WALTHAM International Science Symposium: Nature, Nurture, and the Case for Nutrition

Bacteria in the Gut: Friends and Foes and How to Alter the Balance[1]

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ABSTRACT The activities of the bacteria resident in the colon of companion animals can have an impact upon the health of the host. Our understanding of this microbial ecosystem is presently increasing due to the development of DNA-based microbiological tools that allow identification and enumeration of nonculturable microorganisms. These techniques are changing our view of the bacteria that live in the gut, and they are facilitating dietary-intervention approaches to modulate the colonic ecosystem. This is generally achieved by the feeding of either live bacteria (probiotics) or nondigestible oligosaccharides (prebiotics) that selectively feed the indigenous probiotics. Feeding studies with a *Lactobacillus acidophilus* probiotic have shown positive effects on carriage of *Clostridium* spp. in canines and on recovery from *Campylobacter* spp. infection in felines. Immune function was improved in both species. Prebiotic feeding studies with lactosucrose and fructo-oligosaccharides in both cats and dogs have shown positive effects on the microflora balance. Recently synbiotic forms (a probiotic together with a prebiotic) targeted at canines have been developed that show promise as dietary-intervention tools. J. Nutr. 134: 2022S–2026S, 2004.

KEY WORDS: • *bacteria* • *colonic microflora* • *probiotics* • *prebiotics* • *Bifidobacterium* • *Lactobacillus*

Introduction

The link between the barrier function of colonic microflora and susceptibility to disease (1) is an area of great interest. This has led to a vibrant, global, functional food industry that is introducing new products for gut health into markets targeted to humans and companion animals.

The gut microflora

Most of our knowledge of gut microflora comes from studies on humans (2). Microbiologically, the gut can be thought of in terms of three principal regions: the stomach, small intestine, and colon. In terms of microbial population, the stomach has very low bacterial numbers; facultative anaerobes such as lactobacilli, streptococci, and yeast are present at ~100 colony-forming units (CFU)[3] per milliliter due to the low environmental pH.[3] The small intestine has a larger bacterial load that consists of facultative anaerobes such as lactobacilli, streptococci, and enterobacteria as well as anaerobes such as *Bifidobacterium* spp., *Bacteroides* spp., and clostridia at levels of $\sim 10^4$–10^8 CFU/ml. The most heavily colonized region, however, is the colon, with a total population of 10^{11}–10^{12} CFU/ml of contents (3). The colonic microflora is the predominant target for dietary intervention in the gut ecology, and it is this region that is the subject of this article. Consisting of higher levels of obligate anaerobes and lower levels of facultative aerobes (**Fig. 1**), the colonic microflora is very complex.

The colonic microflora is dominated by strict anaerobes such as *Bacteroides* spp., the clostridia and other families within the *Clostridium* mega-genus (including *Ruminococcus* spp., *Butyrovibrio* spp., *Fusobacterium* spp., *Eubacterium* spp., and *Peptostreptococcus*), *Bifidobacterium* spp., *Atopobium* spp., and the peptococci. Facultative anaerobes occur in numbers ~1000-fold lower and include lactobacilli, enterococci, streptococci, and *Enterobacteriaceae*. Yeasts are present only at relatively low numbers of 10^2–10^4 CFU/ml (4).

In terms of health, the most significant organisms are believed to be the bifidobacteria (4). Bifidobacteria are the major component of the microbial barrier to infection. Bifidobacteria produce a range of antimicrobial agents that are active against Gram-positive and -negative organisms (5). Lactobacilli are also health positive and produce a range of antimicrobial agents, but they are present in much lower levels in the human colon. In addition to producing antimicrobial agents, a large population of beneficial bacteria competitively excludes pathogens by occupying receptor sites and competing for space, nutrients, etc.

Much of the information presently available regarding colonic microflora comes from studies that employed classical microbiological techniques based on agar plates. This poses

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[3] Abbreviations used: CFU, colony-forming unit; FISH, fluorescent in situ hybridization; FOS, fructo-oligosaccharides; GI, gastrointestinal.

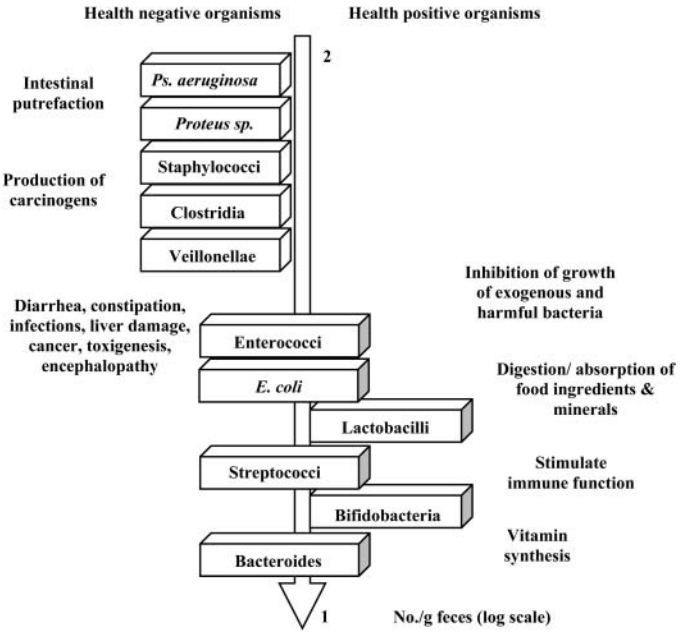

FIGURE 1 Overview of the human colonic microflora. Bacterial genera were classified as health positive, health negative, or health neutral. Bacterial enumeration was by selective media (4). Ps, *Pseudomonas*.

a problem, however, as colonic microflora are thought to contain a high level of biodiversity including many species that cannot be cultured using present techniques. This unculturable flora can only be characterized using DNA-based microbiology methods (6–8). Most of these methods rely on amplification, detection, and/or sequencing of diagnostic regions of 16S rRNA genes (8).

This culture problem is particularly acute in studies on canines. A recent study (9) illustrates the unreliability of apparently selective agar media for enumeration of canine fecal bacteria: many of the selective media used did not support the growth of the target population (**Table 1**).

Our image of the canine gut flora, then, is based largely on traditional methods of investigation (**Fig. 2**) (10). In one study (11), fluorescent in situ hybridization (FISH) was used to describe the flora on one Labrador dog (Fig. 2). The most significant aspect of the canine microflora is the much lower level of bifidobacteria found in canines than in other animals. The feline colonic flora (**Fig. 3**) is even less well characterized (12), and the bifidobacteria levels are probably even lower than in canines. In fact, bifidobacteria are only intermittently isolated from felines.

Dietary tools for changing the balance

Probiotics have been investigated as dietary management tools for many years (13) in human as well as livestock animal studies. The concept is that ingestion of beneficial bacteria leads to colonization of the gut with the added strain, and this then strengthens the gastrointestinal (GI) barrier to disease. Although bifidobacteria are the most significant health-positive organism in the colon, their obligate anaerobic nature has hindered commercial development. Most commercial probiotics are lactobacilli, and several species have been developed for application in humans and livestock animals (13).

There are few studies on probiotics in companion animals. One recent study (14) investigated the application of *Lactobacillus acidophilus* DSM 13241 in canines. This strain

TABLE 1

Selectivity of media used in canine colonic microflora studies[1]

Agar	Selective for	Organism identified, %
Nutrient	Total aerobes	98 *E. coli,* 2 *Str. bovis*
MacConkey	Coliforms	98 *E. coli,* 2 ruminal bacteria
Wilkins-Chalgren	Total anaerobes	60 *Collinsiella intestinalis* 27 *Pectinatus*-like sp. 11 *Streptococci bovis* 1 *E. coli* 1 *Rothia*-like sp.
Rogosa	Lactobacilli	65 *Str. bovis* 24 *L. animalis* 11 *L. ruminus*
Beerens	Bifidobacteria	67 *Str. bovis* 18 *L. ruminus* 15 *Staphylococcus epidermidis*
Azide	Gram-positive cocci	93 unknown 7 *Str. bovis*
Reinforced clostridial	Clostridia	80 *Staphylococcus* sp. 20 unknown
Bacteroides	Bacteroides	56 unknown, 33 *E. coli* 6 *Staph. haemolyticus* 5 *L. animalis*

[1] Fecal samples from 1 healthy adult Labrador dog. Microbial identification was performed by sequencing of the 16S rRNA genes (9).

was chosen on the basis of its growth characteristics, antimicrobial activity toward pathogens, and survival rate in gut models. Feeding of 2×10^9 CFU/d to 15 healthy dogs resulted in a significant increase in the population of recoverable lactobacilli in the feces with a concomitant decrease in the clostridia population (determined by FISH). The animals displayed no significant changes in blood biochemistry, body temperature, or fecal quality. Immune-function studies showed no significant changes in haptoglobin level or white blood cell

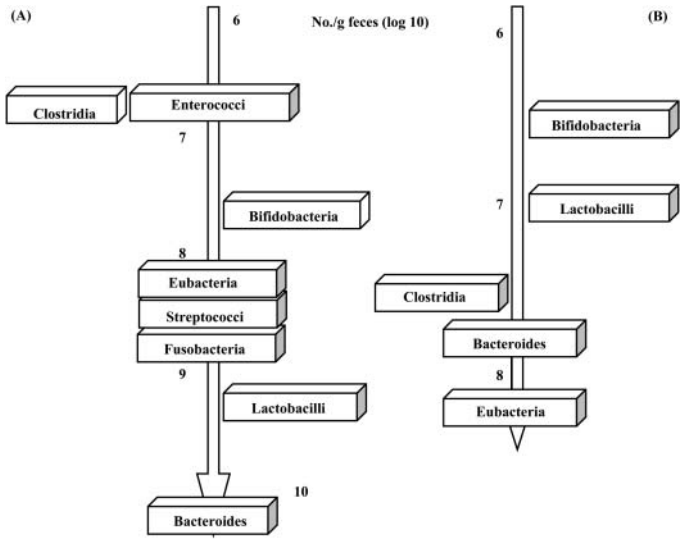

FIGURE 2 Overview of the canine colonic microflora. In a study of healthy adult Shepherd dogs (*A*), bacterial groups were enumerated by selective media and confirmed by denaturing gradient gel electrophoresis (10). In a study of one healthy adult Labrador dog (*B*), bacterial groups were enumerated by FISH (11).

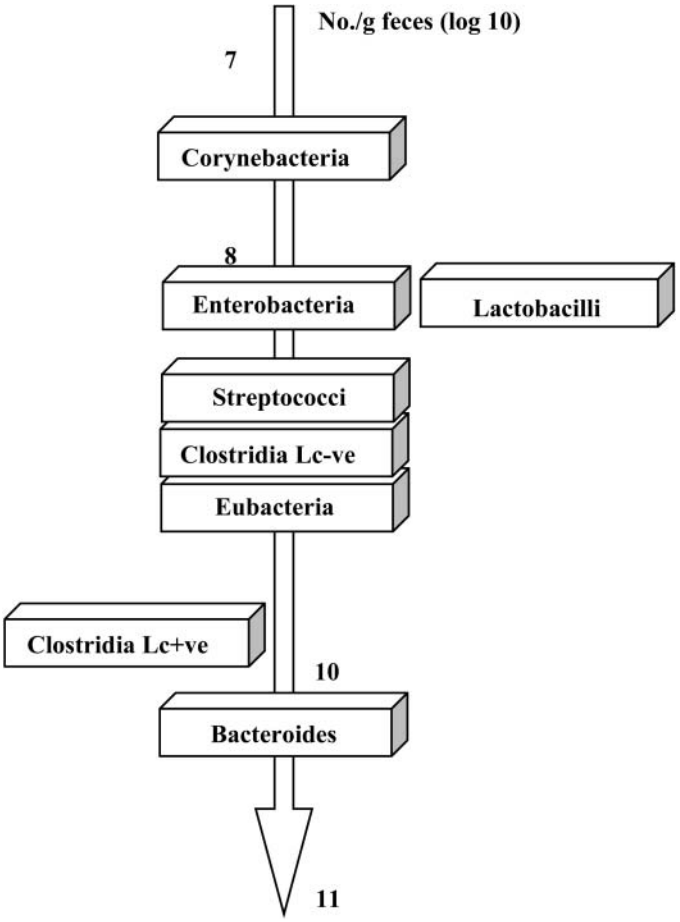

FIGURE 3 Overview of the feline colonic microflora in a study of 8 healthy adult cats. Bacterial enumeration was by selective media (12).

count but significant increases in serum IgG, monocytes, and neutrophils. Significant decreases in plasma nitric oxide levels and the osmotic fragility of red blood cells were observed. The researchers concluded that feeding of the probiotic resulted in positive changes in the gut microbiology and in systemic effects that suggested immune system stimulation as observed in humans after they consumed *Lactobacillus* spp. (13).

An attractive alternative to the feeding of probiotics is the use of prebiotics. A prebiotic is a nondigestible food ingredient that is selectively metabolized by the indigenous probiotic bacteria in the gut (4). Presently all prebiotics are carbohydrates (15), and a range of carbohydrates exist on the market around the world (**Table 2**).

Using prebiotics is attractive, because we can avoid the drawbacks of using probiotic bacteria such as maintaining viability. Prebiotics can thus be incorporated into a wider range of products and are stable to heat treatment.

There is relatively little work published on the use of prebiotics in companion animals (16). Most of the research to date has focused on lactosucrose and fructo-oligosaccharides (FOS). Studies on feeding of lactosucrose have been performed on dogs (17) and cats (12). Feeding of 1.5 g of lactosucrose/d to 8 healthy dogs for 2 wk resulted in statistically significant desirable changes to the gut flora as determined by fecal microbiological analysis (based on selective media). A 0.5-log increase in bifidobacteria was seen together with a 1.6-log decrease in clostridia levels. Decreases were also seen in toxin levels and fecal odor. Lactosucrose was also fed to 8 healthy cats at a level of 750 mg/d for 2 wk (12). This resulted in an increase

TABLE 2

Oligosaccharide prebiotics on the world market

Oligosaccharide
Lactulose[1]
Galacto-oligosaccharide
Fructo-oligosaccharide
Inulin
Isomalto-oligosaccharide[1]
Soybean oligosaccharide[1]
Lactosucrose
Gentio-oligosaccharide[1]
Xylo-oligosaccharide[1]

[1] Found only on the Japanese market (15).

in the incidence of recovery of bifidobacteria and a significant 0.9-log increase in lactobacilli numbers. Significant decreases of 0.4 log were seen with levels of clostridia and *Enterobacteriaceae*. Toxin levels and fecal odor were also reduced.

Several studies are available on FOS consumption in companion animals (16). A representative study (18) fed FOS at 4 g/d to 20 adult dogs. Fecal bacteriology was investigated by selective media, and bacterial metabolites were measured. Statistically significant increases in bifidobacteria (0.58 log) and lactobacilli (0.86 log) numbers were seen together with a small but significant decrease in clostridia level of 0.11 log. Increases were seen in lactate and butyrate quantities but increases were also observed in ammonia, isovalerate, dimethylsulfide, and hydrogen sulfide levels.

A study in cats (19) was performed by feeding a diet that contained 0.75% FOS for 12 wk to 12 adult cats. Bacteriology

TABLE 3

Growth rate of canine probiotics on various carbohydrates[1]

	Growth rate, μ $(\times 10)^1$		
Substrate	*L. acidophilus*	*L. mucosae*	*L. reuteri*
Actilight fructo-oligosaccharides[2]	5.58 ± 0.24	2.76 ± 0.27	9.90 ± 0.28
Biotose high-maltose syrup[3]	11.70 ± 0.67	8.10 ± 0.21	6.54 ± 0.30
Cellobiose	11.46 ± 0.63	7.62 ± 0.15	1.26 ± 0.19
Gentiobiose	4.74 ± 0.45	5.88 ± 0.16	10.26 ± 0.25
Glucose	12.06 ± 0.49	8.40 ± 0.24	6.90 ± 0.29
Isomalto-oligosaccharides	4.20 ± 0.69	6.12 ± 0.19	5.04 ± 0.21
Lactose	10.26 ± 0.55	8.28 ± 0.30	7.26 ± 0.29
Laevan	0.06 ± 0.03	0.06 ± 0.02	1.68 ± 0.17
Maltose	10.20 ± 0.37	6.96 ± 0.12	5.88 ± 0.26
Melezitose	0.78 ± 0.17	3.24 ± 0.17	1.86 ± 0.20
Melibiose	11.34 ± 0.42	7.98 ± 0.24	4.74 ± 0.25
Palatinose	9.48 ± 0.50	9.36 ± 0.17	9.06 ± 0.20
Panorich high-panose syrup[3]	8.34 ± 0.34	8.28 ± 0.19	5.82 ± 0.11
Raffinose	10.86 ± 0.46	7.68 ± 0.20	7.02 ± 0.27
Stachyose	7.44 ± 0.32	6.66 ± 0.19	5.76 ± 0.33
Sucrose	10.32 ± 0.48	6.84 ± 0.23	5.64 ± 0.27
Tagatose	0.06 ± 0.03	1.92 ± 0.27	3.84 ± 0.23
Xylo-oligosaccharides	4.14 ± 0.19	3.84 ± 0.18	7.50 ± 0.33
Xylan	0.96 ± 0.17	0.06 ± 0.03	4.44 ± 0.25

[1] Values are means ± SD of five replicates (11).
[2] Eridania Beghin-Say, Vilvoorde, Belgium.
[3] Nihon Shokuhin Kako, Tokyo, Japan.

TABLE 4

Antimicrobial activity of selected probiotics against GI pathogens[1]

	Inhibition zone (*diameter, mm*)								
	VTEC		EPEC			*S. enterica* serotype *typhimurium*			
Growth substrate	*L. mucosae*	*L. acidophilus*	*L. reuteri*	*L. mucosae*	*L. acidophilus*	*L. reuteri*	*L. mucosae*	*L. acidophilus*	*L. reuteri*
FOS[2]	NG	NG	—	NG	NG	6.5 ± 0.5	NG	NG	—
High maltose syrup[3]	NG	7.0 ± 0.3	4.2 ± 0.5	NG	5.3 ± 0.4	3.0 ± 0.2	NG	6.1 ± 0.5	7.1 ± 0.4
Cellobiose	—	—	NG	—	—	NG	3.8 ± 0.5	—	N/A
Gentiobiose	6.1 ± 0.6	—	—	5.0 ± 0.4	—	—	6.0 ± 0.3	3.2 ± 0.3	3.2 ± 0.4
Isomalto-oligosaccharides	1.4 ± 0.5	4.2 ± 0.5	4.2 ± 0.6	4.6 ± 0.4	—	—	—	1.7 ± 0.4	4.5 ± 0.9
Lactose	—	—	—	—	—	6.2 ± 0.5	—	4.3 ± 0.5	5.9 ± 0.9
Maltose	3.8 ± 0.7	—	6.8 ± 0.8	3.4 ± 0.5	—	1.3 ± 0.4	—	—	6.5 ± 0.7
Melibiose	—	—	—	—	—	5.6 ± 0.5	—	—	—
Palatinose	—	—	—	—	—	—	1.3 ± 0.4	1.0 ± 0.5	—
High panose syrup[3]	6.2 ± 0.4	—	—	5.0 ± 0.3	—	—	9.0 ± 0.4	—	—
Raffinose	6.8 ± 0.5	—	—	—	—	5.8 ± 0.7	3.4 ± 0.5	1.9 ± 0.5	—
Stachyose	—	—	NG	—	—	NG	—	—	N/A
Sucrose	—	1.3 ± 0.5	—	—	—	—	—	3.5 ± 0.4	—
Xylo-oligosaccharides	NG	NG	—	NG	NG	3.7 ± 0.5	NG	NG	—

[1] Inhibition zones around paper disks soaked in cell-free culture supernatants, pH adjusted to pH 7.00. Data are the mean ± SD of five replicates. NG, no or little growth on this substrate; N/A, not tested; —, no inhibition (11).
[2] Actilight, Eridania Beghin-Say, Vilvoorde, Belgium.
[3] Nihon Shokuhin Kako Co., Ltd, Tokyo, Japan.

was performed by selective media, and bacterial metabolites were measured. Only one isolation of *Bifidobacterium* sp. was made, but a significant increase in lactobacilli number was seen (0.22 log). Significant decreases in clostridia (1.47 log) and *Escherichia coli* (0.52 log) numbers and an increase in bacteroides level (0.56 log) were also noted.

The combination of a probiotic with a prebiotic to support its viability and activity has been termed a synbiotic (4). An exciting development in the field of companion animals is that of synbiotics targeted to particular species. This has been attempted for the first time with canine synbiotics. Five candidate lactobacilli, *L. acidophilus*, *L. murinus*, *L. reuteri*, *L. mucosae*, and *L. rhamnosus* were isolated from a Labrador dog (11). It is generally held in the context of human and livestock animal nutrition that probiotic strains should originate from the species in which they are to be used (13). Three of these strains, *L. mucosae*, *L. acidophilus*, and *L. reuteri*, were then evaluated (11) for their growth on various carbohydrates (**Table 3**) and antimicrobial activity (**Table 4**) against *Salmonella enterica* serotype Typhimurium, enteropathogenic *E. coli*, and the toxin-negative mutant of *E. coli* O157:H7. On the basis of these data, candidate synbiotic forms can be identified with activity against specific target pathogens (**Table 5**).

This canine synbiotic concept was taken further in an attempt to manufacture a prebiotic targeted to a particular probiotic organism. Most of the prebiotic oligosaccharides presently on the market are synthesized using enzymatic methods (15). For instance, galacto-oligosaccharides, which are a mixture of β-linked di- to pentasaccharides, are manufactured by the action of β-galactosidase on lactose. The enzyme catalyzes a glycosyl transfer reaction at high lactose levels and transfers galactose (Gal) from lactose, thereby acting

TABLE 5

Effective synbiotic combinations against GI pathogens[1]

		Pathogen		
Probiotic	Carbohydrate	Veritoxigenic *E. coli*	Enteropathogenic *E. coli*	*Salmonella enterica,* serotype Typhimurium
L. reuteri	Maltose	√	—	√
	Lactose	—	√	√
	Actilight fructo-oligosaccharides[2]	—	√	—
L. mucosae	Gentiobiose	√	—	√
	Panorich high-panose syrup[3]	√	—	√
	Raffinose	√	—	—
L. acidophilus	Biotose high-maltose syrup[3]	√	—	√

[1] Combinations yielded a zone of inhibition >6.00 mm.
[2] Eridania Beghin-Say, Vilvoorde, Belgium.
[3] Nihon Shokuhin Kako, Tokyo, Japan.

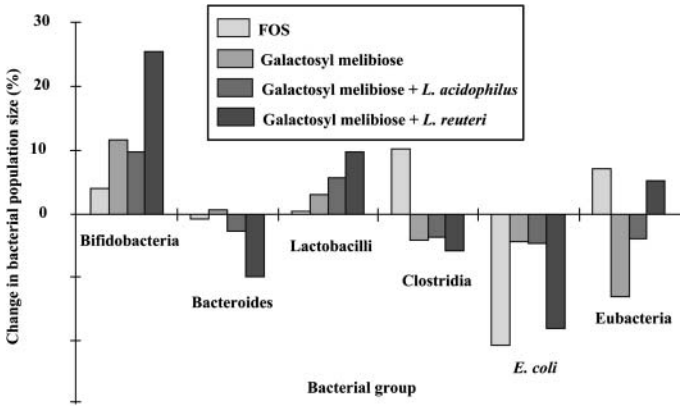

FIGURE 4 Fermentation properties of canine-targeted synbiotics. Bars represent percentage changes from the inoculum level in triplicate pH-controlled fecal batch cultures. Bacterial groups were enumerated by FISH (22).

as a glycosyl donor to other lactose molecules (which are acting as glycosyl acceptors) and thus building up higher oligosaccharide levels (15). This manufacturing technology can be modified to utilize enzymes from bifidobacteria on the assumption that the resultant products might have enhanced selectivity for the producing organism (20). This approach has been used in an attempt to develop highly targeted synbiotics for canine application. One of the canine probiotics discussed above, *L. reuteri*, was selected for further study: α-galactosidase enzyme activity was extracted from cultures of the organism and used to synthesize oligosaccharide mixtures from melibiose (Galα1 $\rightarrow$ 4 glucose) as a glycosyl donor (21). The resultant oligosaccharide mixtures were then evaluated in mixed fecal pH-controlled batch cultures (22). The oligosaccharides were found to be prebiotic in their own right and to act synergistically with added *L. reuteri* to a greater extent than with added *L. acidophilus* (**Fig. 4**). Interestingly, the synbiotic of *L. reuteri* and its synthesized oligosaccharides also stimulated bifidobacterial populations, presumably by inhibiting other species that inhibit the bifidobacteria.

Concluding remarks

The bacterial population within the GI tract of mammals constitutes a metabolically active organ that acts as a significant barrier to infection by exogenous pathogenic microorganisms. At present, our picture of human GI-tract ecology is far from complete, even less so for companion animals such as cats and dogs. Rapid development of new DNA-based methods is under way for studying the composition of complex microbial ecosystems such as the colonic microflora, and these have not yet been systematically applied to the study of companion animals. Such studies are required if we are to realize the potential of dietary manipulation of this barrier effect.

Dietary-management tools already exist in the shape of probiotic microorganisms, prebiotic oligosaccharides, and synbiotic mixtures of the two. There is evidence that these tools do work in dogs and, to a lesser extent, in cats. However, much more research is needed on the effects of the various probiotic strains and prebiotic oligosaccharides in a wide range of breeds. Well-designed feeding studies are required that use molecular microbiology techniques ideally coupled with more fundamental studies on the mechanisms of action of these agents. Such studies will lead to many new product opportunities in the pet-care field.

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