Research and Cinical Experience with Probiotics

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Abstract

There are many products in the veterinary market purported to contain probiotics that exert a beneficial effect on dogs and cats. *Enterococcus faecium* SF68 (FortiFlora,® Nestlé Purina PetCare Co., St. Louis, MO) is one of the most widely studied products. Administration of this product has been shown to have immunomodulating effects in dogs and cats. In addition, use of SF68 has been shown to aid in the management of dogs and cats with diarrhea in animal shelters. This paper will detail several studies describing the use of SF68 in dogs and cats with an emphasis on gastrointestinal diseases.

Glossary of Abbreviations
FHV-1: Feline Herpesvirus 1
IFA: Immunofluorescent Antibody Testing

Introduction

Probiotics are live microorganisms that when administered in adequate amounts confer a health effect on the host.[1] There have been many studies of the effects of probiotics on the health of humans but few in small animals. In a recent review of human studies involving probiotics,[2] it was stated that well-established probiotic effects include:

1. Prevention and/or reduction of duration and complaints of rotavirus-induced or antibiotic-associated diarrhea as well as alleviation of complaints due to lactose intolerance.
2. Reduction of the concentration of cancer-promoting enzymes and/or putrefactive (bacterial) metabolites in the gut.
3. Prevention and alleviation of unspecific and irregular complaints of the gastrointestinal tract in healthy people.
4. Beneficial effects on microbial aberrancies, inflammation and other complaints in connection with inflammatory diseases of the gastrointestinal tract, *Helicobacter pylori* infection or bacterial overgrowth.
5. Normalization of passing stool and stool consistency in subjects suffering from obstipation or an irritable colon.
6. Prevention or alleviation of allergies and atopic diseases in infants.
7. Prevention of respiratory tract infections (common cold, influenza) and other infectious diseases as well as treatment of urogenital infections.

Infectious diseases are common in small animals, so the potential beneficial effects of probiotics could significantly impact veterinary practice. All mechanisms of immune modulation have not been characterized, and it is likely these effects vary by probiotic. It is known that many probiotics in the lactic acid bacteria group help balance the endogenous microbiota, and some can inhibit replication of pathogenic bacteria. The proposed mechanisms of action include competition for essential nutrients or receptor sites, binding with pathogenic bacteria, and production of inhibitory substances. It also is known that some probiotics can beneficially influence innate and acquired immunity systemically by a variety of proposed mechanisms, including inducing cytokine production, natural killer cell activity, and specific and nonspecific immunoglobulin production.[2]

Several review articles in human medicine recently have suggested evidence that probiotics have provided a beneficial effect for a variety of conditions, such as *Clostridium difficile* diarrhea and hospital-acquired pneumonia, suggesting that larger, more rigorously controlled multicenter studies should be performed. These findings emphasize that the biological effects of individual probiotics vary and that each probiotic introduced should be rigorously evaluated in a controlled fashion to define the potential for clinical utility.[3-5] In addition, the source of the probiotic should be considered. For example, in recent veterinary studies, the majority of products claiming to contain probiotics generally did not meet the label claim when evaluated.[6,7] One exception is the Nestlé Purina PetCare probiotic, *Enterococcus faecium* SF68 (FortiFlora®).

The potential benefit of probiotics to animal health could be considerable.[8] There are several commercially available probiotics marked for use in dogs or cats in the United States. Several veterinary probiotic manufacturers have funded and continue to fund research studies evaluating the clinical effect of their products.[9-16]

Enterococcus faecium strain SF68 (NCIMB10415) was originally isolated from the feces of a healthy baby and was initially shown to inhibit the growth of a number of enteropathogens.[17] The purpose of this paper is to summarize key studies regarding the potential effects of this probiotic in the management of different canine or feline clinical syndromes.

From the Purina Companion Animal Nutrition Summit: *The Gastrointestinal Tract in Health and Disease*; Lisbon, Portugal; March 22-24, 2012.

Immune Modulation Studies

In one study, *Enterococcus faecium* strain SF68 was fed to a group of puppies vaccinated for canine distemper virus and compared over time to a control group that was similarly vaccinated but not fed the probiotic.[12] A number of findings suggested an immune-modulating effect of the probiotic. The puppies supplemented with SF68 had increased serum and fecal total IgA concentrations, increased CDV-specific IgG and IgA serum concentrations, and increased percentage of circulating B lymphocytes when compared to control puppies. The effect on canine distemper virus-specific IgG and IgA antibodies in serum was seen only after the puppies had been supplemented for 31 and 44 weeks, and it was believed that SF68 prevented the decline in antibody titers observed in the controls by maintaining high levels of antibodies.

In a follow-up study, a similar experimental design was applied to kittens. In that study, it was hypothesized that feeding *E. faecium* SF68 to kittens would enhance nonspecific immune responses; FHV-1-, FCV- and FPV-specific humoral immune responses; and FHV-1-specific cell-mediated immune responses.[10] Twenty 6-week-old SPF kittens were purchased from a commercial vendor and divided into two groups. One group was fed SF-68 daily, and the other group was fed the placebo starting at 7 weeks of age.

Table 1. Microbiota stability before and during supplementation with SF68 or a placebo[10]

Group	Equilibration	Supplementation	*P* value (vs equilibration)
Number of bands			
SF68 supplemented	22.40	22.09	0.880
Placebo supplemented	24.40	20.53	0.092
P value	0.449	0.593	
Simpson's index of microbiota diversity			
SF68 supplemented	0.863	0.869	0.851
Placebo supplemented	0.899	0.839	0.050
P value	0.114	0.513	
Shannon–Wiener index of microbiota diversity			
SF68 supplemented	2.457	2.538	0.624
Placebo supplemented	2.689	2.385	0.046
P value	0.079	0.492	

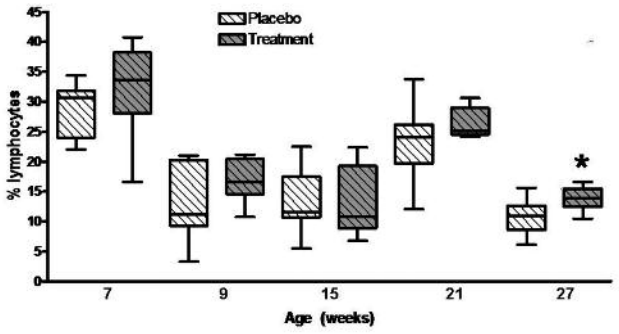
The equilibration period was 14 days and the supplementation period was 140 days. Results of supplementation samples were pooled within cat.

At 9 and 12 weeks of age, a commercially available FVRCP modified live vaccine was administered SQ, and the kittens were followed until 27 weeks of age. The attitudes and behaviors of the kittens were monitored daily throughout the study. Body weight was measured weekly. Blood, saliva, and feces were collected from all cats prior to starting the probiotic or placebo supplementation, at 7 weeks of age, and at 9, 15, 21 and 27 weeks of age. In addition, feces were collected from kittens in the treatment group after the study was completed at 28 weeks of age. For each group of kittens, five fecal samples per day were randomly selected from the shared litter box and scored using a standardized graphic scoring card.

Fecal extracts from samples taken at 9 and 27 weeks of age were analyzed for total IgA and total IgG. Other parameters monitored include randomly amplified polymorphic DNA RAPD-PCR on feces to determine if viable *E. faecium* SF68 was in the stools of treated cats and to assess whether the probiotic was transmitted from the treated kittens to the control kittens. Commercially available ELISAs were used to determine whether *Clostridium perfringens* enterotoxins or *C. difficile* toxins A/B were present in the feces of the kittens. Routine aerobic fecal cultures for *Salmonella* spp. and *Campylobacter* spp. were performed. Complete blood counts, serum biochemical panels and urinalyses were performed to assess adverse events induced by the probiotic. Antigen-specific humoral immune responses were estimated by measuring serum FHV-1-specific IgG, FHV-1-specific IgA, FCV-specific IgG and feline panleukopenia-specific IgG in sera as well as FHV-1-specific IgG and IgA levels in saliva using adaptations of previously published ELISA assays. Total IgG and IgA concentrations in sera, fecal extracts and saliva were estimated using commercially available ELISA assays or radial immunodiffusion assay. Cellular immune responses were assessed via flow cytometry and whole blood proliferation assays. Lymphocytes were stained for expression of CD4, CD8, CD44, MHC Class II, and B cells. In addition, lymphocyte proliferation in response to concanavalin A and FHV-1 antigens was assessed.

Body weight and fecal scores were not statistically different between the two groups over time or at individual time points. Feces from seven of nine treatment cats were positive for SF68 at least at one time point during the study, whereas feces from all control cats were negative for SF68 at all time points. SF68 DNA was not detectible from the feces of any treated cat one week after stopping supplementation (week 28). All samples from placebo cats were negative for SF68 by RAPD-PCR. Neither *Salmonella* spp. nor *Campylobacter* spp. was grown from feces. Numbers of positive samples for *C. difficile* toxins A/B or *C. perfringens* enterotoxin were not significantly different between the groups over the course of the study.

Complete blood counts and biochemical profiles were within normal limits for the age groups of all cats at all time points.



Percent of gated lymphocytes positive for CD4 in peripheral blood by flow cytometry

***Denotes time points at which treatment group was significantly higher than placebo group (p=0.022)**

Figure 1. CD4+/CD8+ cell counts over time in kittens fed either FortiFlora® or a placebo.[10]

A number of immune markers were numerically greater in the SF68 kittens versus the placebo group but did not reach statistical significance. For example, at 21 and 27 weeks of age, the mean levels of FHV-1-specific IgA in serum and saliva were greater in the treatment group when compared to the placebo group. Moreover, the mean FHV-1-specific serum IgG levels were greater in the treatment group when compared to the placebo group at 15, 21 and 27 weeks of age. At 15 weeks of age, the treatment group serum mean FPV-specific IgG levels were greater than those of the placebo group. There were no statistical differences between the groups for any cell surface markers at the first four time points. However, at 27 weeks of age, the treatment group had a significantly higher percentage of gated lymphocytes positive for CD4 (mean 13.87%) than the placebo group (mean 10.61%, p=0.0220, Figure 1).

In this study, we concluded that SF68 was safe to administer to cats and that the increase in CD4+ cell counts in the treatment group compared to the placebo group without a concurrent increase in CD8+ counts at 27 weeks of age demonstrated a systemic immune-modulating effect by the probiotic. Because we did not show a significant increase in lymphocyte stimulation by FHV-1 or an increase in the expression of the memory cell marker CD44 on the CD4+ lymphocytes in the treatment group, the increase in CD4+ T lymphocytes may have been nonspecific as the cells appeared to be unprimed. As the CD4+ T lymphocytes of kittens in this study were not additionally characterized via cytokine production profiles or additional cell surface marker characterization, it could not be determined whether a Th1 or Th2 response predominated. We believed that sample size and/or the duration of the study may have precluded detection of statistical differences between the groups in regard to FPV, FCV and FHV-1 antibody titers.

Chronic Feline Herpesvirus 1 Study

Feline herpesvirus 1 (FHV-1) is extremely common in cats and is frequently associated with morbidity because of recurrent ocular and respiratory disease. In addition, there is no known drug therapy that consistently eliminates the carrier state and vaccination does not provide sterilizing immunity. In this study, it was hypothesized that feeding SF68 would decrease clinical disease, episodes of FHV-1 shedding and numbers of FHV-1 DNA copies shed over time in cats with chronic FHV-1 infection.[11]

Overall, 12 cats with chronic FHV-1 infection were administered either SF68 or a palatability enhancer as a placebo. The cats were monitored for clinical signs of disease and FHV-1 shedding, and evaluated for FHV-1-specific humoral and cell-mediated immune responses as well as for fecal microbiome stability. After an equilibration period, mild stress was induced by changing the housing of the cats from cages to group housing multiple times over a five-month period.

The SF68 was well-tolerated by all cats. Fecal microbial diversity was maintained throughout the study in cats supplemented with SF68 but decreased in cats fed the placebo, indicating a more stable microbiome in cats fed SF68. Upper respiratory signs of disease were not exacerbated in this model of stress. While results varied among cats, those administered SF68 had fewer episodes of conjunctivitis than the placebo group during the supplementation period, suggesting that administration of the probiotic lessened morbidity associated with chronic FHV-1 infection exacerbated by stress (Figure 2).

Murine Acute *Giardia* Study

In previous work, mice administered SF68 and then infected with *Giardia intestinalis* shed fewer trophozoites and less *Giardia* antigen than the placebo group.[14] In addition, supplemented mice had increased CD4+ cells in Peyer's patches and the spleen as well as increased anti-*Giardia* intestinal IgA and serum IgG when compared to untreated mice.

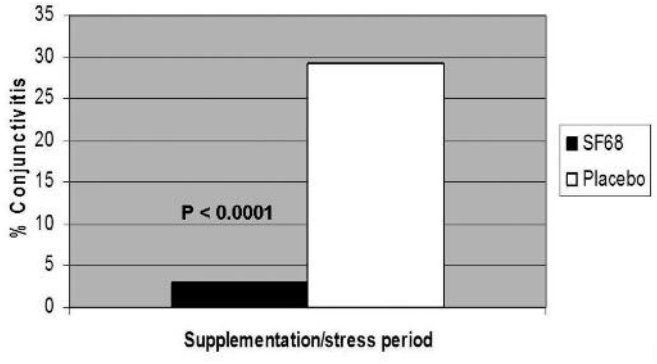


Figure 2. Differences in cumulative conjunctivitis scores from chronic feline herpesvirus 1 infection in cats administered either SF68 (FortiFlora®) or a placebo during the time periods where housing stress was induced as described.[11]

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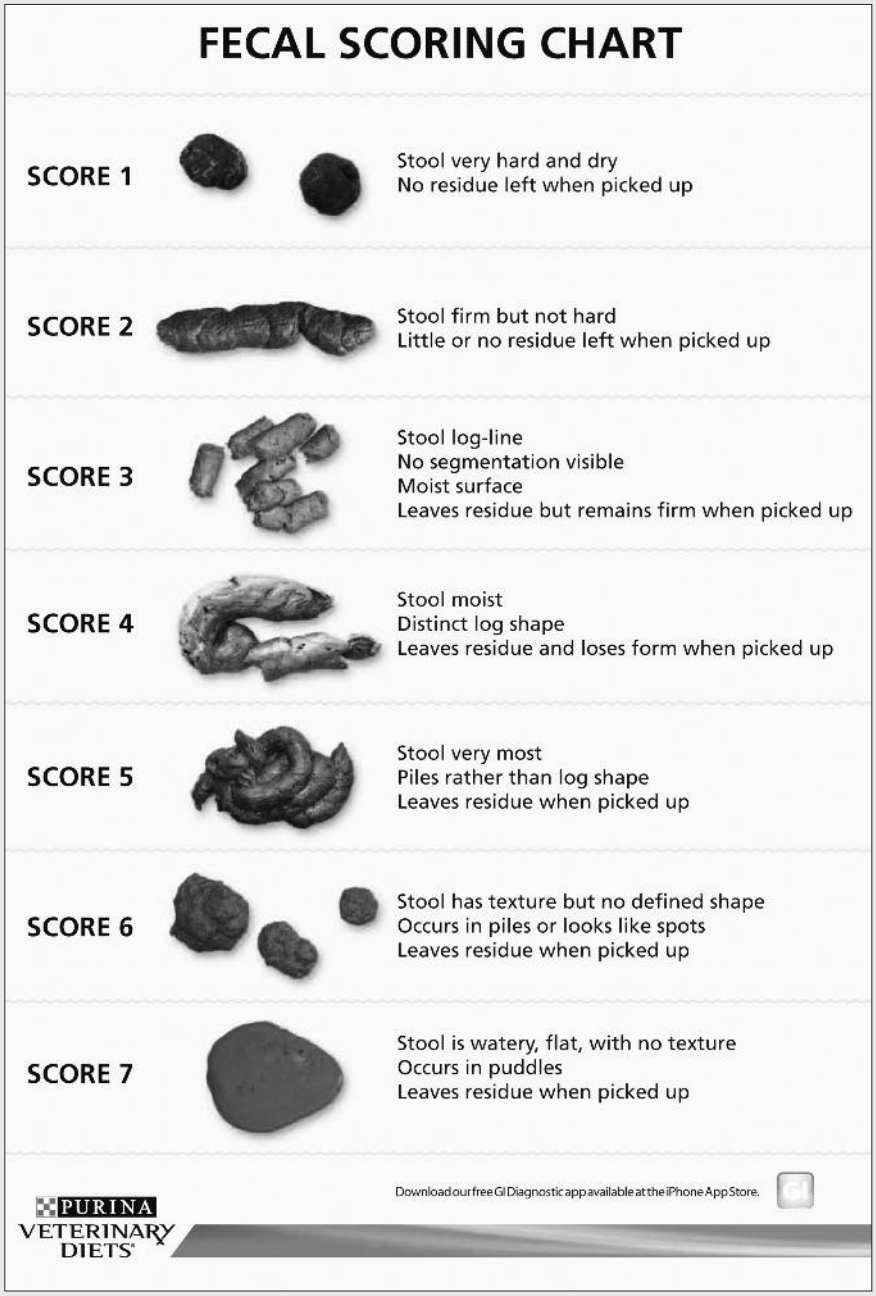
Chronic Subclinical *Giardia* Study in Dogs

When SF68 was administered to 10 adult dogs with chronic subclinical *Giardia* infection, no differences in cyst shedding or fecal antigen testing were found when compared to 10 placebo-treated dogs.[9] There also were no differences between groups in fecal IgA concentrations. In contrast to the mouse study, the dogs were previously infected by *Giardia*, which may have affected the results.[14] In addition, the study was only for six weeks; in the previously discussed puppy study, some of the significant immune-modulating effects were not seen until later in the supplementation period.[12]

Table 2. The Purina Fecal Scoring System for Dogs and Cats[13]



Shelter Animal Acute Nonspecific Diarrhea Study

In a recent study, we hypothesized that cats and dogs housed in an animal shelter that were fed SF68 would have decreased episodes of diarrhea and improved fecal scores compared to untreated cats and dogs in the same environment.[13] The cats and dogs were housed by species in two different rooms in a northern Colorado animal shelter. The cats and dogs were all fed a standardized diet by species. Animals in one room were supplemented daily with FortiFlora,® and animals in the alternate room were supplemented daily with a placebo. Otherwise, management of the rooms was identical for the duration of the study. To reduce risk of a room influence on the results of the study, the room in which cats or dogs were being supplemented with FortiFlora® was switched after one month, with a one-week washout period to lessen the possibility that SF68 surviving in the environment could influence the results of the study.

During the study, routine shelter cleaning and disinfection protocols were being followed. Prior to cleaning the room each morning, feces in each animal's cage were scored by an investigator using the Purina Fecal Scoring System for Dogs and Cats. This person was blinded to the treatment groups. After scoring, feces from dogs with scores from 4 to 7 (indicating mild to severe diarrhea) were collected and transported to Colorado State University for infectious disease testing, which included microscopic examination for parasite eggs, cysts and oocysts after zinc sulfate centrifugation flotation and immunofluorescent antibody testing (IFA) for *Cryptosporidium* oocysts and *Giardia* cysts (Merifluor® *Cryptosporidium/Giardia*, Meridian Bioscience Inc., Cincinnati, OH). The percentages of dogs and cats with diarrhea of >2 days duration were calculated over the course of the study. A generalized linear mixed model using a bionomial distribution with treatment being a fixed effect and the room being a random effect was used to assess for statistical differences between treatment groups. Presence of parasites was included as a covariate. Significance was defined as $p<0.05$.

Diarrhea prevalence rates were low for all dogs in the study, so statistical differences were not detected. However, the percentage of cats with diarrhea >2 days was 7.7% for the probiotic group and 20.7% for the

placebo group (Figure 3). This result was significantly different (p=0.0297). These results suggest that administration of SF68 to cats housed in shelters may lessen the number of days with diarrhea. As this was a short-term study, this effect was likely from probiotic influences on intestinal flora rather than systemic immune-enhancing effects.

Metronidazole and SF68 Study

In one study, dogs with *Giardia* were administered metronidazole alone or with silymarin.[18] While all dogs ceased shedding *Giardia* cysts, the dogs treated with metronidazole and silymarin had several positive clinical findings compared to dogs treated with metronidazole alone, suggesting a beneficial effect for dual therapy.

Based on that study, our research group hypothesized that dogs with nonspecific diarrhea administered SF68 with metronidazole would have better clinical outcomes than dogs administered metronidazole alone.

In the first experiment, we showed that SF68 is resistant to metronidazole, so the two compounds were administered together in the subsequent experiment. In the second experiment, a physical examination was performed on all dogs reported to have a fecal score >4 (Table 2) in an open admission shelter. Stray dogs with diarrhea without vomiting that had a fecal score of >4, interest in food and no clinical findings suggesting a foreign body were included. The fecal score was determined daily by a person masked to the treatment groups. All dogs were fed a standardized diet and were administered metronidazole USP at 25 mg/kg, PO twice daily for seven days. The dogs were randomized to be administered SF68 (treatment) or a placebo mixed with their food daily for seven days. SF68 and the placebo were provided in separate coded and marked capsules, and none of the investigators at the research facility knew which capsule contained which product.

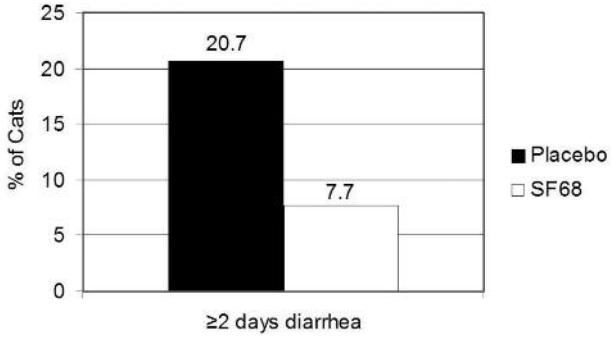


Figure 3. Differences in the prevalence rates of diarrhea for two days or longer in cats fed either FortiFlora® or placebo in an animal shelter in north central Colorado. There was a lower prevalence of diarrhea of two days or longer in the cats fed FortiFlora® in the stray room and when the results from the two rooms were combined. The results between groups are significantly different.[13]

Feces collected prior to treatment were analyzed by fecal flotation, fluorescent antibody assay for *Giardia* cysts and *Cryptosporidium* spp. oocysts, and *Clostridium perfringens* enterotoxin assay. Proportions of dogs in each group to have a fecal score of <4 by day seven were compared by Fisher's Exact Test. Speed to improvement was defined as the first day the score dropped two points from day 0 or a fecal score of 4 was reached and sustained for two consecutive days. Mean values were compared by two-tailed T test. Significance was defined as P<0.05 in both analyses.

A total of 48 dogs were entered into the study at the time this paper was submitted. Thirty-three dogs (16 treatment, 17 placebo) completed the study. Overall, 50% of the treatment group and 29.4% of the placebo group had fecal scores <3 by day seven (p=0.3). However, speed to improvement was faster (p=0.036) for the treatment group (mean=2.8 days) compared to the placebo group (mean=4.4 days). In these dogs, administration of SF68 resulted in a faster speed to improvement than administration of metronidazole alone, suggesting a positive effect induced by the probiotic.

Conclusion

The evidence gathered to date suggests that FortiFlora® has immune-modulating effects in dogs and cats and can be used to aid in the management of select gastrointestinal disorders.

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