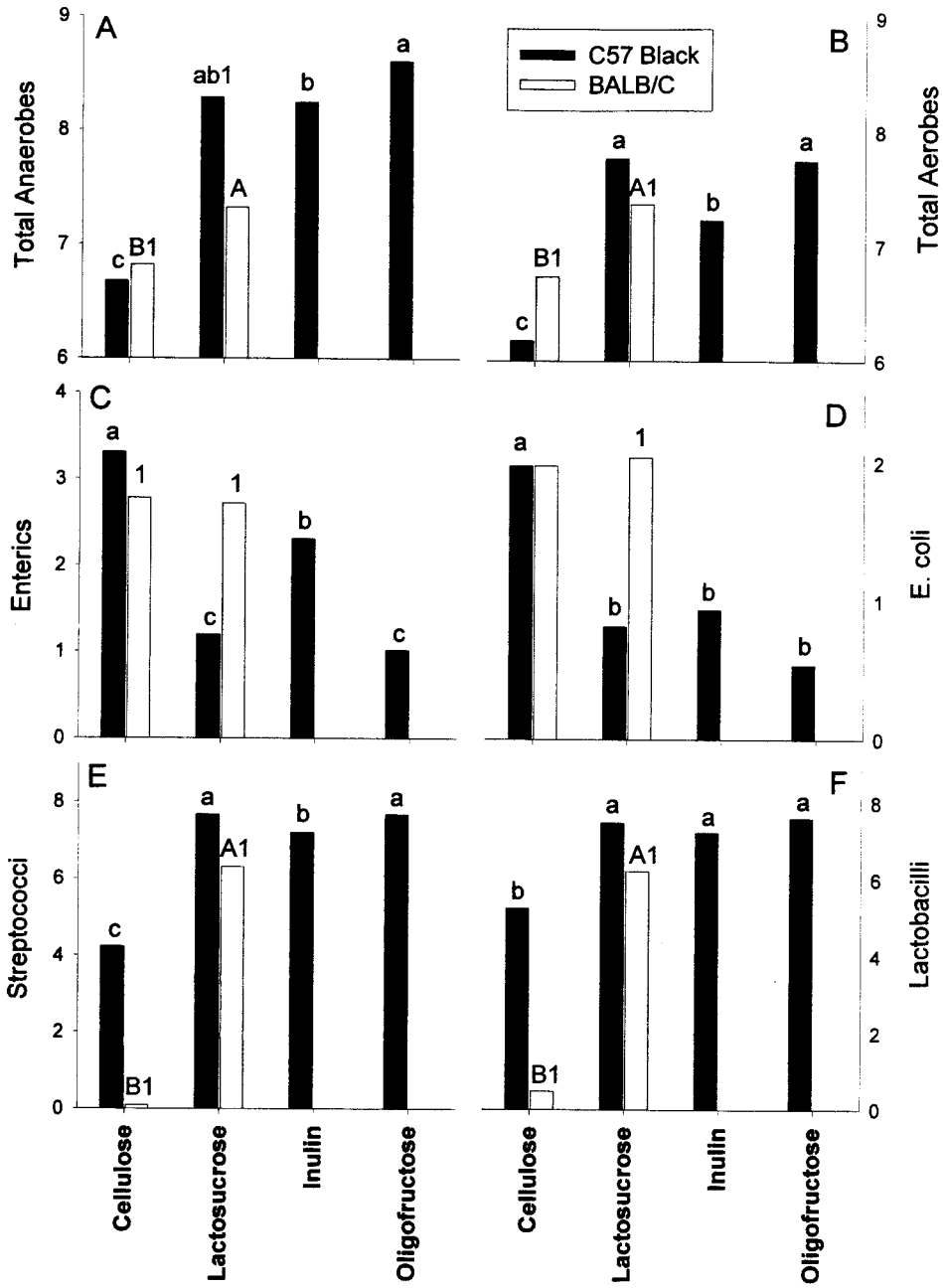

Fig. 2. Densities (log colony forming units per gram) of total anaerobes (A), aerobes (B), enterics (C), *E. coli* (D), streptococci (E), and lactobacilli (F) in the colon contents of C57 Black and BALB/C mice fed the four diets with cellulose or the three NDO at a level of 10%. Values for C57 Black mice are pooled results from both shipments. Bars not sharing the same letter superscripts are significantly different, with small letters used for interdiet comparison of C57 Black mice and capital letters used for BALB/C mice. The numeral denotes a significant difference was detected between C57 Black and BALB/C mice fed the same diet.

Among the three experimental diets fed to the C57B mice, streptococci densities were lower in mice fed the diet with inulin compared to those fed lactosucrose and oligofructose.

Lactobacilli

The main effects of strain, site, and diet, and interactions between diet and strain for the lactobacilli were virtually identical to those for the streptococci. The only difference of note is that inulin did not result in significantly lower ($P = 0.07$) densities than lactosucrose and oligofructose in the C57B mice (Fig. 2F).

DISCUSSION

Although the bacterial populations present in the different regions of the GIT are often considered to be relatively stable, it is possible to change the relative proportions of the different groups using dietary inputs. In a similar manner, the use of fertilizers can alter the proportions of different organisms present in other ecosystems. In both cases, the total number of species and total densities of all organisms do not necessarily change. Instead, there are shifts in the relative abundance of the various species that are present.

The present findings confirm many other reports about how dietary inputs can be used to manage the relative abundance of various species of bacteria resident in the different regions of the GIT. By doing so, it is also possible to alter metabolic activities (11) and influence intestinal structure and functions (15). Our results provide three additional insights about prebiotics, and the first was partly unexpected.

Animal models

The present study was not designed to examine if there were differences between individuals. However, the use of two shipments did reveal variation among groups of individuals that are virtually identical with respect to genotype and environmental conditions. Furthermore, both strains of mice were from colonies that were founded by individuals inoculated with the Altered Schaedler Flora (ASF), which includes eight species (16). The ASF does not include bifidobacteria, and corresponding with this, this group was not detected in any of the mice. Although streptococci are also not included in the ASF, since they were detected in the mice representatives of this group, must have 'invaded' the GIT ecosystem, perhaps from the food, water, or the handlers.

The ecological literature provides ample, incontrovertible evidence about how large, and even subtle, differences in the physical and chemical characteristics of environments and their evolutionary past result in different assemblages of organisms. Similarly, because of variation in GIT structure and functions, which provides different physical and chemical environments, the bacterial assemblages present in the GIT differ among species. Although the variation should be less among individuals of a single species or strain, we still detected different assemblages and responses of the GIT bacteria present in mice from the two shipments of the C57B strain. Therefore, even when members of a population are nearly identical, subtle differences among individuals and the environment during their development can be sufficient to result in different assemblages of bacteria. In a similar manner, individual variation is usually quite high for bacteriologic results associated with clinical studies, sometimes making it difficult to detect diet effects.

The differences detected between the two strains of mice (C57B and BALB/C) are less surprising, even though both were from colonies started with the ASF. However, the differences are of interest in that the two strains of mice differed in how the GIT bacteria responded to the diets. The lactosucrose diet resulted in densities of lactobacilli and streptococci that were several orders of magnitude higher in both strains of mice when compared to those enumerated in mice fed the control diet. However, the increase in LAB was associated with lower densities of enterics and *E. coli* in C57B mice, but not in the BALB/C mice. It is possible that the species/strains of lactobacilli and streptococci, and perhaps other bacteria, present in the GIT of the C57B mice differed from those in the BALB/C mice in their abilities to repress growth of enterics and *E. coli*. Collectively, these findings indicate that the responses of the GIT bacteria to diet seen in one animal model may not apply to another species, and perhaps not even to strains of the same species. Moreover, caution needs to be exercised when trying to extrapolate findings from animal models to human subjects.

The present study also shows that the responses of the GIT bacteria can be variable even when animals from the same population are used, but are obtained at different times. Similarly, results from clinical studies using humans from the same 'population' show variable responses to dietary supplements (11, 17). It needs to be considered that although absolute densities can vary, the patterns of responses are more consistent

Different types of fibers

Adding fiber to the diet is widely regarded as being beneficial by improving 'colonic health'. Whereas the specific influences of insoluble, poorly fermented fibers (e.g., cellulose) are uncertain, soluble NDO that are fermented readily by bacteria resident in the GIT are known to alter the absolute densities and relative proportions of the bacterial groups that constitute the microbiota (3). The NDO used for the present study meet the criteria established for prebiotics (18). They are not digested or absorbed by the GIT, each serves as a substrate for some, but not all, GIT bacteria, and when all three are included in diets they influence the composition of the bacterial assemblages.

A consistent finding among numerous studies is that fermentable fibers increase the absolute and relative densities of anaerobes and LAB, and decrease the proportion of aerobes. The present study shows that different types of NDO have variable influences on the specific responses of the GIT bacteria. These findings extend previous reports that the diverse forms of fermentable fiber do not elict consistent responses among the various GIT bacteria (19, 20). Therefore, it is not surprising that the responses to lactosucrose differed in some cases from those seen for inulin and oligofructose. The lower magnitude of responses by the bacteria to inulin compared to oligofructose is interesting in that these two prebiotics differ only in degree of polymerization (inulin > oligofructose).

Another consistent finding among reported studies is that NDO increase the concentrations of short chain fatty acids (SCFA). Although not examined in the present study, the proportions of the various SCFA that are produced by bacterial fermentation (e.g., acetate, butyrate, propionate) are not consistent for the various types of NDO (19). Although the increases in lumenal SCFA elicited by including NDO in the diet is known to stimulate growth and the functional capacities of the intestinal mucosa and are associated with induction of gene expression

(21), the specific proportions of the different SCFA that induce the 'optimal' response have not been determined.

Regional responses

The responses to NDO are generally based on changes in fecal bacteria. This is especially true for clinical trials using human subjects. However, *in vitro* fermentation studies have suggested that the responses vary and may be more profound in the upper reaches of the GIT (22). Corresponding with this, we detected differences among the five sample sites in how the resident bacteria responded to the four diets. For example, the replacement of cellulose by oligofructose resulted in a 37-fold increase in anaerobes in the small intestinal contents of C57B mice, with aerobes increasing less (13-fold) and streptococci more so (616-fold). In the contents of the proximal colons of the same mice, the values were 165-, 53-, and 2750-fold for anaerobes, aerobes, and streptococci, respectively. Oligofructose also resulted in lower enteric counts in all regions (compared to mice fed the control diet with cellulose), but more so in the contents of the small intestine (0.002) compared to the two segments of the colon (0.015 and 0.32). Regional differences can also be seen for the responses to the other two NDO. However, the magnitude of differences were not consistent for the three NDO. Similarly, responses of the cecal and fecal bacteria of rats are not consistent among various NDO (19). Collectively, these findings indicate that stool samples will provide only partial insights into the responses to NDO in more proximal regions. This highlights a need to identify indicators that can be used to better understand events occurring throughout the GIT.

Another important consideration is how the assemblages of mucosal (adherent or resident) bacteria respond to NDO relative to those that are present in the contents (transient). The bacteria that are closely associated with the mucosa are likely to exert greater health effects, whether beneficial or detrimental, yet less is known for the bacteria present in this 'habitat'. The present study shows that the adherent bacteria differ from those in the lumen with respect to the relative proportions of the bacterial groups and the responses to NDO.

Perspectives and implications

The GIT can be considered as a small, but complex ecosystem that shares many similarities with streams (23, 24). Like streams, changes in the proportions of the resident bacteria can alter the functional attributes of the system by altering energy and materials flow, thereby influencing the productivity of the entire system. This is evident from shifts in the concentrations and relative proportions of SCFA (19) and the stimulation of intestinal growth and gene expression (21). Additional implications with the host include nutrient utilization and enhancement of immune functions.

NDO can be considered as 'management tools' that can be used to optimize the GIT ecosystem. It is now clear that the different types of NDO will elicit variable effects on the ecosystem, much like different fertilizers will elicit different responses in other ecosystems. Further consideration must be given to the likelihood that not all members of the LAB will provide the same health benefits (25), and that different species and even strains may not respond similarly to NDO. Therefore, it will be important to identify specific NDO, or combinations, that will encourage the proliferation and metabolic activities of the LAB that will provide the greatest health benefits.

Animal models will continue to be used to provide insights about responses of the GIT ecosystem to NDO. However, the present study in conjunction with results from numerous other investigations demonstrate how care must be used in comparing data from different studies. Ecologists face the same dilemma when trying to extrapolate findings from one stream ecosystem to another. Specifically, differences in the characteristics or past history of an ecosystem that may be too subtle to detect can result in different species assemblages and how they respond to inputs. As a result, it is likely each ecosystem (i.e., GIT) will respond in a unique manner and will need to be considered individually.

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