The Role of Probiotics in GI Tract Health

PURINA VETERINARY DIETS®

Stress comes in many forms for your patients. Changes in diet and environment, medications, pathogens and parasites—all are stressors that can lead to an imbalance of intestinal microflora, resulting in gastrointestinal (GI) upset.

Probiotics provide a safe, effective and natural way to help promote normal intestinal microflora. That's why our international team of microbiologists, nutritionists, immunologists, veterinarians, food scientists and stability specialists spent more than eight years studying the probiotic used in Purina Veterinary Diets® FortiFlora™ brand Canine and Feline Nutritional Supplements.

It's rewarding to work for a company that is willing to break new ground and put the necessary time and resources into development of a product like this. And as a pet owner myself, it's exciting to be part of an effort that will help pets live healthy lives.

This monograph provides the information you need to make probiotic supplementation a vital component of your protocol for managing GI upset in canine and feline patients. By using FortiFlora as a tool, you can make a real difference for your patients.

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Gut Microflora and Intestinal Health

While the main function of the gastrointestinal (GI) tract is digestion and absorption of nutrients, the GI tract's important role as a body's natural defense system is often overlooked. The intestine is home to thousands of species of bacteria collectively referred to as the intestinal microflora. Helpful microorganisms contribute to intestinal mucosal integrity, metabolism and immune status, both local and systemic. Beneficial bacteria are paramount to the health and well-being of the host animal.

Introduction to Gut Microflora

Initially sterile, the GI tract is colonized within hours of birth. This well-controlled colonization involves a variety of organisms, which find their own particular niches along the GI tract so that different groups of microorganisms colonize various locations of the intestinal tract. During the colonization process, these bacteria are organized into a state of equilibrium — a balance between beneficial and harmful bacteria — so health of the animal is maintained.

In the stomach, because of its oxygen content and acidity, resident bacteria numbers are limited to 10^3–10^4 bacteria per gram of stomach content. The primary bacteria populating the stomach are *Lactobacilli* and *Enterococci.*

Mucous membranes lining the GI tract contain beneficial bacteria that compete with pathogens and nutrients. A number of functions occur here to maintain a state of equilibrium as part of the body's natural defense system:

- The mucus layer traps microorganisms.
- Cilia on the surface of epithelial cells help propel pathogenic microorganisms out of the body.
- Antimicrobial substances produced by cells and beneficial bacteria in mucous membranes help kill pathogenic bacteria on mucosal surfaces.
- Beneficial bacteria lower pH in the GI tract by secreting organic acids, which antagonize the growth of certain microorganisms.
- Physiological barriers, such as the low pH of the GI environment, discourage growth of some bacteria.

Bacteria in the small intestine are facultative anaerobes whose growth is limited by peristaltic activity. Populations of these microorganisms are low in the upper small intestine, but increase throughout the tract from the duodenum to the ileum.

The greatest colonization of the GI tract (approximately 200 species or 400 different strains of bacteria) occurs in the colon, where decreased intestinal transit time allows bacteria to reach numbers of 10^{11}–10^{12} organisms per gram. Bacterial flora in the colon are dominated by anaerobes such as *Bacteroides, Eubacterium, Bifidobacterium, Lactobacillus* and *Enterococcus.*

Functions of Intestinal Microflora

Nutritional Benefits

The intestinal microflora is important in digestion and absorption of several nutrients. These organisms may:

- Act on nitrogen and lipid-containing substances;
- Degrade complex polysaccharides to allow colonocytes to recover energy provided by these substances;
- Hydrolyze urea via endogenous urease;
- Modify the metabolism of cholesterol and bile salts;
- Modulate mineral absorption;
- Synthesize vitamins such as B_{12}, folic acid, biotin, pantothenic acid and vitamin K; and
- Help nourish the GI tract.

Disease Resistance

The most important function of the intestinal microflora is protection against infection and colonization by harmful and sometimes pathogenic bacteria. This protection is provided by physical barriers and resistance to pathogen colonization. Both of these protective mechanisms can be altered by several factors.

The balance of the intestinal ecosystem can be changed and the barrier efficacy reduced by pathological states such as congenital or acquired immunodeficiency diseases, systemic disease or gastric hypochlorhydria. Aging and stress associated with traveling, kennel boarding, changes in environment or diet, and poor nutrition also can affect the balance. One study that investigated the effects of aging on the intestinal microflora of dogs found that young adult dogs had higher levels of *Lactobacilli* and lower levels of *Clostridium perfringens* compared to elderly dogs[1] (Figure 1).

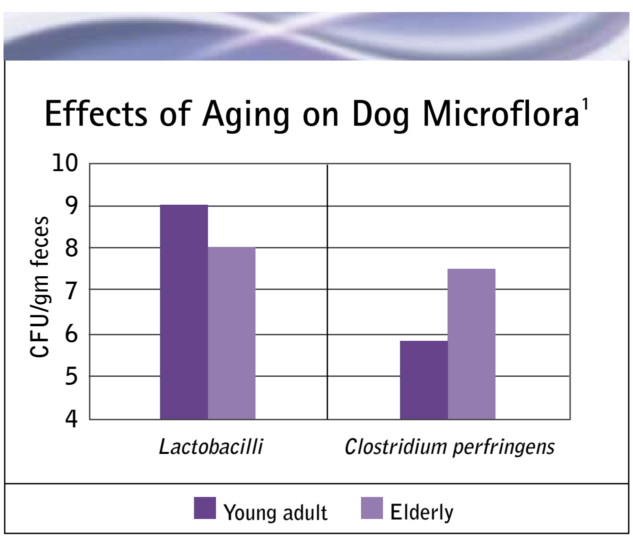

Figure 1: Levels of various intestinal microorganisms shift as dogs age; Clostridia *levels increase;* Lactobacilli *and* Bifidobacteria *levels decrease. Similar changes are noted in humans.*

Stress can trigger changes in intestinal pH, contractility and transit time, resulting in an imbalance in microflora. Shifts in microbial populations can result in increased shedding of pathogenic bacteria and reduced performance of the protective barrier of the gut, which can lead to GI upset and systemic problems. Even "good" stresses, such as traveling for a competitive show dog, can upset this delicate balance.

Composition of the microflora can change because different bacterial species have synergistic or antagonistic effects on one another. Competition between species can occur, for example, due to demand for the same metabolic substrates or adhesion sites on the intestinal wall, or by production of inhibitory substances such as short-chain fatty acids.

Another well-recognized cause of disturbance of the intestinal microflora is the use of wide-spectrum antibiotics to treat infection. Beyond killing pathogenic bacteria, these antibiotics also kill certain commensal organisms, upsetting the intestinal ecology. When this occurs, potentially harmful and pathogenic organisms (e.g., *Clostridium difficile, Klebsiella oxytoca*) can become dominant and cause antibiotic-induced diarrhea. Some medications, including antibiotics, also cause shifts in the intestinal pH, which can exacerbate issues created by a microflora imbalance.

The Immune System of the Intestine

The gut is the largest immune system organ in the body.[2,3] In fact, about 70% of the body's immune system is located in the GI tract. The mucosal barrier helps to block most pathogenic bacteria from entering the body while remaining permeable to nutrients. Since some antigenic molecules can pass across this barrier, host defense mechanisms must be working optimally to cope with the many foreign substances and pathogens to which the mucosa is constantly exposed.

Mucus in the GI tract is constantly renewed due to the destructive effects of gastric acidity, bile salt toxicity, pancreatic secretions and peristaltic activity. This provides nonspecific protection, since the renewed mucus secretions can counteract negative effects of harmful and pathogenic bacteria that diminish the integrity of the barrier by degrading intestinal glycoproteins and mucins.[4,5] In addition, the colonic microflora controls development of pathogens and regulates the intestinal immune response.

Because the digestive tract is a major defense system of the body, it performs many functions that are essential for good health. The GI tract has three main lines of defense, working in concert with one another:

- Intestinal microflora, which competes with pathogens and provides an environment that favors beneficial bacteria;
- Intestinal mucosa, which provides a protective barrier against pathogenic bacteria; and
- Gut-associated lymphoid tissue (GALT).

In the GI tract, specialized cells are the first line of defense against pathogenic bacteria and environmental insults. The mucosa comprises organized lymphoid aggregates (Peyer's patches) and isolated lymphoid follicles, which extend through the mucosa and submucosa of the small intestine. Together they are known as the gut-associated lymphoid tissue (GALT).

The mucosal system is part of a large immune network that permits mucosal T and B cells to circulate in the blood or lymph system and to communicate with cells in other tissues. These connections allow the animal to rapidly respond to primary infections and exhibit immunological memory upon reinfection.

IgA is the major class of immunoglobulin secreted by B lymphocytes at the intestinal mucosa. IgA has several ways of counteracting bacterial infection:

- blocking bacterial adhesion at the mucosal surface;
- agglutinating the bacteria for easier elimination;
- neutralizing bacterial toxins; and
- disturbing bacterial growth.

Key Points

- Bacteria in the GI tract are organized into a balance between beneficial and harmful bacteria.
- Mucous membranes lining the GI tract help defend the body against harmful organisms.
- Many factors can upset the balance of the intestinal ecosystem, including disease, age, stress and even certain drugs, such as antibiotics.

Overview of Probiotics

The microflora in the GI tract includes both beneficial and pathogenic microorganisms, sometimes referred to as good and bad bacteria. While research continues, the scientific community has identified bacterial strains that have beneficial health effects. Given the importance of maintaining a healthy intestinal balance of these microbial populations to good health, it is valuable to know that considerable scientific evidence demonstrates this microflora environment can be influenced by what an animal eats.

Probiotic bacteria are microorganisms that, when ingested, have a beneficial effect on intestinal function that maintains or promotes good health. In 1989, Fuller defined probiotics as:

> "*A live microbial feed supplement, which beneficially affects the host animal by improving its intestinal microbial balance.*"

In the last few years, many scientific studies have focused on lactic acid bacteria (LAB), especially the *Lactobacilli, Bifidobacteria* and *Enterococci.* These bacteria use fermentation to transform some sugars into organic acids (particularly lactic and acetic acids), which lower pH in the gut and inhibit the growth of certain harmful microorganisms. Moreover, it has been demonstrated that ingesting these types of bacteria as probiotics can increase the number of beneficial bacteria in the intestine throughout the period of consumption and ultimately can have a positive effect on the host.

Certain probiotics increase fecal bacterial counts of good bacteria.

LAB have long been used to produce yogurt and other fermented dairy products. Bacteria are sometimes intentionally added to other foods by food manufacturers to enhance flavor, digestibility or shelf life of the products. In fact, much of the scientific interest in the role of intestinal *Lactobacilli* in prevention of human diseases originated in 1907 from the observation by Metchnikoff that longevity and good health of Bulgarian peasants resulted from the high levels of yogurt consumption, which is fermented in part by *Lactobacilli.* Consumers now generally accept that certain yogurts and other fermented dairy foods that contain probiotics exert protective effects and promote good health.[6]

Characteristics of Beneficial Probiotics[7]

Probiotics must have certain qualities and be consumed in sufficient quantities to gain their protective and beneficial effects. To be of value, a probiotic must:

- Survive in the GI tract;
- Adhere to intestinal cells or transiently localize;
- Exclude or reduce pathogenic adherence (sometimes called pathogen exclusion);
- Produce acids, hydrogen peroxide and/or bacteriocins that are antagonistic to pathogen growth;
- Coaggregate to help achieve normal balanced microflora populations; and
- Be safe, noninvasive, noncarcinogenic and nonpathogenic to the animal.

Effects of Probiotics on Intestinal Microflora

The beneficial effects of probiotics depend on the number of live bacteria that transiently colonize the GI tract. Survival of the probiotic will vary depending on the strain and location of colonization in the tract. Careful studies must be performed to determine a candidate probiotic's stability and resulting value to the animal.

Studies in both humans and animals have shown that certain probiotics increase fecal bacterial counts of good bacteria (e.g., *Enterococcus faecium, Lactobacillus* and *Bifidobacterium*), while decreasing the numbers

Intestinal Bacteria

■ BENEFICIAL	■ HARMFUL
Lactobacilli	*P. aeruginosa*
Eubacteria	*Proteus* spp.
Bifidobacteria	*Staphylococci*
Enterococci	*Clostridia*
	Veillonellae

Figure 2: Various types of intestinal bacteria play different roles in the GI tract.

of pathogenic bacteria (e.g., *Clostridium*) (Figure 2). As stated earlier, a balanced intestinal microflora helps ensure optimal intestinal function and transit time, so the animal may be better able to avoid the GI effects of stress, including stress-induced diarrhea.

Protection Against Pathogenic Adherence

Some probiotics have been shown to minimize adherence and establishment of pathogenic bacteria. This is accomplished through several mechanisms:

- Reducing intestinal pH and destroying pathogenic bacteria and toxins;
- Producing short-chain fatty acids, lactic acid and acetic acid, which lower pH in the GI tract and nourish intestinal cells (beneficial bacteria generally thrive in more acidic environments, while pathogenic bacteria prefer environments with higher pH levels);
- Producing antimicrobials (bacteriocins and peroxides) that inhibit pathogens;
- Depleting and/or competing for nutrients pathogens require; and
- Tying up binding sites, making them unavailable to pathogenic bacteria.

Probiotics must be able to adhere to intestinal epithelial cells.[4,7] Compared to the highly colonized large bowel, there is poor microfloral localization in the small bowel and limited barrier protection against pathogens. On the other hand, the majority of the GALT is located in the small bowel, with immune stimulation taking place preferentially in a specific compartment of the small intestinal mucosa. Consequently, probiotics exert an important beneficial effect in the small intestine.

Much evidence suggests that a mechanism of action of probiotics is their ability to inhibit harmful pathogens from colonizing in the gastrointestinal tract. Probiotics attach to the enterocytes and produce inhibitory substances such as bacteriocins, lactic acid and toxic oxygen metabolites, which inhibit binding of enteric pathogens to the intestinal mucosa. This mechanism is referred to as competitive exclusion of pathogens (Figure 3).

Effect of Microflora on Pathogen Function

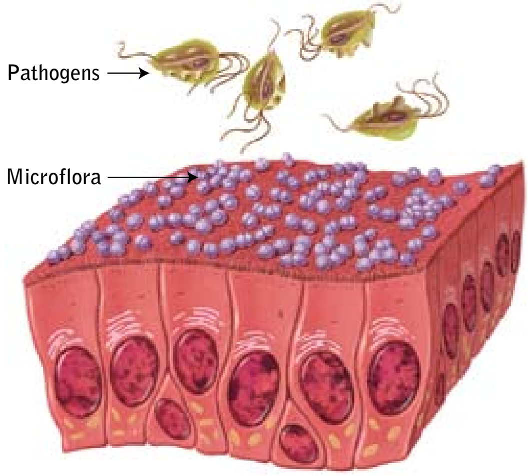

Figure 3: Probiotics inhibit pathogens from colonizing the GI tract.

Beneficial Effects Against Diarrhea

In addition to stress, many infectious agents cause diarrhea, including rotaviruses, *Escherichia coli, Salmonella, Shigella, Campylobacter* and *Vibrio cholera.* In dogs, the parasitic causes of diarrhea are led by *Cryptosporidium* and *Giardia.* Researchers have demonstrated a rationale for the use of probiotics to help control infectious diarrhea, since probiotics employ competitive exclusion, compete for available nutrients and binding sites, and increase specific and nonspecific immune responses.[8,9,10]

Promotes a Strong Immune System

Some species and strains of LAB have immunopotentiating activities, including enhancement of nonspecific anti-infective mechanisms (e.g., phagocyte and natural killer [NK] cell activities in peripheral blood, cytokine and antibody production, and lymphocyte proliferation). *Enterococcus faecium* SF68 has been shown to increase production of antibodies in mice exposed to *Giardia*.[11] The mice fed probiotics had decreased levels of active trophozoites in the small intestine and decreased fecal shedding of *Giardia.* The ability of *E. faecium* SF68 to strengthen the immune system at both the mucosal and systemic levels demonstrates the mechanism by which probiotics may be able to antagonize pathogens *in vivo* to reduce the risk of protozoan infection.

Since important changes in microflora also occur at weaning, events during this period may have a strong effect on the overall health of the dog. Probiotics have been shown to have a positive effect on the immune system of puppies.[12,13]

Key Points

- Lactic acid bacteria (LAB) have long been recognized to support good health as part of the diet.
- A probiotic must survive in the GI tract in sufficient quantities to provide beneficial effect.
- Some probiotics minimize adhesion of pathogenic bacteria in the intestinal tract.

Probiotic Value for Dogs and Cats

Lactobacilli are part of the natural microflora of healthy dogs and cats. These organisms are found all along the gastrointestinal tract, with their numbers increasing from the stomach to the colon[1,14] and in the feces.[15,16] As the animal ages, shifts in microflora populations (e.g., reduced levels of *Lactobacilli* and *Bifidobacteria,* increased levels of *Clostridia*), can lead to the animal being less tolerant of dietary changes and stress and more likely to experience stress-induced diarrhea.

Lactobacilli and *Bifidobacteria* are also common in cats, but less is known about their intestinal microflora. Previous reports[17,18,19] on fecal microflora of cats revealed a diverse group of anaerobic bacteria. Other studies have demonstrated that *Lactobacilli* were present in the duodenum[20] and feces[21] of healthy cats. Patil et al[22] demonstrated that fecal *Bifidobacteria* levels were significantly ($p<0.06$) lower in old cats (9 to 14 years of age) than in young cats (1 to 9 years of age).

Enterococcus faecium SF68

Nestlé Purina has selected a special strain of *Enterococcus faecium* (strain SF68) as the probiotic agent for use in Purina Veterinary Diets® FortiFlora™ brand Canine and Feline Nutritional Supplements. *Enterococci* are typical lactic acid bacteria that occur naturally in a wide variety of environments, including on plants, in surface water, in human foods, and in the GI tracts of humans[23] and animals.[24] They are found in raw and processed meats, plus many fermented foods, such as traditional European cheeses and sausages (e.g., chorizo).

E. faecium SF68 strain was originally isolated in Sweden in 1968. Since that time, the use of a "master culture" and strict quality control procedures by the supplier of *E. faecium* SF68 has guaranteed that the strain used in the original form has not been genetically altered or contaminated.

From many potential probiotic strains, Nestlé Purina selected *E. faecium* SF68 for a number of reasons:

- Excellent biological activity:
  - Rapid growth and transient colonization in the GI tract;
  - Inhibits growth of pathogenic organisms such as *Salmonella, E. coli* and *Shigellae;*
  - Produces lactic acid and bacteriocidal-like substances; and
  - Promotes a strong immune system.
- A long history of safe use in both humans and animals:
  - Not resistant to antibiotics;
  - Not transferred antibiotic resistance;
  - Nonpathogenic and nontoxic; and
  - Not absorbed into the bloodstream.
- The potential benefits of *E. faecium* SF68, based on a large number of trials in humans and animals, were consistent with desirable benefits in pets.
- *E. faecium* SF68 was demonstrated to survive the acidic conditions of the GI tract in pets in well-controlled *in vitro* and *in vivo* tests.
- The chosen *E. faecium* SF68 is microencapsulated (Figure 4) to offer:
  - Stability of the microorganism through the production process;
  - Viability of the microorganism for the stated shelf life of the product; and
  - Proven viability of the organism through the GI tract of the dog and cat (*in vivo*).

Microencapsulated
***Enterococcus faecium* SF68**

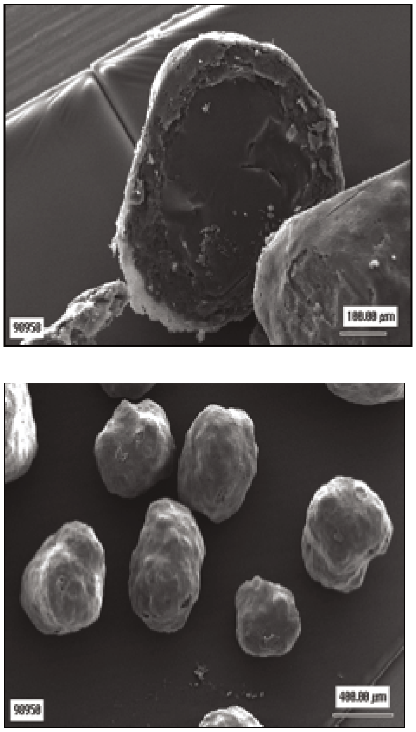

Figure 4: E. faecium *SF68 in microencapsulated form offers increased stability and viability.*

Microencapsulation

Most commercially available probiotics suffer tremendous loss of activity during storage, so after five to six months of storage, virtually no live organisms are present. In fact, a recent published article indicated that only 10% of the commercial probiotic products analyzed contained the minimum guaranteed level of active microorganisms.[25] However, in the development of FortiFlora, Nestlé Purina formulated the product with a proprietary microencapsulated microorganism. This microencapsulation process conserves and protects the biologically active *E. faecium* SF68 so it can withstand handling, processing and storage. The organisms are encapsulated with a protective coating that is safe for humans and animals. In the intestinal tract, this coating completely dissolves and the probiotic becomes available to the animal. As a result, at the end of shelf life, physiologically significant levels of *E. faecium* SF68 are consumed by the pet. In addition, mandatory testing validates probiotic levels and the environment is carefully controlled during production, distribution and commercial storage to help minimize losses of *E. faecium* SF68.

Safety and Tolerance of E. faecium **SF68**

E. faecium SF68 has a long history of safe use in companion animals and humans. Since 1985, veterinarians in Austria and Switzerland have recommended the probiotic for dogs and cats to help maintain and restore healthy gut microflora in animals showing disturbances caused by changes in diet, stress, antibiotic therapy and other factors. It is estimated that 18,000 pets have received the probiotic in those countries.

In the United States, *E. faecium* is considered GRAS (generally recognized as safe) by the U.S. Food and Drug Administration (FDA) and is listed as an approved additive as a "direct-fed microorganism" by the Association of American Feed Control Officials (AAFCO), based on a safety review by the FDA's Center for Veterinary Medicine.

To address potential concerns regarding development of resistance to antibiotics when probiotics are part of a long-term diet, European Union researchers reviewed extensive antibiotic resistance tests and concluded *E. faecium* SF68 poses no risk when fed. It has been shown that *E. faecium* SF68 only transiently colonizes the intestinal tract and disappears from the tract within a few days after consumption stops, further emphasizing its safety.

When puppies and kittens were fed either a control diet or the same diet supplemented with *E. faecium* SF68, there were no differences in shedding of potential pathogens such as haemolytic *E. coli, Salmonella* and *Campylobacter* (Nestlé Purina, internal data). Hence, *E. faecium* SF68 is not only safe for the pet, but also for the pet owner who is handling the product.

See Kayser (2003)[26] for additional information on the safety of *E. faecium* SF68.

E. faecium SF68 Efficacy Studies in Dogs and Cats

Weiss[13] investigated the effects of *E. faecium* SF68 on the development and health of hand-reared Beagle puppies. The control and experimental groups consisted of six puppies each, with the trial beginning three days after birth to ensure intake of colostrum and ending on Day 98. Puppies fed the probiotics demonstrated higher phagocytic activity of neutrophils, indicating that the probiotics enhanced immune response. Probiotic-fed puppies showed higher IgA levels in Weeks 2 through 4, indicating possible gentle stimulation of mucosal immunity. The effect of *E. faecium* SF68 on intestinal microflora was also demonstrated in this study, with higher total counts of *Lactobacilli,* lower levels of *E. coli* and fewer *C. perfringens*–positive animals in the group fed *E. faecium* SF68.

While not a controlled study, Vahjen and Manner[27] recently published a study detailing the effects of *E. faecium* SF68 on fecal bacteria of healthy dogs under various food and environmental conditions. Twelve healthy household dogs were supplemented for 18 days with a commercially available strain of *E. faecium* SF68. The probiotic was fed once daily before meals (2 g per dog; 9.2×10^9 CFU). Rectal fecal samples were taken before the study began and on Day 18. *E. faecium* SF68 supplementation significantly reduced *Clostridium* spp. counts in 10 of the 12 dogs. These results demonstrate that *E. faecium* SF68 can reduce the level of potentially pathogenic bacteria in the GI tracts of healthy dogs.

Similar changes in intestinal microflora have been demonstrated in cats fed *E. faecium* SF68. Feeding the probiotic reduced the fecal concentration of *C. perfringens* ($p<0.059$) (Figure 5) and significantly reduced the number of cats in which *C. perfringens* could be detected ($p<0.05$), which is often considered a positive indicator of colon health.[28] No adverse effects were observed during supplementation of *E. faecium* SF68.

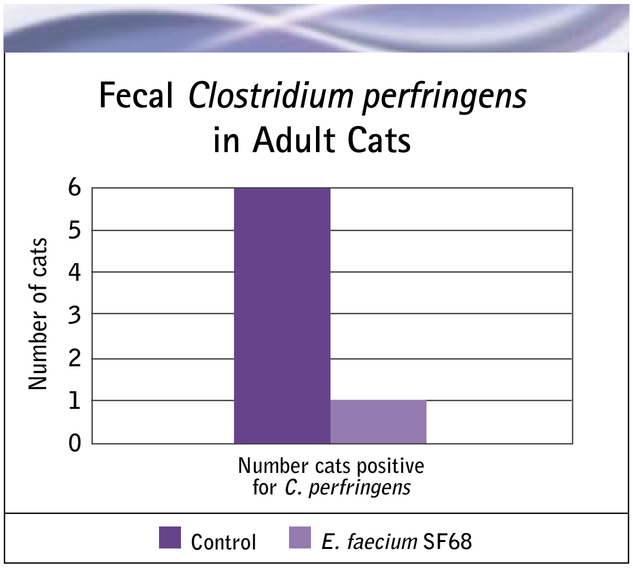

Figure 5: Effects of feeding E. faecium *SF68 on* C. perfringens *detection and fecal concentration in adult cats (p<0.05).*

When *E. faecium* SF68 was fed to Labrador Retriever, Shih Tzu and Miniature Schnauzer puppies from 3 weeks of age to 1 year of age, increased levels of fecal *Bifidobacteria* ($p<0.05$) and *Lactobacilli* ($p<0.015$) were detected (Figure 6).

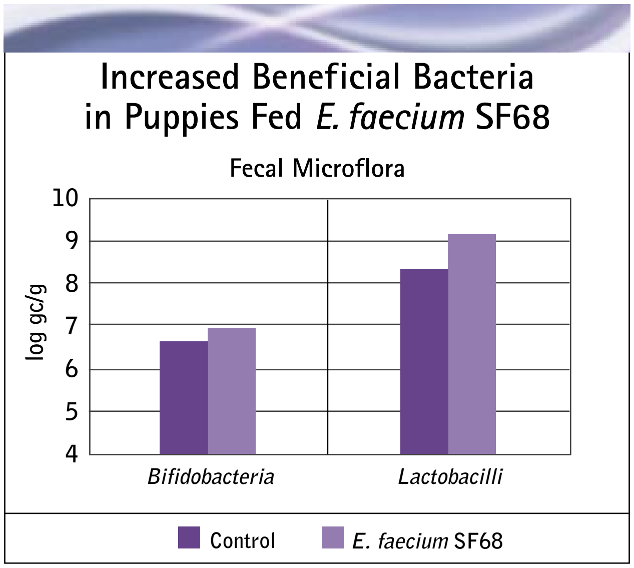

Figure 6: Beneficial bacteria concentrations in puppies fed E. faecium *SF68; significantly different from control (p<0.05).*

Benefits on Gut Microflora Balance

Other studies conducted by Nestlé Purina researchers have examined the specific effects of E. faecium *SF68 on gut microflora populations. Two of those studies are described below.*

Potential of* E. faecium *SF68 to Promote and Maintain a Balanced Gut Microflora in Healthy Dogs

F. Rochat, C. Cavadini, Nestlé Research Centre

The objective of this research was to examine the potential of *E. faecium* to temporarily survive in the GI tract of healthy adult dogs. In this prospective trial, 21 healthy adult dogs were kept on a standard diet without probiotics for the baseline period, then were switched to the test diet containing a daily dose of *E. faecium* SF68 (5×10^8 CFU) given each morning for two weeks of supplementation. Fecal analysis was performed to measure *Enterococci* levels. The study was conducted at Nestlé Purina R&D Centre, Amiens, France.

No adverse effects were observed during supplementation with *E. faecium* SF68 (NCIMB 10415). *E. faecium* SF68 was detected at physiologically relevant concentrations in feces of the dogs, suggesting the microbe survived passage through the canine GI tract.

Effect of* E. faecium *SF68 on Fecal Microflora in Puppies

G.L. Czarnecki-Maulden, C. Cavadini, Nestlé Purina PetCare Research

A trial was conducted to determine the potential benefits of feeding *E. faecium* SF68 on the microflora balance of puppies. In the trial, 41 large breed (Labrador Retriever) and small breed (Schnauzer, Shih Tzu) puppies were fed either a control diet (nutritionally complete dry puppy food) or the same diet supplemented with *E. faecium* SF68. Diets were fed from the initiation of solid food (approximately 3 weeks of age) until 1 year of age. Puppies were fed to maintain ideal body condition and water was supplied ad libitum. Puppies were housed in pairs in environmentally enriched pens, but were separated for feeding and microflora collection. Fresh fecal samples were collected throughout the trial and analyzed for *Bifidobacteria, Lactobacilli, Clostridium perfringens, Campylobacter, Salmonella* and *E. coli.*

Increased levels of fecal *Bifidobacteria* ($p<0.05$) and *Lactobacilli* ($p<0.015$) were detected in probiotic-fed puppies compared to control puppies. This beneficial effect was sustained throughout the first year of life. There were no differences in shedding of hemolytic and nonhemolytic *E. coli, Campylobacter* or *Salmonella* in probiotic-fed puppies, indicating *E. faecium* SF68 is safe for both the pet and the pet owner handling the probiotic. Consumption of *E. faecium* SF68 throughout the first year of growth helped establish a healthy microflora balance in puppies.

Benefits on Diarrhea and Immune Function

Several studies demonstrated the value of probiotics on controlling diarrhea and enhancing immune response in cats and dogs.

Effect of* E. faecium *SF68 on Chronic, Intractable Diarrhea in Adult Cats

G.L. Czarnecki-Maulden, C. Cavadini, J. Mrkvicka, Nestlé Purina PetCare Research

E. faecium SF68 was fed with a complete and balanced diet for 25 days to eight adult cats with chronic, intractable, idiopathic diarrhea. Control cats (n=7) were fed complete and balanced feline food without the addition of *E. faecium* SF68. Food and water were provided ad libitum.

Probiotics-fed cats demonstrated statistically significant improvements in average fecal scores versus baseline values ($p<0.0005$). Fecal consistency improved in seven out of eight cats fed *E. faecium* SF68 as indicated by decreased incidence of severe diarrhea ($p<0.02$) (Figure 7). These results demonstrate *E. faecium* SF68 may be a beneficial nutritional supplement to help decrease the incidence and severity of chronic, idiopathic diarrhea in cats.

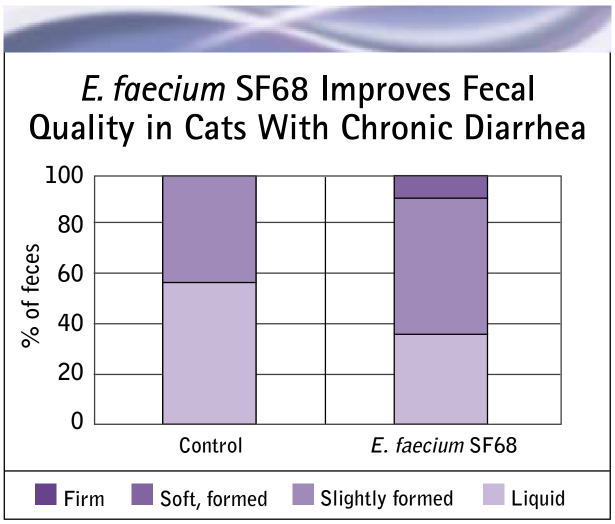

Figure 7: Fecal quality of cats with chronic diarrhea fed E. faecium *SF68; significantly different from control (p<0.0005).*

E. faecium *SF68 Helps Minimize Naturally Occurring Diarrhea in Kittens*

G.L. Czarnecki-Maulden, C. Cavadini, D. Lawler, Nestlé Purina PetCare Research

In this trial, 31 male and female kittens were fed a control diet (nutritionally complete dry kitten food) or the same diet supplemented with *E. faecium* SF68 from 8 to 14 weeks of age (postweaning) to 1 year of age. Food and water were supplied ad libitum. Kittens were housed in small groups in environmentally enriched pens. Approximately three months after the study began, an outbreak of diarrhea occurred. Severity of symptoms determined whether individual kittens were treated for diarrhea, with the knowledge that poor fecal quality and diarrhea occasionally occur in growing kittens.

While 60% of the kittens fed the control diet developed diarrhea severe enough to require treatment, only 9.5% of the probiotics-fed kittens developed diarrhea that required treatment ($p<0.05$). For those probiotic-fed kittens requiring treatment, the amount of time necessary to resolve the diarrhea was significantly ($p<0.045$) shorter than in control kittens (45 versus 18 days) (Figures 8 and 9). There was also a significant increase ($p<0.02$) in *Bifidobacteria,* decreased *C. perfringens* ($p<0.01$) and increased blood IgA ($p<0.05$) in kittens fed *E. faecium.* In this trial, consumption of *E. faecium* SF68 during the early postweaning growth period helped decrease incidence and duration of diarrhea in growing kittens.

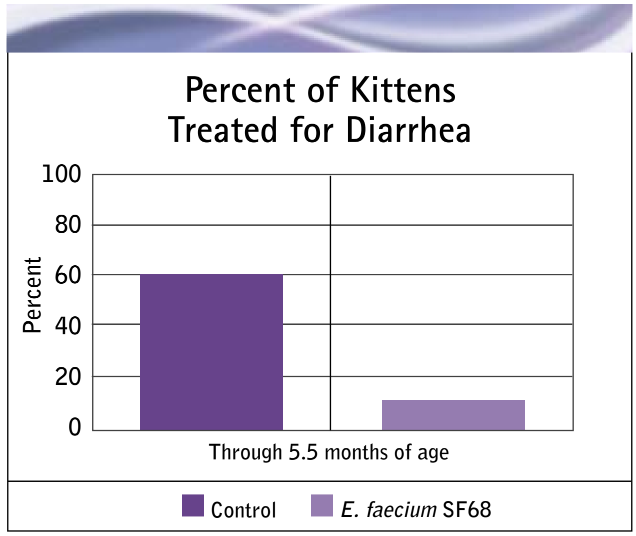

Figure 8: Percentage of kittens treated for diarrhea (p<0.01).

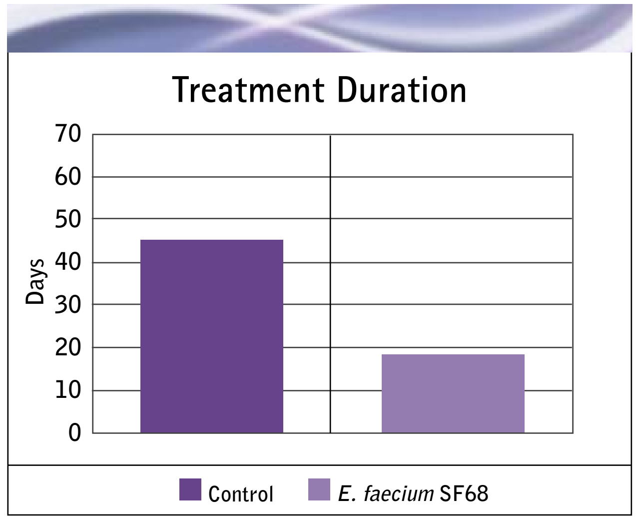

Figure 9: Treatment duration required for kittens with diarrhea (p<0.045).

E. faecium *(SF68) Supplementation Stimulates Immune Functions in Young Dogs* [12]

J. Benyacoub, G.L. Czarnecki-Maulden, C. Cavadini, T. Sauthier, R.E. Anderson, E.J. Schifrin, T. von der Weid

The objective of this research was to determine if supplementing food with *E. faecium* SF68 helps encourage formation of a healthy immune system in young dogs. *E. faecium* SF68 was fed to Labrador Retriever, Fox Terrier, Manchester Terrier and Beagle puppies from weaning to 1 year of age. Fecal and blood samples were collected at different time points and analyzed for fecal IgA, circulating IgG and IgA, and proportions of lymphoid cell subsets.

Results demonstrated a statistically significant increase in plasma IgA in probiotic-fed puppies, indicating that *E. faecium* SF68 enhanced immune function. A higher proportion of mature B cells and a statistically significant increase in canine distemper virus-specific IgG and IgA were found in probiotic-fed puppies compared to control puppies (Figure 10). These data demonstrate that dietary *E. faecium* SF68 strengthens specific immune function in young dogs.

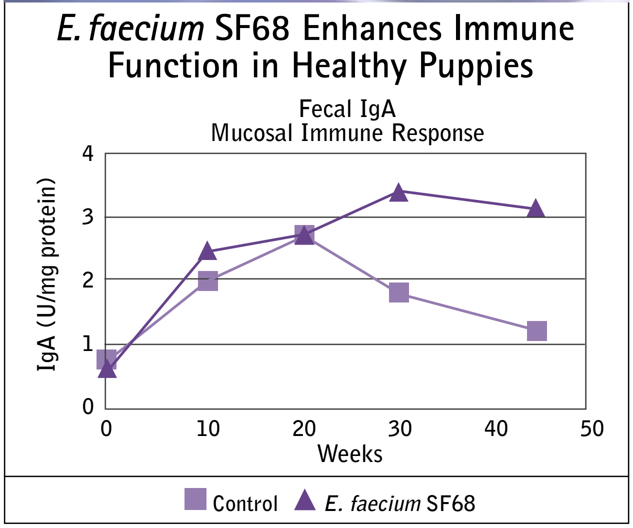

Figure 10: Immune function of puppies fed E. faecium *SF68 ($p<0.05$).*

Key Points

- Nestlé Purina has chosen *Enterococcus faecium* SF68 to include in FortiFlora.
- *E. faecium* SF68 is a safe, effective probiotic for use as a supplement for dogs and cats.
- *E. faecium* SF68 has been proven to promote normal intestinal microflora in dogs and cats.
- *E. faecium* SF68 helps nutritionally manage dogs and cats with diarrhea.

Purina Veterinary Diets® FortiFlora™ brand Canine and Feline Nutritional Supplements

Clinical Considerations

Gastrointestinal tract conditions such as diarrhea are commonly seen in dogs and cats and are often associated with an imbalance in intestinal microflora. Restoring microflora balance is a key component of the effective management of these conditions. ForiFlora is a nutritional supplement that contains a probiotic, *Enterococcus faecium* strain SF68, for the dietary management of dogs and cats with diarrhea. This probiotic has been shown to be safe, stable and effective in restoring normal intestinal health and balance.

Characteristics

Purina Veterinary Diets FortiFlora brand Canine and Feline Nutritional Supplements have been formulated to achieve the following characteristics:

- Contain a guaranteed level of viable microorganisms
- Proprietary microencapsulated microorganisms for enhanced stability
- Proven to promote normal intestinal microflora
- Help promote a strong immune system
- Shown to be safe for use in dogs and cats
- Contain high levels of antioxidant Vitamins A, E and C
- Excellent palatability

Medical Indications

FortiFlora is recommended as a nutritional supplement to help with:

- Diarrhea associated with microflora imbalance
- Acute enteritis
- Diarrhea associated with stress, antibiotic therapy and diet change
- Poor fecal quality in puppies and kittens
- Promoting a strong immune system

Medical Contraindications

- Dogs and cats with food allergies
- Severely immune-compromised animals

Guarantee

Each packet contains a minimum of $1 x 10^8$ CFU/g *Enterococcus faecium.* Average nutrient content: 5×10^8 CFU/g *Enterococcus faecium.*

Feeding Instructions

Feed 1 FortiFlora packet daily.

Calorie Content

Metabolizable energy (ME) (calculated): Each 1-gram packet contains 4 kcal.

Ingredients (Canine)

Animal digest, *Enterococcus faecium,* L-ascorbyl-2-polyphosphate (source of Vitamin C), Vitamin E supplement, zinc proteinate, beta-carotene, salt, manganese proteinate, ferrous sulfate, copper proteinate, calcium iodate, sodium selenite.

Ingredients (Feline)

Animal digest, *Enterococcus faecium,* L-ascorbyl-2-polyphosphate (source of Vitamin C), Vitamin E supplement, beta-carotene, zinc proteinate, taurine, salt, manganese proteinate, ferrous sulfate, copper proteinate, calcium iodate, sodium selenite.

Average Nutrient Content

	Feline Formula		Canine Formula	
Nutrient	As Fed	Amount per Packet (1 gram)	As Fed	Amount per Packet (1 gram)
Protein	47.1%	470 mg	45.2%	450 mg
Fat	18.1%	180 mg	17.6%	180 mg
Fiber	0.3%	3 mg	0.3%	3 mg
Calcium	0.3%	3 mg	0.4%	4 mg
Phosphorus	2.1%	21 mg	2.1%	21 mg
Potassium	0.9%	9 mg	0.9%	9 mg
Chloride	0.5%	5 mg	0.3%	3 mg
Sodium	1.1%	11 mg	1.7%	17 mg
Magnesium	0.1%	1 mg	0.1%	1 mg
Selenium	1.8 mg/kg	0.002 mg	2.6 mg/kg	0.003 mg
Vitamin E	11,100 IU/kg	11 IU	12,900 IU/kg	13 IU
Vitamin C	8,670 mg/kg	9 mg	10,700 mg/kg	11 mg
Taurine	0.7%	7 mg		
Enterococcus faecium		5×10^8 CFU/g		5×10^8 CFU/g

Glossary

Adherence The ability of a bacterium to attach itself to the intestinal wall. Normally associated with specific binding sites.

Aerobic bacteria Bacteria that require oxygen for their growth and survival. The requirement can be total (obligate aerobes) or partial (facultative aerobes).

Anaerobic bacteria Bacteria that are killed or inhibited by exposure to oxygen. As with aerobes, this can range from total inhibition (obligate anaerobes) to partial inhibition (facultative anaerobes).

Antibacterial substances Substances produced by a type of bacteria that inhibits growth and development of another type.

Bacteriocins Compounds produced by certain bacteria that have antimicrobial activity.

Bacteroides Genus of obligate anaerobic bacteria normally found in the intestinal tract.

Bifidobacterium Genus of obligate anaerobic bacteria common in the colon and feces; considered one of the most important, beneficial bacterial genera in the large intestine.

Clostridia Genus of obligate anaerobic, gram-positive spore-forming bacteria. Many species are known; those most commonly found in the GI tract are *C. septicum* and *C. difficile*. Some species are potentially pathogenic and have been associated with diarrhea in pets.

Colicins Specific proteins produced by some strains of *E. coli* that are able to kill other strains of the same species.

Coliform A general term to describe fermentative gram-negative rod-shaped bacteria. Examples of various genera are *Escherichia, Klebsiella* and *Enterobacter*.

Colonization The ability of bacteria to inhabit and establish themselves in a particular region of the body. The term is most frequently used with respect to the gastrointestinal tract, but can equally be applied to other regions (e.g., oral cavity and urogenital tract). Transient colonization indicates that, when consumed, a probiotic species can inhabit a region of the intestinal tract, however, the probiotic does not remain in the system after probiotic consumption stops.

Colony-forming units (CFU) A colony of bacteria on a semi-solid culture medium that is derived from reproduction of a single bacterium; normally referred to as CFU per unit volume or weight (mL or g).

Commensal This describes the ability of an organism to live on or in another host organism and derive benefit from it without harming the host in any way. It is one form of symbiosis. If the association is beneficial to both organisms, it is known as mutualism; if only one benefits without harm to the other, it is known as commensalism.

Competitive exclusion Mechanism whereby beneficial probiotic bacteria attach to the intestinal epithelial cells and inhibit the attachment of potential pathogens by production of inhibitory compounds (bacteriocins, lactic acid).

E. coli *Escherichea coli*, a species of enterobacteria that constitutes a large part of the intestinal flora of humans and other animals. Some strains are important pathogens.

Enterobacteria A family of bacteria consisting of gram-negative, rod-shaped organisms. Included in this family are the genera *Escherichia* and *Salmonella*.

Enterococci **Lactic** acid bacteria that are a normal component of the GI tract of humans and animals, used in the production of many foods (cheeses, fermented meats).

Enterococcus faecium A species of lactic acid bacteria that is a normal component of the intestinal tract. Many strains of *Enterococcus faecium* exist, some of which have beneficial probiotic effects. A beneficial strain of *Enterococcus faecium* is commonly referred to as SF68 and has been used as a probiotic for many years in humans and animals. *E. faecium* SF68 was isolated in 1968 from a healthy human infant in Sweden.

Eubacteria Gram-positive, anaerobic bacilli found in several areas of the body, including the intestinal tract; normally nonpathogenic.

Facultative aerobes Organisms normally existing in environments containing high levels of oxygen, but adaptable to lower amounts if necessary.

Facultative anaerobes Organisms normally existing in environments containing no oxygen, but capable of adjusting to one with low levels of oxygen.

Fusobacteria Obligate anaerobic filamentous bacteria occurring as normal microflora in the mouth and intestinal tract.

Genus (species/strain) A taxonomic category (method of biological classification). The order of classification, in increasing order of specificity: Order, family, tribe, genus, species and strain.

Gram-positive/-negative A method of classifying bacteria, based on the composition and structure of their cell wall. The method relies on the ability of an organism to retain a crystal violet dye (Gram's stain) after washing in alcohol. Those that retain stain (and are, therefore, violet/blue in color) are referred to as gram-positive. Those that do not retain stain are called gram-negative.

Gut-associated lymphoid tissue (GALT) The intestinal tract is protected by an extensive immune system that makes it the largest immune organ in the body. The GALT is composed of cells found in the lamina propria regions of the gut, cells interspersed between gut lining epithelial cells (intraepithelial cells, IEC, and intraepithelial lymphocytes, IEL) and immune cells residing in lymphatic tissue, such as Peyer's patches and mesenteric lymph nodes. These immune cells are critical in maintaining the mucosal barrier.

Lactic acid bacteria (LAB) *Lactobacilli*, *Enterococci* and *Bifidobacteria*; through fermentation, convert sugars (primarily lactose) into organic acids such as lactic and acetic acid.

Lactobacilli Genus of large, gram-positive, primarily anaerobic, organisms that normally inhabit the mouth, intestine and urogenital tract; nonpathogenic.

Microencapsulation Process whereby a live microorganism is treated to provide a safe, protective coating that extends its stability and improves its processing characteristics. The coating then dissolves in the GI tract, making the active, live microorganism available to the animal.

Microflora Total community of microorganisms in a given, defined environment (e.g., body).

Peyer's patches Organized lymphoid tissues found along the mucosal surface of the intestinal tract.

Prebiotic Nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, which can improve the host health.

Probiotic Particular strains of microbes that, when consumed in a living form as part of the diet, beneficially affect the health of the host by improving its intestinal microbial balance.

Putrefactive factors By-products of protein breakdown typically found in feces, responsible for the aversive odor of stools.

Short-chain fatty acids (SCFAs) Most commonly acetic, butyric and propionic acid, produced mainly by anaerobic microflora–generated fermentation in the colon; can be used as energy sources by the cells of the colonic epithelium and are positively associated with intestinal health.

Staphylococci Round, gram-positive bacteria that grow in clusters and resemble bunches of grapes (the name *staphylococcus* is derived from Greek and refers to this grape-like structure); part of the normal flora of the skin and upper respiratory tract. Some species are nonpathogenic (e.g., *S. epidermidis*) and some are pathogenic (e.g., *S. pyogenes*).

Streptococci Round, gram-positive bacteria that are facultative anaerobes. Species can be pathogenic (e.g., *S. pneumoniae*), part of the normal nonpathogenic flora of humans (e.g., *S. faecalis*) or associated with the souring of milk (e.g., *S. thermophilus*).

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For More Information

Dogs and cats with gastrointestinal tract conditions may benefit from long-term dietary management with Purina Veterinary Diets® FortiFlora™ brand Canine and Feline Nutritional Supplements. For additional assistance with nutritional management of GI patients, contact our Veterinary Resource Center toll-free at 1-800-222-VETS (8387) Monday–Friday, 8:00 a.m. to 4:30 p.m. CT or at purinavets.com.

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