Effects of dietary fructooligosaccharide on selected bacterial populations in feces of dogs

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Objective—To evaluate fecal concentrations of selected genera of colonic bacteria in healthy dogs, and to investigate effects of dietary fructooligosaccharides (FOS) on those bacterial populations.

Animals—6 healthy adult Beagles.

Procedure—Dogs were randomly assigned to 2 groups of 3 and fed an unsupplemented diet for 370 days. After 88 days, fecal samples were collected. Another fecal sample was collected from each dog 282 days later. Group A then received a diet supplemented with FOS, and group B continued to receive the unsupplemented diet. Twenty-eight to 29 days later, fecal samples were collected. Diets were switched between groups, and fecal samples were collected 31 and 87 days later. Concentrations of Bifidobacterium spp, Lactobacillus spp, Clostridium spp, Bacteroides spp, and Escherichia coli in freshly collected feces were determined. Effects of diet and time on bacterial concentrations were compared between groups.

Results—Bifidobacterium spp and Lactobacillus spp were inconsistently isolated from feces of dogs fed either diet. Sequence of diet significantly affected number of Bacteroides spp subsequently isolated from feces, but diet had no effect on numbers of Clostridium spp or E coli.

Conclusions and Clinical Relevance—Some genera of bacteria (eg, Bifidobacterium) believed to be common components of colonic microflora may be only sporadically isolated from feces of healthy dogs. This deviation from expected fecal flora may have implications for the effectiveness of supplementing diets with prebiotics. (Am J Vet Res 2000;61: 820–825)

The colonic microflora of dogs, like that of people, is varied and complex. Perturbations of this microflora may, at least in some circumstances, cause or contribute to alimentary tract disease. In particular, diet can affect the colonic microflora, and understanding the relationships between diet, change in colonic microflora, and alimentary tract disease may facilitate treatment and help ameliorate clinical signs of disease.

Because the colonic microflora is so complex, it is difficult, if not impossible, to isolate all genera of bacteria expected to be in feces. Bacteria that are expected to be in feces may not be detectable in some animals, using standard bacteriologic culturing techniques. Furthermore, there is an increasing awareness that our views of what constitutes normal colonic microflora may not be accurate, especially when feces from an individual animal are evaluated. For example, anaerobic bacteria from the genera Bacteroides, Lactobacillus, and Bifidobacterium are considered normal inhabitants of the intestinal tract of all but neonatal dogs and believed to be easily isolated from feces of healthy adults. Sometimes these types of statements appear to be extrapolated from results of studies in people, but results of some studies in dogs also seem to support these statements. In one study, Bifidobacterium spp were consistently isolated from the feces of 8 German Shepherd Dogs on 4 separate occasions, and in a second study, this genus was cultured from fecal samples collected from 16 Beagles. However, other investigators identified Bifidobacterium spp in feces from most, but not all, dogs, whereas others have identified this genus in feces of only a minority of dogs examined. Particularly noteworthy are the results of Mitsuoka et al, who isolated Bifidobacterium spp from feces of 12 of 14 dogs < 24 months old and consistently failed to culture this genus from feces of 5 dogs > 4 years old. These findings support those of another study suggesting that old (> 11 years old) Beagles had significantly fewer Bifidobacterium spp in their feces than young (< 1 year old) Beagles. However, in that study, old dogs also had fewer Bacteroides spp, Eubacterium spp, Peptostreptococcus spp, Lactobacillus spp, and Staphylococcus spp than young dogs.

The lack of consistent results among investigators may reflect differences in sampling and culturing techniques, differences in breeds or housing conditions, differences among individual dogs, or differences between geographic areas. Five of 6 studies investigating fecal bacterial populations in healthy dogs were done in Japan, and only 1 was done in the United States, thus, differences in origins of dogs or geographical location may also contribute to inconsistent results of bacteriologic culture. Such disparity in fecal and, presumably, colonic bacterial populations may have important clinical ramifications for the use of prebiotics designed to target specific bacteria such as Bifidobacterium spp.

Prebiotics are foodstuffs that either suppress or enhance replication of specific colonic bacteria indigenous to the host and are fed to attain a specific clinical effect, as opposed to probiotics, which are bacteria or yeast that are fed to alter colonic bacterial populations and cause a specific clinical effect. Fructooligosaccharides (FOS) may have prebiotic effects.
Fructooligosaccharides are composed of sucrose oligomers with additional fructose units. They are not digested by mammals but rather pass undigested through the small intestine until they reach the large intestine. When FOS reach the large intestine, they serve as a substrate for some bacteria but not others, thus promoting select bacteria to proliferate at the expense of others. In people fed FOS, the colonic bacteria that subsequently proliferate may have beneficial effects (eg, they affect fecal concentrations of specific volatile fatty acids, ammonia, and putrefactive substances such as p-cresol and indole as well as stimulate immune function and synthesize vitamins) that are a result of metabolic products or suppression or proliferation of detrimental bacteria. Therefore, to assess the effects of dietary supplementation with prebiotics, it is important to have an understanding of the normal colonic microflora.

The purpose of the study reported here was to evaluate selected genera of colonic bacteria in healthy dogs in the United States and to investigate effects of dietary FOS on those populations. We chose to evaluate *Clostridium* spp, *Escherichia coli*, *Bacteroides* spp, *Lactobacillus* spp, and *Bifidobacterium* spp in fecal samples collected from healthy adult dogs, because these genera may cause either detrimental or beneficial effects.

### Materials and Methods

#### Animals

Six female Beagles between 7 and 8 years old were used. These dogs were originally obtained from a commercial breeder but had been housed at the Texas A&M University Laboratory Animal Resources and Research Facility for more than 2 years and were used in a study that investigated the effects of otic medications. Prior to the present study, physical examinations were performed, blood samples were collected for CBC, serum biochemical analyses, and determination of serum IgA concentrations, and feces were collected for parasitologic analysis by direct microscopic examination and fecal flotation with zinc sulfate solution. Dogs were found to have dental problems, which were resolved before beginning the study.

Dogs were individually housed in stainless steel cages in the same room at the Texas A&M University Small Animal Clinic for the entire study. Each dog occupied the same cage throughout the study. Dogs were allowed into a common area when the cages were cleaned, at which time they had contact with one another. Dogs were walked outside twice daily, cared for by the same technician throughout the study, periodically examined and weighed, and fed an unsupplemented diet consisting primarily of corn starch and chicken protein (Table 1). Food intake was adjusted to maintain appropriate body weight. During the study, feces were collected 6 or 7 times from each dog for parasitologic analysis by fecal flotation. Otitis and keratoconjunctivitis sicca were detected in 1 dog, and a mammary nodule was found in a second dog. The project was approved by the University Laboratory Animal Care Committee.

#### Diet formulation

The diets fed to the dogs (Table 1) were specifically formulated for this study and were designed to be as identical as practically possible. The diets were formulated with the same amount and source of chicken, chicken by-product meal, and mineral supplements. One percent FOS was added to the supplemented diet on a weight per weight basis (ie, wet powdered FOS to wet ingredients in the formula). Differences in gross energy between the 2 diets were attributed to analytical variation in the bomb calorimeter used to generate gross energy values. Differences detected in the 2 diets were minor and considered unlikely to affect colonic microflora. Fructooligosaccharide content in the final diets was not measured; however, FOS is only found in trace amounts in the ingredients used in these diets.

### Project design

Eighty-eight days after starting the unsupplemented diet, fecal samples were collected from each dog (prestudy collection period). Dogs were randomly assigned to 2 groups of 3 dogs each, and both groups continued to receive the unsupplemented diet for an additional 282 days, after which individual fecal samples were again collected (study collection period 1). We had originally planned the first study collection to take place 6 weeks after dogs were assigned to treatment groups. However, because of difficulties with the bacteriologic culturing techniques, the first study collection was delayed until day 282. Five days after the first study collection, group A received a FOS-supplemented diet (Table 1), and group B continued to receive the unsupplemented diet. Twenty-eight to 29 days later, fecal samples were collected from all dogs (study collection period 2). Three days later, diets were again changed. Group A now received the unsupplemented diet, and group B received the FOS-supplemented diet. Thirty-one days later, fecal samples were collected from individual dogs (study collection period 3). Because the cultures for *Clostridium* spp from study collection period 3 were overgrown and unreadable, and because time was available in the study design, dogs in group A continued to be fed the unsupplemented diet and dogs in group B the FOS-supplemented diet for an additional 36 days, at which time fecal samples from each dog were collected for the final time (study collection period 4). The entire study lasted approximately 17 months.

#### Collection of feces

Feces were digitally removed from the rectum and anal canal, using a sterile glove lubricated with water. Approximately 5 to 10 g of feces was routinely obtained. The samples were immediately placed in plastic bags and emulsified by squeezing the bag repeatedly between the finger tips until homogeneity seemed assured. One gram of feces was immediately added to 9 ml of prerduced phosphate-buffered saline solution (pH 7.3). Remaining clumps of fecal material were disrupted with a sterile loop, and samples were mixed well by vortexing. Three sets of serial ten-fold dilutions (range, $10^{-2}$ to $10^{-7}$) in a total volume of 10 ml each were made from initial emulsions.

#### Bacteriologic techniques

Briefly, 0.05 ml of appropriate dilutions of emulsified feces were inoculated in triplicate on the appropriate media, and plates were incubated at 37°C for 48 hours in an anaerobe jar with indicator or for 24 hours under aerobic conditions.

### Table 1—Analysis of 2 diets fed to 6 healthy adult Beagles

<table>
<thead>
<tr>
<th>Component</th>
<th>Unsupplemented</th>
<th>FOS supplemented</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fructooligosaccharide (%)</td>
<td>0</td>
<td>1.0</td>
</tr>
<tr>
<td>Corn starch (%)</td>
<td>49.8</td>
<td>48.9</td>
</tr>
<tr>
<td>Protein, primarily as chicken (%)</td>
<td>29.23</td>
<td>27.81</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>7.47</td>
<td>9.71</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>6.17</td>
<td>5.77</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>7.26</td>
<td>8.14</td>
</tr>
<tr>
<td>Crude fiber (%)</td>
<td>1.14</td>
<td>1.61</td>
</tr>
<tr>
<td>Nitrogen-free extract (%)</td>
<td>48.73</td>
<td>46.96</td>
</tr>
<tr>
<td>Calcium (%)</td>
<td>1.00</td>
<td>0.78</td>
</tr>
<tr>
<td>Phosphorous (%)</td>
<td>0.75</td>
<td>0.71</td>
</tr>
<tr>
<td>Gross energy (cal/g)</td>
<td>4,409.1</td>
<td>4,312.7</td>
</tr>
</tbody>
</table>

FOS = Fructooligosaccharide.
For isolation of Bifidobacterium spp, samples of $10^2$ through $10^6$ dilutions were inoculated on commercially prepared Bifidobacterium isoacetic acid media (BIM-10) plates, and plates were incubated anaerobically. Each morphologic variant was then inoculated individually on fresh BIM-10 (anaerobically incubated) and bovine blood agar (aerobically incubated) plates. Strict anaerobic isolates were Gram-stained, and isolates that did not produce spores were grown in trypticase-yeast extract-broth and tested for fructose-6-phosphate phosphoketolase activity to confirm their identity as Bifidobacterium spp.

For isolation of Bacteroides spp, samples of $10^2$ through $10^6$ dilutions were inoculated on commercially prepared neomycin-brilliant green-taurolactate agar plates, and plates were incubated anaerobically. Each morphologic variant was individually inoculated on bovine blood agar plates that were then incubated aerobically and anaerobically. Strict anaerobic isolates were Gram-stained, and gram-negative rods and pleomorphs were biochemically tested with a commercial system to confirm their identity as Bacteroides spp.

For isolation of Lactobacillus spp, samples of $10^2$ through $10^6$ dilutions were inoculated on commercially prepared sucrose-lactobacillus agar plates, and plates were again incubated anaerobically. Each morphologic variant was individually inoculated on Rogosa agar plates, and plates were incubated anaerobically. Isolates were Gram-stained, and non-spoor forming, gram-positive rods were examined, using gas layer chromatography, to confirm their identity as Lactobacillus spp.

For isolation of Clostridium spp, samples of $10^2$ through $10^6$ dilutions were inoculated on commercially prepared reinforced clostridial agar plates, and plates were incubated anaerobically. Each morphologic variant was individually inoculated on bovine blood agar plates that were then incubated aerobically and anaerobically. Strict anaerobic isolates were Gram-stained, and gram-positive rods were examined using a commercial system to confirm their identity as Clostridium spp.

After preparation of all plates for anaerobic culture, dilutions of emulsified fecal samples (ie, $10^2$ through $10^6$ dilutions) were plated on MacConkey agar and incubated aerobically. Individual colonies were Gram-stained, and gram-negative rods were further examined with a commercial system to confirm their identities as E coli.

To ensure that the bacteriologic techniques would consistently allow growth of Bifidobacterium spp and Lactobacillus spp in fecal material, positive control emulsions were prepared by adding a well-characterized suspension of bacteria to the first dilution of emulsified feces from each of 2 or 3 dogs to yield a final concentration of $10^5$ colony-forming units (CFU)/ml of emulsified feces. The suspension of known bacteria had a turbidity that was visually equivalent to a No. 2 MacFarland standard and contained at least 2 of the following Bifidobacterium species: B animalis (ATCC 27536), B pseudolongum (ATCC 25526), and B adolescentis (ATCC 15705); and 1 of the following Lactobacillus species: L fermentum (ATCC 9338) and L acidophilus (ATCC 4356). Control emulsions were diluted and inoculated, as described, for the isolation of Bifidobacterium spp and Lactobacillus spp from experimental samples. These control bacteria were consistently grown from the spiked fecal emulsions.

Specifically identified colonies on plates with an optimal number of bacterial colonies for enumeration (ie, $>10$ but $<10^5$) were counted. Only the plates with the dilution resulting in this number of colonies per plate were evaluated. Concentration of bacteria per gram of feces was calculated as follows:

\[
\text{Number of colonies per gram of feces} = \left( \frac{\text{Number of colonies at dilution}}{10} \right) \times 10^{(\text{dilution exponent} - 5)}
\]

The concentration of each of the 5 genera of bacteria in an individual fecal sample was expressed as the median value (CFU per gram of feces) determined from triplicate plates. Fecal samples from each dog were analyzed also for Clostridium perfringens enterotoxin A by use of a reversed passive latex agglutination test.

### Statistical analyses

Data (CFU per gram of feces) for each genera of bacteria isolated from feces collected at the first 3 study collection periods were plotted, and graphs were visually inspected to determine whether data followed a Gaussian distribution. To approximate a Gaussian distribution, data for E coli and Bacteroides spp were transformed, using the natural logarithm function. However, because of several observed values of 0 in data for Lactobacillus spp, Bifidobacterium spp, and Clostridium spp, these data were transformed, using the sin function. The effects of diet on fecal bacterial counts were examined by use of a generalized linear model approach to ANOVA for repeated measures, because all dogs were receiving the unsupplemented diet when fecal samples were first collected, and data were unbalanced. The statistical model also allowed us to use data collected from an individual dog at study collection period 1 as the control for data from that same dog collected at study collection periods 2 and 3. The sequence that diets were fed and time periods were modeled as fixed effects, and individual dogs nested within a sequence were modeled as random effects. Type-III sums of squares were used to assess significance of the random effects generalized linear model. Median number of Clostridium spp isolated from feces at the presudy collection period and study collection period 4 were compared by use of the sign test for all tests, $P < 0.05$ was considered significant.

### Results

#### Presudy examinations

When dogs were initially examined, all had substantial dental plaque, and some had loose teeth. Seven days before dogs received the unsupplemented diet for the first time and 95 days before the prestudy collection of feces, dogs were anesthetized, dental plaque removed, and loose teeth extracted. Dogs received amoxicillin (22 g/kg of body weight, PO, q 12 h) the day before and the day after dental prophylaxis. Other than those 4 doses of amoxicillin, dogs received no antibacterial drugs or steroids for the duration of the study.

Three dogs had marginally low PCV, and 1 had a moderately high serum alkaline phosphatase activity (478 U/L; reference range, < 130 U/L). These tests were not repeated. Serum IgA concentrations were between 24 and 40 mg/dl (reference range, 30 to 120 mg/dl). No parasites or parasite ova were detected by microscopic examinations of fecal flotations. There were no obvious contraindications for inclusion of any dog in the study. Additional fecal floatation examinations performed during the study failed to detect parasites, ova, or cysts.

One dog in group B was treated with chlorhexidine aural flushes and petrolatum ophthalmic ointment twice a day for persistent otitis externa and keratoconjunctivitis sicca. The ophthalmic ointment was begun 1 year before the first study collection period and continued throughout the study. The aural flushes began 17 days after the first study collection period and continued throughout the study. One dog in group A had a mammary mass that was surgically removed 8 months before the first study collection period.
samples ranged from $10^7$ to $10^9$ CFU/g of feces. Median concentration of Escherichia coli in fecal samples ranged from $10^4$ to $10^8$ CFU/g of feces.

Lactobacillus spp were isolated from 12 of 18 fecal samples obtained at study collection periods 1, 2, and 3. Only 2 dogs consistently had Lactobacillus spp isolated from feces. Median concentration of Lactobacillus spp in fecal samples ranged from $<10^4$ to $1.5 \times 10^8$ CFU/g of feces.

Escherichia coli was isolated from 17 of 18 fecal samples obtained at study collection periods 1, 2, and 3. Escherichia coli was not grown from 1 fecal sample from study collection period 3. Concentration of E. coli in fecal samples ranged from $<10^4$ to $1.5 \times 10^8$ CFU/g of feces.

Bifidobacterium spp were isolated from feces of only 2 of 6 dogs. This bacteria was isolated from 1 of 6 fecal samples obtained at the pre-study collection period and from 4 of 18 fecal samples during study collection periods 1, 2, and 3 (ie, 2 dogs each had Bifidobacterium spp isolated twice). Concentrations of Bifidobacterium spp in these 4 samples ranged from 0.03 to $2.26 \times 10^8$ CFU/g of feces. Bifidobacterium spp ($0.8 \times 10^8$ CFU/g of feces) was isolated from the pre-study fecal sample from 1 of these 2 dogs but was never isolated from feces of the 4 remaining dogs.

Clostridium spp were isolated from feces from each dog at every collection period. However, quantitative data could not be determined from all the fecal samples obtained at study collection periods 2 or 3, because plates were overgrown, and we were unable to accurately count individual colonies. Because plates prepared from feces collected at study collection period 3 were overgrown and there was time at the end of the experiment to repeat these cultures, Clostridium spp were isolated from feces obtained 56 days later (study collection period 4). Concentrations of Clostridium spp in all fecal samples obtained at study collection period 4 (median, $110 \times 10^6$ CFU/g of feces; range, $<10^4$ to $200 \times 10^6$ CFU/g of feces) did not significantly differ from concentrations in feces obtained at the pre-study collection period ($0.57 \times 10^6$ CFU/g of feces; range, 0.26 to $8 \times 10^6$ CFU/g of feces). However, in 5 of 6 dogs, median concentration of Clostridium spp was greater at the end of the study than at the beginning. Clostridium perfringens enterotoxin A was not detected in feces at any time.

Effect of diet on bacterial populations—Because Bifidobacterium spp and Lactobacillus spp were inconsistently isolated from fecal samples, statistical analysis of the effect of diet on their numbers was not considered reliable. The effects of diet on Clostridium spp, likewise, could not be meaningfully analyzed because of missing quantitative data from study collection periods 2 and 3.

Analysis of the number of Bacteroides spp isolated from fecal samples revealed that diet and time had no effect on fecal bacterial concentrations. However, there was a significant ($P = 0.002$) effect of sequence of diet on the number of Bacteroides spp isolated from feces. With the exception of 1 dog at study collection period 3, dogs in group A (ie, those that received the FOS-supplemented diet first) had higher fecal concentrations of Bacteroides spp than did dogs in group B at each study collection period. Fructooligosaccharide supplementation did not have a significant ($P = 0.07$) effect on fecal concentrations of E. coli (Table 2).

Discussion

Because all dogs had moderate to severe periodontal disease and dental calculus, teeth were cleaned and loose teeth removed before the study began. This was done for 2 reasons. First, human care of the dogs dictated that oral disease should not be allowed to progress. Second, cleaning the teeth would theoretically result in lower numbers of bacteria being swallowed, which may have an effect on number of bacteria in the colon. Although gastric acid should eliminate most bacteria that are swallowed, such bacteria do reach the colon, especially if protected by food, which is the basis of probiotic treatment.

Prior to study collection period 1, we were unable to isolate Bifidobacterium spp from the feces of most of the dogs, and we assumed our culturing technique for this genus was invalid. Thus, we delayed further collection of fecal samples and altered our culturing techniques until we felt confident that our inability to consistently isolate Bifidobacterium spp reflected the true prevalence of this genus in the large intestine of these dogs.

**Table 2—Median concentration (range) of Escherichia coli (colony-forming units $\times 10^7$/g of feces) in feces of 6 healthy adult Beagles fed an unsupplemented or FOS-supplemented diet**

<table>
<thead>
<tr>
<th>Collection period*</th>
<th>Dog No. 1</th>
<th>Dog No. 2</th>
<th>Dog No. 3</th>
<th>Dog No. 4</th>
<th>Dog No. 5</th>
<th>Dog No. 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study 1</td>
<td>0.038</td>
<td>2.4</td>
<td>5.4</td>
<td>3.8</td>
<td>2.6</td>
<td>8.8</td>
</tr>
<tr>
<td></td>
<td>(0.032–0.048)</td>
<td>(2–2.4)</td>
<td>(4.2–5.8)</td>
<td>(2.8–4.4)</td>
<td>(2.6–3.6)</td>
<td>(8.4–13.2)</td>
</tr>
<tr>
<td>Study 2</td>
<td>2.3</td>
<td>0.03</td>
<td>0.16</td>
<td>0.36</td>
<td>12</td>
<td>07</td>
</tr>
<tr>
<td></td>
<td>(1.4–3)</td>
<td>(0.032–0.032)</td>
<td>(0.036–0.164)</td>
<td>(0.32–0.4)</td>
<td>(6–40)</td>
<td>(0.0–0)</td>
</tr>
<tr>
<td>Study 3</td>
<td>0.8</td>
<td>4</td>
<td>0.16</td>
<td>0.36</td>
<td>12</td>
<td>07</td>
</tr>
<tr>
<td></td>
<td>(0.7–1)</td>
<td>(3.4–4.4)</td>
<td>(0.036–0.164)</td>
<td>(0.32–0.4)</td>
<td>(6–40)</td>
<td>(0.0–0)</td>
</tr>
</tbody>
</table>

*Study collection period 1, both groups fed an unsupplemented diet for 370 days. Study collection period 2, group A fed a FOS-supplemented diet and group B fed an unsupplemented diet for 28 to 29 days. Study collection period 3, group A fed an unsupplemented diet and group B fed a FOS-supplemented diet for 31 days. **Actual concentration was $< 10^7$ colony-forming units/g of feces.
Dogs that did not receive lactosugar. Finally, Hidaka et al. found that concentrations of Enterobacteriaceae were not significantly different from concentrations in German Shepherd Dogs fed lactosugar were not significantly different from concentrations in German Shepherd Dogs. Although our results are consistent with those of some investigators, a, b they contradict results of one study c that indicated Bifidobacterium spp were consistently isolated from feces of adult male Beagles. Similarly, we believe that our inability to consistently isolate Lactobacillus spp from feces of healthy dogs also reflects the prevalence of that genus in the intestinal tract. Thus, it may be difficult to predict the effects of specific nutrients or feed additives on colonic microflora, because the prevalence of at least some genera of bacteria in individual dogs varies from what is commonly expected.

Concentration of Clostridium spp in feces did not differ significantly over time. However, in 5 of 6 dogs, median concentration was greater at the end of the study, which was suggestive of an effect that we may not have been able to detect because of the limited number of dogs in the study. This difference in median concentration is consistent with our need to reculture Clostridium spp from feces collected at the third study collection period. It would have been interesting to continue the study to determine whether there was an effect of time on fecal concentrations of Clostridium spp independent of the effect of diet. Balish et al. reported that dogs housed in strict confinement appeared to have differences in fecal flora. Therefore, the housing conditions under which our dogs lived for > 300 days may have had an effect on the fecal concentrations of Clostridium spp.

The significant effect that sequence of feeding diets had on fecal concentrations of Bacteroides spp could reflect delayed effects of dietary changes, or it may be attributable to differences in the intestinal microflora between the 2 groups of dogs. Finding increased numbers of Bacteroides spp in feces from dogs in group A in all but one instance is suggestive of an intrinsic difference in the fecal and, hence, colonic concentrations of this bacteria between the 2 groups. Randomly assigning dogs to 2 groups does not ensure uniformity. The difference we found between the 2 groups seems likely a result of inherent differences between the dogs rather than an implication that a new diet must be fed for > 3 to 4 weeks before effects of prior diets on bacterial populations disappear. However, until this issue is resolved, it is best to feed a diet for a prolonged time prior to collection of feces for bacterial culture to ensure that there are no remaining effects of the previous diet. Our dogs were fed each diet for at least 28 days before we collected feces. We chose this duration because other investigators reported changes in fecal concentrations of Bifidobacterium spp and Lactobacillus spp within 7 to 14 days, respectively, of changing diet. d, e

Other investigators have also reported that fecal concentrations of E coli did not decrease when dogs ate a FOS-supplemented diet. Ogata a found that fecal concentrations of Enterobacteriaceae were not affected by feeding dogs a commercial mixture of FOS. Terada et al. found that fecal concentrations of E coli in German Shepherd Dogs fed lactosugar were not significantly different from concentrations in German Shepherd Dogs that did not receive lactosugar. Finally, Hidaka et al. stated that dogs fed FOS had fewer Enterobacteriaceae, but specific concentrations were not given in that report. Differences in E coli concentrations between groups in our study were not significant (P = 0.07) but may have been had we used more dogs.

In a recent report, feeding a FOS-supplemented diet to cats was associated with decreased numbers of E coli in their feces.14

Prebiotics may be potentially beneficial for people and animals.10,12,21 However, to expect useful effects of prebiotics, intestinal tract microflora should be at least somewhat predictable. Our data and those of others a, b suggest that, at least in dogs, this is not always the case for Bifidobacterium spp. Bifidobacterium spp have been inconsistently isolated from fecal samples of healthy adult dogs in Japan and the United States. This is potentially important, because Bifidobacterium spp are reported to be beneficial colonic bacteria a, b and prebiotic treatment has often been designed specifically to increase their numbers. However, FOS supplementation had no effect on our ability to isolate Bifidobacterium spp from feces of these healthy dogs. Our results contradict those from studies in humans in which FOS supplementation resulted in increased colonic concentrations of Bifidobacterium spp.13,14,21

If our findings are reproducible, then several points must be considered. First, our failure to see an effect of diet on Bifidobacterium spp might be attributable to the amount of FOS added to the diet. Adding more FOS may have resulted in an effect on fecal concentrations of Bifidobacterium spp. Second, if FOS supplementation has a prebiotic effect primarily as a result of increasing colonic and, thus, fecal concentrations of Bifidobacterium spp, then such dietary manipulations might not be effective in all dogs, especially older dogs. Finally, even if FOS, regardless of dose, does not alter colonic populations of Bifidobacterium spp in dogs, dietary FOS supplementation could induce clinically beneficial effects if it alters other bacterial populations. Therefore, studies evaluating dogs with gastrointestinal tract disorders are needed to determine the clinical usefulness of FOS supplementation.

If FOS or other dietary substances are found to have prebiotic effects in dogs, then it may be possible to prevent or control various gastrointestinal tract maladies by feeding appropriately supplemented diets instead of administering drugs. Such therapeutic benefits have been realized in people with gastrointestinal tract disease (ie, antibiotic-associated colitis,15 rotavirus diarrhea16) treated with probiotics, and successful probiotic treatment suggests that prebiotic treatment may also be useful. If effective prebiotics can be identified, their value may lie as much in preventing alimentary tract diseases as in treating already existing ones.

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2 The IAMS Co, Lewisburg, Ohio.
3 Edge Biologicals, Memphis, Tenn.
4 Vitek, bioMerieux Vitek Inc, Hazelwood, Mo.
5 BBL, Cockeysville, Md.
References


