Effect of feeding a selected combination of galacto-oligosaccharides and a strain of *Bifidobacterium pseudocatenulatum* on the intestinal microbiota of cats

Giacomo Biagi, PhD; Irene Cipollini, PhD; Alessio Bonaldo, PhD; Monica Grandi, PhD; Anna Pompei, PhD; Claudio Stefanelli, PhD; Giuliano Zaghini

**Objective**—To evaluate the growth kinetics of a strain of *Bifidobacterium pseudocatenulatum* (BP) on 4 oligo- or polysaccharides and the effect of feeding a selected probiotic-prebiotic combination on intestinal microbiota in cats.

**Animals**—10 healthy adult cats.

**Procedures**—Growth kinetics of a strain of cat-origin BP (BP-B82) on fructo-oligosaccharides, galacto-oligosaccharides (GOS), lactitol, or pectins was determined, and the combination of GOS and BP-B82 was selected. Cats received supplemental once-daily feeding of 1% GOS–BP-B82 (10^10 CFUs/d) for 15 days; fecal samples were collected for analysis the day before (day 0) and 1 and 10 days after the feeding period (day 16 and 25, respectively).

**Results**—Compared with the prefeeding value, mean fecal ammonia concentration was significantly lower on days 16 and 25 (288 and 281 µmol/g of fecal dry matter [fDM], respectively, vs 353 µmol/g of fDM); fecal acetic acid concentration was higher on day 16 (171 µmol/g of fDM vs 132 µmol/g of fDM). On day 16, fecal concentrations of lactic, n-valeric, and isovaleric acids (3.61, 1.52, and 3.65 µmol/g of fDM, respectively) were significantly lower than on days 0 (5.08, 18.4, and 6.48 µmol/g of fDM, respectively) and 25 (4.24, 17.3, and 6.17 µmol/g of fDM, respectively). A significant increase in fecal bifidobacteria content was observed on days 16 and 25 (7.98 and 7.52 log10 CFUs/g of fDM, respectively), compared with the prefeeding value (5.63 log10 CFUs/g of fDM).

**Conclusions and Clinical Relevance**—Results suggested that feeding 1% GOS–BP-B82 combination had some positive effects on the intestinal microbiota in cats (*Am J Vet Res* 2013;74:90–95).

Organisms that compose the intestinal microbiota have an important influence on the health of mammals by modulating the immune system and providing pathogen resistance. Among the dietary strategies that can be used to modulate intestinal microbiota in humans and other animals, probiotics (live microorganisms administered to colonize the large intestine and confer physiological benefits to the recipient), prebiotics (food or dietary supplement products that selectively stimulate activity or growth of beneficial bacteria already residing in the intestines), and symbiotics (probiotic-prebiotic combinations that have a synergistic intestinal health benefit) have gained attention. Because of their beneficial effects, bifidobacteria have been studied in humans and other animals, including calves and nonruminants such as pigs. Among prebiotics, GOS have attracted particular attention because they are present in human milk and have an enhancing effect on the growth of bifidobacteria. Recently, the characterization of feline intestinal microbiota has been considerably refined, revealing that cats host a variety of bacterial populations.

**ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
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<tr>
<td>FDM</td>
<td>Fecal dry matter</td>
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<tr>
<td>FOS</td>
<td>Fructo-oligosaccharides</td>
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<td>GOS</td>
<td>Galacto-oligosaccharides</td>
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<td>OD</td>
<td>Optical density</td>
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<td>SCFA</td>
<td>Short-chain fatty acids</td>
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From the Department of Veterinary Medical Sciences, University of Bologna, 40064 Ozzano Emilia, Italy (Biagi, Cipollini, Bonaldo, Grandi, Zaghini); and the Departments of Pharmaceutical Sciences (Pompei) and Biochemistry (Stefanelli), University of Bologna, 40100 Bologna, Italy.

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Address correspondence to Dr. Biagi (giacomo.biagi@unibo.it).
including bifidobacteria. Moreover, it is known that the composition of feline intestinal microbiota can be influenced by the administration of a probiotic Lactobacillus strain. However, little is known about the effect of bifidobacteria strains in association with prebiotics on intestinal microbiota in cats. The objective of the study reported here was to evaluate in vitro the growth kinetics of a strain of Bifidobacterium pseudocatenulatum (previously isolated from an adult healthy cat) on 4 oligo- or polysaccharides and investigate the effect of feeding 1 selected probiotic-prebiotic combination on the composition and metabolism of intestinal microbiota in adult cats.

Materials and Methods

Isolation of the probiotic strain—Within 30 minutes after collection from a litter box, fecal samples from 6 healthy adult cats (European domestic shorthair cats [between 1 and 6 years of age] fed various commercial dry diets and living in various environments) were each homogenized and serially diluted in an enrichment broth for anaerobic bacteria supplemented with L-cysteine HCl (0.5 g/L). Dilutions were plated on a selective medium for bifidobacteria containing raffinose, and plates were incubated in an anaerobic cabinet under an 85% N₂, 10% CO₂, and 5% H₂ atmosphere at 37°C for 48 hours.

The taxonomy of the colonies isolated on selective raffinose-Bifidobacterium plates was determined at the genus level on the basis of fructose-6-phosphate phosphoketolase activity and confirmed by amplification with the 16S rDNA primer set Bif164/Bif662. Species attribution of the colonies was achieved with an automated ribotyping device, and the speciation was confirmed by rDNA sequence analysis, as described by other authors.

After speciation, one of the isolated strains (B82) of the species B. pseudocatenulatum was used because it was the only Bifidobacterium strain to be recovered at concentrations > 10⁵ CFUs/g of feces (B82 was recovered in the feces of only 1/6 cats from which samples were collected). Bifidobacterium pseudocatenulatum B82 was subcultured on an enrichment broth for lactic bacteria containing 0.5 g of L-cysteine HCl/L and anaerobically incubated at 37°C for 24 hours. Cells from the broth cultures were inoculated (5% [vol/vol]) into the following complex medium: soy peptone (10 g/L), casein hydrolysat (10 g/L), sodium acetate (2.5 g/L), yeast extract (10 g/L), polysorbate 80 (1 mL/L), L-cysteine HCl (0.5 g/L), MnSO₄•7H₂O (7 mg/L), KH₂PO₄ (0.15 g/L), MgSO₄•7H₂O (0.5 g/L), and glucose (20 g/L); the pH was adjusted to 6.8. After incubation at 37°C in anaerobic conditions for 48 hours, the biomass was harvested via centrifugation, resuspended in a preservation suspending fluid (skim milk [50 g/L], lactose [30 g/L], yeast extract [50 g/L], and ascorbic acid [5 g/L]; pH adjusted to 7.0), frozen at −80°C, and freeze dried.

Concentration of the B. pseudocatenulatum strain in the final freeze-dried product was assessed by plating different dilutions on a selective medium for bifidobacteria containing raffinose, according to the method described. The identity of colonies was confirmed via optical microscopy. The freeze-dried probiotic contained approximately 10⁸ CFUs/g. The purity of the bacterial culture was confirmed via optical microscopy and by culturing the organisms on selective media for Clostridium perfringens, coliforms, and enterococci. Plates for coliforms and enterococci were incubated aerobically at 37°C for 24 and 48 hours, respectively; plates for C. perfringens were incubated anaerobically at 37°C for 48 hours.

Selection of the prebiotic—The growth kinetics of B. pseudocatenulatum B82 was determined on glucose and each of 4 oligo- or polysaccharides substrates: GOS (whey product containing 28.5% GOS and 36% lactose), FOS (from partially hydrolyzed inulin from chicory; extent of polymerization between 3% and 7%), lactitol, and pectins (from citrus fruit; extent of esterification between 70% and 74%).

Carbohydrate fermentation by B. pseudocatenulatum B82 was investigated in an enrichment broth for lactic bacteria. The sugar-free broth was autoclaved for 30 minutes at 120°C. Carbohydrates were filter-sterilized (0.22 µm) and added to the sterile medium to achieve a concentration of 10 g/L. Finally, the sterile medium was dispensed into 10-mL tubes and inoculated with B. pseudocatenulatum B82. Sugar-free broth was used as a negative control. During incubation of the tubes for 24 hours at 37°C, bacterial growth was monitored by measuring changes in turbidity every 2 hours with a spectrophotometer (OD, 600 nm). All fermentations were performed in triplicate.

Cats and feeding trial—The feeding trial was conducted with client-owned cats that were regularly vaccinated and periodically treated for intestinal parasites; cats had no clinical signs of gastrointestinal tract disorders during the previous 12 months. Before the trial started, consent was obtained from each owner. The experimental protocol was reviewed and approved by the Ethical Committee of the University of Bologna.

Ten adult European domestic shorthair indoor-living cats that were between 1 and 6 years old were included in the study. The mean ± SD weight of the cats was 4.9 ± 1.5 kg. Each cat had received a prebiotic- and probiotic-free diet for at least 1 month prior to commencement of the feeding trial.

For the 15-day feeding trial, cats remained at their homes and were fed by their owners; cats received a commercial premium dry food (5% moisture, 31% crude protein, 14% ether extract, 1.4% crude fiber, and 7% ash) that was based on cereals, meat and meat products (minimum, 28% chicken meat), vegetable protein extracts, oils, fats, and egg products. Moreover, once a day, half of a 50-g can of commercial wet food (86% moisture, 11% crude protein, 0.9% ether extract, 0.1% crude fiber, and 1% ash) that was based on tuna (Euthynnus affinis) with no addition of gelling agents was offered to each cat. The commercial diets were chosen on the basis of their low soluble fiber content. After a 10-day period of adaptation to the base diet, cats received a probiotic-prebiotic combination consisting of 0.1 g of the freeze-dried B. pseudocatenulatum B82 strain (corresponding to 10⁸ CFUs/d) and GOS (1% of the diet) for 15 days. Commencing on day 1, the probiotic-prebiotic combination was fed as a single daily oral
supplement mixed with the wet food. The daily food amount for each cat was calculated on the basis of the energy content of the experimental diet and daily energy requirements, according to the recommendation for moderately active adult cats: 60 kcal/kg of body weight.16 Fecal samples were collected for each cat on the day before administration of the probiotic-prebiotic combination started (day 0) and again at 1 and 10 days after treatment ceased (days 16 and 25, respectively); feces were collected from the litter box used by each cat immediately after excretion and frozen within 20 minutes. Only feces excreted during the first evacuation on sample collection days were collected for evaluation.

Chemical and microbiological analyses—Fecal moisture and food chemical analyses were performed according to the Association of Official Analytical Chemists standard methods (method 950.46 for water content, method 954.01 for crude protein, method 920.39 for ether extract, method 962.09 for crude fiber, and method 942.05 for ash).17 For each fecal sample, fecal pH was determined after diluting samples 1:10 with distilled water. The fecal ammonia concentration was measured with a commercial kit.18 For SCFA analysis, fecal samples were homogenized, diluted 1:2 with distilled water, deproteinized with perchloric acid, and analyzed via gas chromatography.19 For the determination of concentrations of polyamines, fecal samples were diluted 1:5 with perchloric acid; polyamines were later separated by high-performance liquid chromatography and quantified via fluorometry, according to the method proposed by other authors.20

Within 10 days after collection, fecal specimens were homogenized, serially diluted with a half-strength enrichment broth for anaerobic bacteria,21 and plated in triplicate onto selective media for C perfringens,22 coliforms,23 and enterococci.24 Plates for coliforms and enterococci were incubated aerobically at 37°C for 24 and 48 hours, respectively; plates for C perfringens were incubated anaerobically at 37°C for 48 hours.

A fluorescence in situ hybridization technique was used to determine counts of Bacteroides spp, bifidobacteria, and lactobacilli. For this purpose, ready-to-use commercial kits containing specific fluorescein isothiocyanate–labeled probes for the enumeration of Bacteroides spp, Bifidobacterium spp, and Lactobacillus spp were used.25 The slides were evaluated with an epifluorescence microscope equipped with a fluorescein isothiocyanate–specific filter.26

Statistical analysis—For each variable of interest, the mean of findings for all cats at each time point was calculated. A modified Gompertz bacterial growth model27 was used to fit data from the in vitro fermentation of different carbohydrates as described;28 turbidity data were analyzed with a 1-way ANOVA. Differences among variable means were analyzed by use of the Student-Newman-Keuls test.29 Results at days 0, 16, and 25 were analyzed via 1-way ANOVA for repeated measurements, with time as the main factor; differences among variable means were analyzed via the Student-Newman-Keuls test.30 Differences were considered significant at values of P < 0.05.

Results

Selection of the prebiotic—Growth curves of B pseudocatenulatum B82 on glucose, GOS, FOS, lactitol, pectins, and sugar-free broth (control) were determined (Figure 1). After 24 hours, turbidity (OD at 600 nm) was higher (P < 0.001) for glucose and GOS (2.80 and 2.76, respectively) than for the other substrates (1.90, 0.75, and 0.47 for FOS, lactitol, and citrus pectins, respectively). The maximum growth rate, which occurs at the point of inflection of the growth curve, was higher with GOS (0.60/h; P < 0.001) than with glucose (0.36/h), FOS (0.24/h), lactitol (0.15/h), or pectins (0.07/h). On the basis of these results, it was decided to use GOS as the prebiotic in the feeding trial with cats.

Feeding trial—All 10 cats remained in good health during the administration of the GOS–B pseudocatenulatum B82 combination. Mean fecal moisture and pH were not influenced by feeding the probiotic-prebiotic combination to the cats (data not shown); values were 560 g/kg and 6.64, respectively. Fecal concentrations of ammonia, SCFA, and polyamines before the start (day 0) and at 1 and 10 days after cessation of feeding the probiotic-prebiotic combination (days 16 and 25, respectively) were determined (Table 1). Ammonia concentration was significantly lower on days 16 and 25 (288 and 281 µmol/g of IDM, respectively) than before the start of the trial (33 µmol/g of IDM). Fecal concentration of acetic acid was significantly higher on day 16 (171 µmol/g of IDM) than before the start of the trial (132 µmol/g of IDM). On day 16, fecal concentrations of n-valeric, isovaleric, and isovaleric acids (3.61, 1.52, and 3.55 µmol/g of IDM, respectively) were significantly lower than on days 0 (5.08, 18.4, and 6.48 µmol/g of IDM, respectively) and 25 (4.24, 17.3, and 6.17 µmol/g of IDM, respectively). Concentrations of polyamines were not influenced by the probiotic-prebiotic treatment.

Fecal counts of bifidobacteria, enterococci, C perfringens, and coliforms before the start (day 0)
Table 1—Mean concentrations (µmol/g of FDM) of ammonia, SCFA, and polyamines in the feces of 10 cats collected before (day 0) and 1 (day 16) and 10 days (day 25) after 15-day administration of a probiotic-prebiotic combination (10^9 CFUs/d of Bifidobacterium pseudocatenulatum B82 strain and GOS at 1% of daily dietary ration).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Day 0</th>
<th>Day 16</th>
<th>Day 25</th>
<th>Pooled SEM</th>
<th>ANOVA P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonia</td>
<td>353^a</td>
<td>288^b</td>
<td>281^a</td>
<td>16.8</td>
<td>0.013</td>
</tr>
<tr>
<td>SCFA: Acetic acid</td>
<td>132^a</td>
<td>171^b</td>
<td>151^a</td>
<td>9.101</td>
<td>0.021</td>
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<tr>
<td></td>
<td>67.4</td>
<td>68.8</td>
<td>65.2</td>
<td>7.231</td>
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<td></td>
<td>5.31</td>
<td>4.17</td>
<td>5.35</td>
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<td>0.270</td>
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<td></td>
<td>35.5</td>
<td>39.3</td>
<td>41.9</td>
<td>4.250</td>
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<tr>
<td></td>
<td>6.48</td>
<td>3.55</td>
<td>6.17</td>
<td>0.738</td>
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</tr>
<tr>
<td></td>
<td>18.4</td>
<td>1.52</td>
<td>17.3</td>
<td>2.045</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>5.08</td>
<td>3.61</td>
<td>4.24</td>
<td>0.231</td>
<td>0.004</td>
</tr>
<tr>
<td>Polyamine: Cadaverine</td>
<td>1.92</td>
<td>2.14</td>
<td>2.44</td>
<td>0.358</td>
<td>0.595</td>
</tr>
<tr>
<td></td>
<td>1.16</td>
<td>1.27</td>
<td>1.52</td>
<td>0.270</td>
<td>0.635</td>
</tr>
<tr>
<td></td>
<td>0.18</td>
<td>0.22</td>
<td>0.26</td>
<td>0.041</td>
<td>0.397</td>
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<tr>
<td></td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.001</td>
<td>0.152</td>
</tr>
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<td></td>
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</tr>
</tbody>
</table>

Figure 2—Mean ± SEM bacterial counts (log₁₀ CFUs/g of FDM) in the feces of 10 cats before (day 0) and 1 (day 16) and 10 days (day 25) after administration of a probiotic-prebiotic combination (10^9 CFUs/d of B. pseudocatenulatum B82 strain and GOS at 1% of daily dietary ration) for 15 days. *Bifidobacteria counts are expressed as log₁₀ cells per gram of FDM. **Within the bifidobacteria population, means without a common letter differ significantly (P < 0.001).

Discussion

In 1 study, B. pseudocatenulatum isolated from humans had excellent properties in terms of ability to survive under gastroenteric conditions. In another study, B. pseudocatenulatum had an inhibitory effect on the transfer of the gene responsible for β-lactam antimicrobial resistance among Enterobacteriaceae. Moreover, the lack of toxic effects observed when a strain of B. pseudocatenulatum was fed at relevant doses to mice, together with the high concentrations of B. pseudocatenulatum B82 detected in the cat feces collected to obtain a suitable organism for the experiment initially in the study reported here (≥10⁸ CFUs/g of feces), justified our choice to use this strain for the feeding trial with cats.

The high growth rate of the B. pseudocatenulatum B82 strain on GOS, compared with growth rates on the other evaluated substrates, during the selection of the probiotic was consistent with the findings of other researchers who have confirmed the bifidogenic properties of this particular oligosaccharide in vitro.

In the present study, water content of the cats’ feces was not affected by administration of the probiotic-prebiotic combination. It is known that diet supplementation with nondigestible oligosaccharides may result in increased fecal moisture in both dogs and cats. However, results of recent studies in cats have indicated that fecal moisture is not affected by diets supplemented with FOS (4%) and FOS or GOS (0.5%), which is in accordance with results of the study reported here.

A reduction in fecal ammonia concentration was observed both at the end of the probiotic-prebiotic supplementation period (day 16) and 10 days afterward (day 25), compared with the value before the beginning of that feeding trial. Ammonia is a toxic and potentially carcinogenic compound produced by proteolytic bacteria, and if present at relevant concentrations, it can seriously damage the enteric mucosa. The reduction in fecal ammonia concentration might have been a consequence of partial inhibition of microbial putrefactive activity by the probiotic-prebiotic combination. Although this latter hypothesis was also supported by the observed reduction in fecal concentration of isovaleric acid, another proteolytic catabolite, inhibition of proteolytic bacterial populations was not confirmed by other fecal variables such as counts of C. perfringens and Bacteroides spp, pH, or polyamine concentrations. These results are in accordance with the findings of another study, in which the investigators did not observe any fecal acidification or modulation of polyamine and SCFA contents after feeding cats with GOS at 0.5% of their daily dietary intake. Regardless, reduction in fecal ammonia concentration still persisted 10 days after withdrawal of the probiotic-prebiotic supplementation in the study reported here.

For the cats of present study, fecal acetic acid concentration increased (30%) as a result of 15-day administration of the probiotic-prebiotic combination. This finding is in agreement with that of other authors.
who reported that acetic acid was one of the most important metabolites derived from GOS fermentation by *Bifidobacterium* spp. Administration of the probiotic-prebiotic combination also resulted in lower fecal lactic acid concentration (~29%). Recently, an in vitro study involving canine fecal inoculum and a combination of GOS and *Bifidobacterium bifidum* revealed an increase in lactic acid concentration after the first 10 hours of fermentation, followed by a decrease thereafter and a concomitant increase in the butyrate concentration; this lead us to hypothesize that there was a conversion of lactate into butyrate by specific enteric butyrate-producing bacteria, an opinion expressed by other authors. In the study reported here, the fecal butyrate concentration did not increase as a result of probiotic-prebiotic treatment; thus, we do not have an explanation for the lower lactate concentration that was evident after treatment. However, it is well-known that the concentrations of bacterial metabolites that are able to cross the intestinal mucosa can vary while digesta move along the intestinal tract. Therefore, feces might not reflect the changes in the concentration of SCFA and other molecules (eg, polypeptides) that the probiotic-prebiotic combination may have induced in the colon.

Mean fecal *Bifidobacterium* spp counts were significantly higher on days 16 and 25, compared with the count before the start of the feeding trial. Unfortunately, the experimental protocol did not allow us to determine the specific contributions of the probiotic strain and GOS. In an in vitro study involving canine fecal inoculum, the incubation of GOS and *B bifidum* was observed to have a synergetic effect on the fecal concentration of bifidobacteria. However, other authors detected only a minor increase in fecal bifidobacteria count in cats receiving GOS at 0.5% of their dietary ration. Because recovery of the probiotic strain in the feces of the cats of the study reported here was not attempted, survival of those organisms through the gastrointestinal tract cannot be confirmed. Nevertheless, on the basis of the high survival rate of *B pseudocatenulatum* under physiologic gastrointestinal conditions, it seems reasonable to suppose that the *B pseudocatenulatum* B82 strain fed to the cats of the study reported here contributed to increases in the number of fecal bifidobacteria. Interestingly, the synergetic effects of administration of the GOS–*B pseudocatenulatum* B82 strain (ie, lower fecal ammonia concentration and higher fecal bifidobacteria count, compared with prefeeding trial values) were still present 10 days after cessation of treatment. This result was unexpected and not in accordance with the findings of most other researchers, who did not observe any persistent effects on human and other animal fecal microbiota after the suspension of probiotic or symbiotic administration. Therefore, the *B pseudocatenulatum* B82 strain appeared to temporarily colonize the intestinal environment in the cats of the study reported here.

Although the present study of cats had some limitations (eg, the short period of adaptation to the base diet and lack of assessment of intestinal survival of the probiotic strain), the results have suggested that some positive effects on feline intestinal ecosystem were exerted by the ingested probiotic-prebiotic combination. In particular, the reduction in fecal ammonia concentration and the increased fecal bifidobacteria count might help promote intestinal health in animals that are fed this probiotic-prebiotic combination.

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