PURINA Institute

Advancing Science for Pet Health

CANINE & FELINE MICROBIOME:

GROUNDBREAKING NUTRITIONAL SCIENCE FROM PURINA

Golden Retriever, Russian Blue, and *Enterococcus faecium*

The Purina Institute is strongly committed to advancing nutritional science to benefit pet health. Purina's team of more than 500 scientists and pet care experts – including nutritionists, veterinarians, behaviorists, molecular nutritionists, biochemists, microbiologists, and more – has a proven track record of making nutritional discoveries that improve the lives of pets around the globe.

The **microbiota** encompasses all of the organisms (bacteria, fungi, viruses and protozoa) that reside within a given space (e.g., gut, skin, mouth), whereas the **microbiome** is the organisms combined with their genetic material. Although the two terms are not technically the same, they are often used interchangeably and the term microbiome is widely accepted in veterinary medicine. Purina continues to be a leader in microbiome research, contributing to the scientific community as well as learning from the research to develop nutritional interventions that benefit pet health.

Mixed Bacterial Species

1. FOUNDATIONS OF MICROBIOME SCIENCE

Before innovations can be made, science begins with foundational studies to establish and validate testing procedures and determine benchmarks for comparison. In collaboration with our external research partners, Purina's research has led to breakthrough advancements in microbiome science by studying how the microbiome changes with age, environment, diet, and health status.

Golden Retriever, Russian Blue, and *Bifidobacterium*

DIET TYPE (WET vs DRY) AFFECTS THE MICROBIOME AND MICROBIAL METABOLISM

The gut microbiome plays important roles in overall health, and a stable, diverse microbiome resists overgrowth of potentially pathogenic bacteria that may cause dysbiosis (an imbalance between beneficial and potentially harmful bacteria) and produce clinical disease. Dietary modifications can alter the microbiome and its metabolic activity. The objective of this study was to evaluate the effect of two distinctly different types of commercial diets (dry and wet) on markers of microbial metabolism pertinent to intestinal health.

Study Design: Eighteen (18) adult dogs (mean age 7.2 years, range 2.7 to 13 years) were allocated to two groups based on breed and age and were fed a dry, extruded diet or a wet, canned diet for 6-week periods in a crossover design (all dogs were switched to the opposite diet for the second feeding period). The dogs were housed individually and fed daily to maintain stable body weight. Fecal samples were collected during the fourth week of each period to determine the apparent digestibility of the diets. Fresh fecal samples were collected in Weeks 5 and 6 of each period for chemical and enzyme activity analysis.

Results: Fourteen (14) dogs completed the study; four dogs were removed from the study for medical reasons unrelated to the study or for refusal to eat the wet diet. All fourteen dogs maintained body weight through the study. No differences in fecal output or fecal moisture were observed between the two groups. Compared to the wet diet, the dry diet reduced fecal pH, fecal indole, sulphide and ammonia concentrations and increased total short-chain fatty acid, acetic and propionic acid concentrations. The microbial enzyme activity of dogs fed the canned diet was significantly higher than that observed when the dogs were fed the dry diet: the enzymes nitroreductase, β-glucosidase, β-glucuronidase and β-galactosidase increased by a factor of 1.5 to 3.5 in the fecal samples collected during wet feeding.

Discussion: Dietary changes can influence the gut microbiome and microbial metabolism, and considerable changes were observed when healthy dogs were fed two different types of commercial dog food. Because the two foods varied in their macro- and micronutrient compositions, the results could not be attributed to any one difference between the foods. The dry diet resulted in dramatic differences in fecal bacterial population, including significantly increased *Lactobacillus* and *Bifidobacteria* and decreased *Clostridia*. Compared to the dry diet, the wet diet was associated with increased concentrations of short-chain fatty acids, which are important modulators of mucosal function. The dry diet was associated with decreased stool odor.

Martineau, B., & Laflamme, D. P. (2002). Effect of diet on markers of intestinal health in dogs. *Research in Veterinary Science, 72*, 223-227. doi: 10.1053/rvsc.2002.0548

BOVINE COLOSTRUM INCREASES MICROBIOME DIVERSITY, STABILITY AND IMPROVES IMMUNE RESPONSE TO VACCINATION IN DOGS

Colostrum is the early milk produced after giving birth, and serves to transfer antibodies to the offspring to provide immunity and promote growth and development of the gastrointestinal tract. Bovine colostrum is known to contain a number of biologically active components with immunomodulatory effects. The goal of this study was to evaluate the immunomodulatory effect of dietary supplementation of bovine colostrum in adult dogs.

Study Design: Twenty-four (24) Husky-mix dogs with a mean age of 2.5 years were allocated to two groups (test and control) balanced for age, sex and baseline fecal IgA levels. During a pre-test phase, all dogs were acclimated to a complete and balanced dry kibble diet. At the end of the 8-week pre-test phase (Week 0 of the test phase), all dogs received a canine distemper virus (CDV) booster vaccine (the dogs had been previously vaccinated). Dogs in the control group remained on the control diet, while dogs in the test group were fed the control diet supplemented with 0.1% spray-dried bovine colostrum (BC). The test phase was 40 weeks in length. Fecal scores were recorded daily and body weights were recorded weekly. Blood and fecal samples were collected every four (4) weeks. Response to the CDV vaccine was evaluated by measuring vaccine-specific plasma IgG levels. Fecal IgA levels were determined as an indication of gut-associated lymphoid tissue (GALT) response. The dogs' microbiomes were also evaluated. C-reactive protein (CRP) was measured near the end of the study as an indicator of generalized inflammation. During Week 38 of the 40-week study, a standard exercise protocol was performed; dogs sprint-raced 10 miles in harness as part of a team pulling an unladen sled. Rectal swabs were collected 24 hours before and 24 hours after the exercise protocol to evaluate the effect of BC on the microbiome and its stability in response to exercise.

Results: Food intake, body weights and fecal scores did not differ between the two groups during the trial. Based on C-reactive protein data, there was no sign of inflammation in either group of dogs at the end of the study. CDV antibody concentration rose gradually following administration of the booster vaccine and peaked at 8 weeks post-vaccination in both groups. In the control group, the antibody levels then declined to baseline values 16 weeks after vaccination. In the BC group, the antibody levels remained high until the end of the trial. Over the course of the study, dogs fed the BC-supplemented diet showed a significantly higher ($p < 0.05$) CDV vaccine response and significantly higher ($p < 0.05$) levels of fecal IgA at 40 weeks compared to the control group. Although the increase in these antibodies did not significantly differ immediately after vaccination, the BC-supplemented dogs' IgG levels remained closer to peak levels throughout the study while the control group's IgG levels declined rapidly from 8 to 16 weeks and remained low through the remainder of the study period. The dogs in the BC group also showed significantly greater species diversity in their microbiome ($p < 0.05$) compared to the control group. When the pre- and post-exercise samples were evaluated, dogs fed the BC-supplemented diet had greater similarity between the pre- and post-exercise samples, indicating a more stable microbiome ($p < 0.05$). The BC-supplemented group showed an approximately 82% similarity between the pre- and post-exercise samples, compared to approximately 46% similarity in the control group.

BOVINE COLOSTRUM INCREASES MICROBIOME DIVERSITY, STABILITY AND IMPROVES IMMUNE RESPONSE TO VACCINATION IN DOGS (CONT.)

Discussion: IgA is a key product of the gut-associated lymphatic tissue (GALT) and has protective effects via preventing pathogen adherence and colonization, blocking viral adhesion, and neutralizing toxins. The results of this study show BC supplementation enhanced GALT function, resulting in higher IgA production. There was a statistically significant increase in specific anti-CDV IgG levels in dogs in the BC group, suggesting that the diet increased priming of the B-cell response to vaccination. The peak value was not significantly higher between the groups, but the BC-supplemented group's IgG response remained closer to peak for the duration of the study while the control group's response reduced after 8 weeks. Dogs fed the BC diet showed higher microbial species diversity, indicating that these dogs had a more stable microbiome that was more resistant to challenge. Based on the results of this study, supplementing dogs' diets with bovine colostrum resulted in increased gut microbiome diversity and stability as well as an improved immune response to CDV vaccination without inducing generalized inflammation.

Satyaraj, E., Reynolds, A., Pelker, R., Labuda, J., Zhang, P., & Sun, P. (2013). Supplementation of diets with bovine colostrum influences immune function in dogs. *British Journal of Nutrition, 110*, 2216-2221. doi: 10.1017/S0007114513001751X

THE DOG MICROBIOME'S RESPONSE TO DIET

Study Design: Lean and obese Beagles and Labradors (32 of each breed, half lean and half obese) were fed a baseline diet for 4 weeks, followed by a 4-week feeding trial of either a high-protein, low-carbohydrate diet (49.4% and 10.9%, respectively) or a lower-protein, high-carbohydrate diet (25.5% protein, 38.8% carbohydrate). The investigators collected fecal samples from the dogs at the end of each phase and analyzed them with 16S rRNA gene sequencing analysis to determine the types of bacteria present.

Results:

- Diet has a large and reproducible effect on the dog microbiome, and these effects are similar to what has been previously observed in human studies.
- The microbiomes of lean and obese dogs are different.
- Obese dogs experienced larger shifts in the microbiome in response to dietary changes. This may indicate that their microbiome is less stable, but it could also present opportunities for nutritional intervention to improve weight management.
- The microbiome of obese dogs fed a high protein, low carbohydrate diet (as opposed to those fed a diet lower in protein and higher in carbohydrates) more closely resembled the microbiome of lean dogs.

Discussion: Diet impacts the microbiome (this is not new, but this investigation adds to the accumulating evidence). The finding that obese dogs experienced larger shifts with dietary change is consistent with the view that overweight dogs' microbiome reside in a less stable state compared to those of healthier, lean dogs. Diet affects the microbiome, which in turn affects animal (and human) health; enhancing the microbiome toward a more favorable (lean) type may provide opportunities for improved weight management approaches. The findings of this study open up the possibility that we can positively impact the microbiome of overweight dogs to make weight management strategies more effective, but we still have a lot to learn about the interactions of diet and the microbiome.

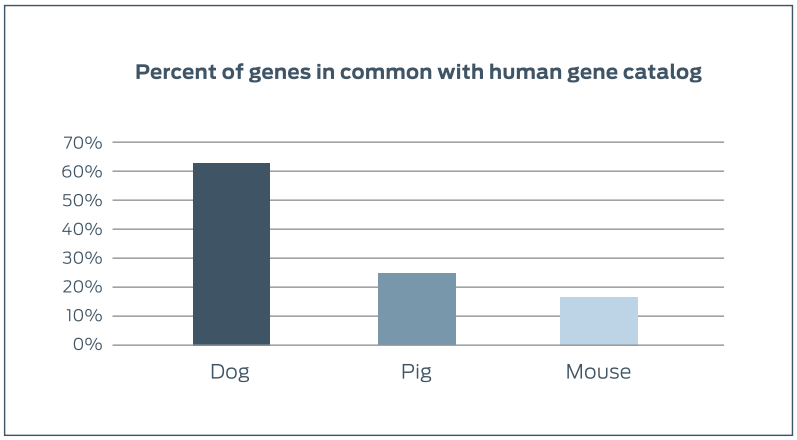
Li, Q., Lauber, C. L., Czarnecki-Maulden, G., Pan, Y., & Hannah, S. S. (2017). Effects of the dietary protein and carbohydrate ratio on gut microbiomes in dogs of different body conditions. *MBio, 8*(1), e01703-16. doi: 10.1128/mBio.01703-16.

THE DOG MICROBIOME'S SIMILARITY TO THE HUMAN MICROBIOME

The genes of the human gut microbiome were compared to those of the dog, pig and mouse. When genes are compared, they are considered "mapped" (or matched) if their genetic sequences are 95% or more identical.

Study Design: Lean and obese Beagles and Labradors (32 of each breed, half lean and half obese) were fed a baseline diet for 4 weeks, followed by a 4-week feeding trial of either a high-protein, low-carbohydrate diet (49.4% and 10.9%, respectively) or a lower-protein, high-carbohydrate diet (25.5% protein, 38.8% carbohydrate). DNA was extracted from the fecal samples to create a "gene catalog" of the dog gut microbiome and compare it to existing gene catalogs of human, pig and mouse gut microbiomes.

Results: The dog microbiome is closer to the human microbiome than the pig or the mouse. The chart below shows the percent of each species' gut microbiomes that mapped with the human gut microbiome. The overlap for the dog is 63%, which is more than twice that of the pig (24%) and almost 4 times that of the mouse (17%).



Discussion: The similarity between the dog and human microbiomes is a new finding. The mouse is the most common animal model used, yet it shares less in common with humans than either dogs or pigs. Although humans and mice are closer from an evolutionary standpoint, it appears that the shared lifestyle and physiology between the dog and human has led to their microbiomes being more similar than mice and humans.
The Purina catalog already contains most of the genomic content of the gut microbiome of dogs in a Western pet care center. According to the authors, it "provides over 1 million taxonomically and functionally annotated genes and can serve as a resource for future studies. Together with the taxonomic census, our study of two dog breeds should provide a baseline for expanding dog microbiome research."

Coelho, L. P., Kultima, J. R., Costea, P. I., Fournier, C., Pan, Y., Czarnecki-Maulden,...Bork, P. (2018). Similarity of the dog and human gut microbiomes in gene content and response to diet. *Microbiome, 6*(72). doi: 10.1186/s40168-018-0450-3

RELATEDNESS, MATERNAL MICROBIOTA AND LIVING ENVIRONMENT AFFECT THE MICROBIOME OF DOGS

The gut microbiome has a significant impact on the health of dogs, as well as people. This study investigated the effects of relatedness, maternal microbiota, and living environment on the microbiome in a large and well-defined population of German Shepherd dogs. They also examined the impact of a probiotic administered during the last trimester of pregnancy and during the first 12 weeks of life.

Study Design: The study involved 30 bred female purebred German Shepherds and their puppies, for a total of 168 dogs. The bitches, part of the Swedish Armed Forces canine program, had been living with families distributed throughout Sweden but were brought into the central kennel facility prior to day 42 of their pregnancy and maintained in that facility until the puppies were 8 weeks of age. At 8 weeks, the puppies were placed with families throughout Sweden but continued to be part of the study until they were 18 months of age. All dogs were fed the same diet throughout the entire study. Either a placebo or a probiotic (*Lactobacillus johnsonii* NCC533) was administered to the bitch during the last trimester of pregnancy and until the puppies were 8 weeks old. The puppies were administered the same treatment as their mothers from 3 to 12 weeks of age. Blood and fecal samples were collected. The bitches were sampled at day 42 of pregnancy, at whelping, and at 4 and 7 weeks after whelping. The puppies were sampled at 7 weeks and at 12-13 and 15-18 months. Fecal samples were analyzed for fecal IgA antibodies against canine distemper virus (CDV) and the microbiome was evaluated. Blood samples were evaluated for IgA, IgE and IgG antibodies against CDV. (The CDV tests were performed to assess the effects of the probiotic on the immune system.)

Results: The predominant bacterial families in the bitches, regardless of whether they were pregnant or lactating, were the same as the predominant phyla in 7-week old puppies: Firmicutes, Fusobacteria and Bacteroidetes. The Firmicutes were the most dominant at all ages, and comprised 78-89% of the population, followed by Actinobacteria at 4-9% of the population. The bitches' bacterial diversity increased from pregnancy to the end of lactation, and the relative proportions of the different bacteria changed during lactation: *Erysipelotrichaceae* and *Lactobacillaceae* species increased during lactation in the bitches, while *Fusobacteriaceae* and *Clostridiaceae* decreased. The diversity and proportions of bacteria in the microbiota varied between 7 weeks and 15-18 months, indicating that the microbiota changes with age and environment. The microbial diversity was similar from 7 weeks to one year of age, but the relative proportions of the bacteria differed. From puppyhood to adult hood, the relative amounts of *Clostridiaceae*, *Erysipelotrichaceae* (unidentified genus) and *Lachnospiraceae* increased, while the relative amounts of *Erysipelotrichaceae* (genus *Allobaculum*), *Lactobacillaceae* and *Bifidobacteriaceae* increased. Littermates' microbiomes were more similar to each other than to unrelated dogs, showing an effect of relatedness (family) on the microbiome. The similarity between littermates was highest at 7 weeks, but was still present (but not as strong) at 18 months.

RELATEDNESS, MATERNAL MICROBIOTA AND LIVING ENVIRONMENT AFFECT THE MICROBIOME OF DOGS (CONT.)

Results (cont.): Puppies' microbiomes were more similar to their mothers than to unrelated bitches at 7 weeks of age, but not at birth; at birth, their microbiomes were no more like their mothers' than an unrelated dog. The dogs' microbial diversity was affected by their living area; dogs that lived in bigger cities had more diverse microbiota than those living in smaller cities or the countryside. (There was no significant difference between their microbiota when they were all housed at the central kennel facility.) The investigators found no effect of the probiotic on the specific parameters tested in this study.

Discussion: The gut microbiome of the bitch changes after whelping and during lactation. The gut microbiome in puppies changes as they mature. The microbiome is affected by geography, with dogs living in larger cities having a more diverse microbiome compared to those living in smaller cities or in the country; the authors suggested that this results from exposure to a wider range of microbes in larger cities. Relatedness has an effect on the microbiome: despite geographical separation, littermates' microbiomes were still more similar to other littermates than to unrelated dogs at 18 months of age.

This study provided a number of important firsts:

- First study to describe fecal microbiota in a large number of dogs of the same breed, from the same kennel under well-controlled natural conditions. This provided a unique opportunity to study the effects of age, relatedness, reproductive stage, living area, and a probiotic.
- First and only study comparing gut microbiota in pregnant and lactating bitches
- First to show that living environment (urban vs. rural) affects the fecal microbiota of dogs

Vilson, A., Ramadan, Z., Li, Q., Hedhammar, A., Reynolds, A., Spears, J.,...Hansson-Hamlin, H. (2018). Disengaging factors that shape the gut microbiota in German Shepherd dogs. *PLoS ONE, 13*(3): e0193507. d10.1371/journal.pone.0193507

2. PREBIOTICS

Prebiotics are non-digestible food ingredients that selectively stimulate growth and/or activity of potentially beneficial bacteria. Early Purina research led to widespread incorporation of prebiotics into pet foods to benefit gastrointestinal health.

Chicory Root

PREBIOTICS AFFECT THE MICROBIOME

Dogs' microbiomes change with age, which can lead to dysbiosis (an imbalance of beneficial and potentially harmful bacteria). Prebiotics such as inulin and mannan-oligosaccharide (MOS) are non-digestible food ingredients that selectively stimulate growth and/or activity of potentially beneficial bacteria. The objectives of this study were to determine the effects of inulin, MOS or a combination of the two on the colonic microbial population and immune parameters of senior dogs.

Study Design: Thirty-four (34) senior dogs (aged 8-11 years) were randomly allocated to four groups. During the four-week baseline period, all of the dogs received the control diet. During the subsequent four-week trial period, one group received a control diet and the test groups received the control diet supplemented with chicory (inulin source) alone, MOS alone, or a combination of chicory and MOS. The dogs were housed individually. Total feces excreted by each dog during a 3-day period at the end of each period (baseline and trial) were collected and analyzed. Fecal scores were recorded based on a scale of 1-5 (a score of 1 was assigned to hard, dry pellets; a score of 5 was assigned to watery liquid feces). A freshly excreted sample was analyzed for *Bifidobacteria* species, *Lactobacillus* species, *Escherichia coli* and *Clostridium perfringens*. Blood samples were collected on the last day of each period and analyzed for serum immunoglobulin concentrations, complete blood count and white blood cell differentiation.

Results: Dietary supplementation with either MOS or the combination of chicory and MOS, but not chicory by itself, tended to increase food intake compared to those fed the control diet. Although wet fecal output was increased for dogs supplemented with chicory or MOS compared to control, the difference was not significant when corrected for the increased intake. Dogs supplemented with the combination of chicory and MOS had significantly higher fecal scores and dogs supplemented with chicory alone tended to have higher fecal scores than dogs on the control diet; although the fecal scores increased, they remained in the desirable range and were not associated with diarrhea. No significant differences were observed in digestibility data, although chicory supplementation tended to increase fat digestibility by a small – but probably not physiologically significant – amount. Fecal samples from dogs supplemented with either chicory or MOS alone had greater concentrations of *Bifidobacteria* compared to dogs fed the control diet, but the same effect was not seen with the combination of chicory and MOS. *Lactobacillus* species did not differ between the groups. Fecal *E. coli* concentrations were reduced only in MOS-supplemented dogs compared to control dogs. Fecal *Clostridium perfringens* concentrations were not different between the groups. Supplementation with chicory or MOS alone or in combination did not affect serum immunoglobulin concentrations compared to controls. Supplementation with MOS or the combination of chicory and MOS, but not supplementation with chicory alone, tended to increase neutrophil concentrations by approximately 20% compared to controls; however, the difference was not significant. Peripheral lymphocyte concentrations were reduced in dogs supplemented with either MOS or the combination of MOS and chicory.

Discussion: Chicory and MOS may work through different mechanisms to alter bacterial concentrations and when combined may result in competition for substrate by gut bacterial species. The prebiotics did not significantly alter the food's digestibility. Supplementation with MOS or chicory increased *Bifidobacteria*, and MOS supplementation decreased *E. coli*. Although individual immune cell concentrations were also affected by the prebiotics, further research is indicated to determine the clinical significance of the changes.

Grieshop, C. M., Flickinger, E. A., Bruce, K. J., Patil, A. R., Czarnecki-Maulden, G. L., & Fahey, G. C. (2004). Gastrointestinal and immunological responses of senior dogs to chicory and mannan-oligosaccharides. *Archives of Animal Nutrition, 58*(6), 483-493.

3. PROBIOTICS

Probiotics are live organisms that, when administered in adequate amounts, confer a health benefit on the host.[1] Purina was the first to offer a shelf-stable probiotic supplement proven to enhance immune function and help manage diarrhea. This probiotic, a specific strain of *Enterococcus faecium* we call *E. faecium* SF68 (NCIMB 10415 4b1705), remains the most studied probiotic in veterinary medicine based on publications to date. The manuscripts presented here document the veterinary scientific community's growing knowledge of the indications and efficacy of this probiotic strain.

1. Hill, C., Guarner, F., Reid, G., Gibson, G. R., Merenstein, D. J., Pot, B.,...Sanders, M. E. (2014). The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology & Hepatology, 11*, 506–514. doi: 10.1038/nrgastro.2014.66

Bifidobacterium

ENTEROCOCCUS FAECIUM *SF68 STIMULATES IMMUNE FUNCTION AND MAY IMPROVE VACCINE RESPONSE IN YOUNG DOGS*

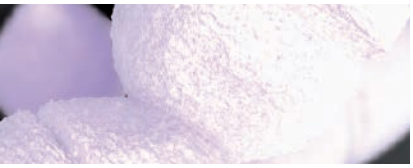
The gut microbiome plays a critical role in host immunity and defense, and probiotics may enhance these functions through direct (via cytokines) or indirect (via balancing the microbiome and mitigating dysbiosis) mechanisms. This study was performed to determine the effects of a specific strain of *Enterococcus faecium*, SF68, on immune function in puppies.

Study Design: Fourteen (14) puppies of four different breeds were fed one of two diets (control diet or the control diet supplemented with *E. faecium* SF68) from weaning (8 weeks) until one year of age. One puppy from each of seven litters was fed the control diet, and one littermate was fed the test diet. The puppies were housed in pairs within each treatment group. Food intake, body weight, and bloodwork (for complete blood and immunologic analyses) were assessed throughout the study. The puppies were vaccinated with a live attenuated canine distemper virus (CDV) vaccine at 9 weeks of age and boosted at 12 weeks of age (during the fourth week of the trial). Feces were collected and analyzed for secretory IgA.

Results: The presence of *E. faecium* SF68 was confirmed in the feces of the test group, and its absence was confirmed in the control group. There were no significant differences between the control and test groups with respect to food intake, body weight, complete blood count and blood chemistry values. No significant differences between groups were observed in total plasma IgG antibody levels or the amounts or ratio of two types of T cells (CD4+ and CD8+), but monocytes were more activated in test dogs' plasma. The increase in these antibodies did not significantly differ immediately after vaccination. However, test group dogs showed significantly higher amounts of specific anti-CDV IgG and IgA antibodies compared to control at Weeks 31 and 44; these higher levels were maintained throughout the remainder of the study. The total fecal IgA in test dogs was greater in the test group at Week 44 ($p = 0.056$). Plasma IgA was higher in test group dogs from Week 18 onward, and test dogs showed a greater proportion of mature B cells at Weeks 31 and 44. Plasma samples from the test dogs were analyzed to confirm there was no evidence of overstimulation or dysregulation of immune system.

Discussion: This was the first study showing that feeding a dry dog food supplemented with *E. faecium* SF68 enhanced long-term immune functions in growing dogs. The results suggest *E. faecium* SF68 provides a mucosal and systemic adjuvant effect without overstimulation, which may improve the protective immune response against various infections during the critical weaning period as well as at later stages in life. In addition, the results suggest that *E. faecium* SF68 increased priming of naïve B cells in response to initial CDV vaccination, which may enhance the effectiveness of the CDV vaccine in preventing CDV infection.

Benyacoub, J., Czarnecki-Maulden, G. L., Cavadini, C., Sauthier, T., Anderson, R. E., Schiffrin, E. J., & von der Weid, T. (2003). Supplementation of food with *Enterococcus faecium* (SF68) stimulates immune function in young dogs. *Journal of Nutrition, 133*, 1158-1162.



ENTEROCOCCUS FAECIUM *SF68 REDUCES INCIDENCE AND SEVERITY OF DIARRHEA IN KITTENS*

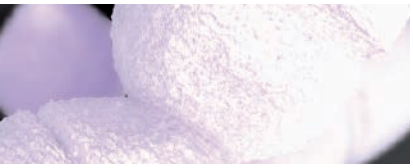
Gastrointestinal issues, such as diarrhea, are common during kittens' first few months of life, and may be at least partially explained by an unstable microbiome. This was a blinded trial to determine the effects of the probiotic strain *Enterococcus faecium* SF68 on diarrhea in kittens.

Study Design: Thirty-one (31) kittens were fed a nutritionally complete dry kitten food ad libitum from weaning (8-14 weeks of age) through 1 year of age. The kittens were divided into two groups, with one group receiving *E. faecium* SF68 and the other kittens receiving a placebo. The veterinary staff was blinded to which kittens were in which group.

Results: Kittens fed the diet supplemented with the placebo were treated for intestinal problems significantly more than kittens fed the *E. faecium* SF68 (60% vs 9.5%, respectively; $p<0.05$). In addition, kittens receiving *E. faecium* SF68 had shorter times to resolution of the intestinal problems (18 days vs 45 days for SF68 and placebo, respectively; $p<0.045$). Kittens receiving *E. faecium* SF68 had significantly higher fecal *Bifidobacteria* and lower fecal *Clostridium perfringens* than the kittens receiving the placebo ($p<0.02$). In addition, kittens fed *E. faecium* SF68 showed higher serum IgA levels than kittens receiving the placebo ($p<0.5$).

Discussion: *E. faecium* SF68 was associated with lower incidence and duration of naturally occurring diarrhea, higher serum IgA antibody levels in kittens. In addition, *E. faecium* SF68 was associated with higher levels of *Bifidobacteria* and lower levels of *Clostridium perfringens*, indicating a more balanced microbiome in kittens receiving the probiotic.

Czarnecki-Maulden, G. L., Cavadini, C., Lawler, D. F., & Benyacoub, J. (2007). Incidence of naturally occurring diarrhea in kittens fed *Enterococcus faecium* SF68. *Compendium: Continuing Education for Veterinarians* (Supplement), *29* (2A), 37.

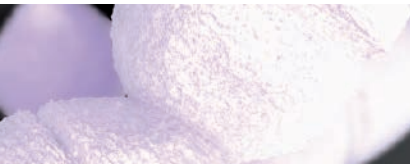


ENTEROCOCCUS FAECIUM *SF68 HAS IMMUNOMODULATORY EFFECTS IN KITTENS*

A previous study[1] showed that supplementation with the probiotic strain *Enterococcus faecium* SF68 led to improved immune function and vaccine response in puppies. This study was performed to evaluate the effect of supplementation with *E. faecium* SF68 in immune function and vaccine response in kittens.

Study Design: This was a blinded, placebo-controlled study. Following a 10-day equilibration period to a standardized diet, twenty (20) 7-week old kittens were randomly allocated to two groups of 10 kittens each and housed by group. One group of kittens received *E. faecium* SF68, while the other received a placebo, for 20 weeks. All of the kittens were vaccinated at 9 and 12 weeks of age with a multivalent vaccine against feline herpesvirus-1 (FHV-1), feline calicivirus (FCV), and feline panleukopenia virus (FPV). The kittens' attitudes and behavior were monitored daily, and body weight was measured weekly. Five fecal samples were collected daily from the shared litterbox for each group and were scored using a standardized fecal consistency scoring system. The daily mean fecal score was determined for each group. Blood, saliva, urine and feces were collected from all cats at 7 weeks of age (prior to administration of the probiotic or placebo) and at 9, 15, 21 and 27 weeks of age. Blood samples were analyzed for complete blood count and serum biochemistry as well as immunologic assays. Humoral immune responses were estimated by measuring IgA and/or IgG against the vaccine components. Lymphocytes from whole blood samples were evaluated for the expression of CD4, CD8 and CD44 markers, and B cells were evaluated for B220, CD21 and major histocompatibility class II (MHC II) markers. Lymphocyte proliferation assays were performed to assess the kittens' active immune response. Specific IgA and IgG against FHV-1 were measured in saliva samples. Fecal extracts from samples taken at 9 weeks and 27 weeks of age were analyzed for total IgA and total IgG. Feces were analyzed to confirm the presence (probiotic group) or absence (placebo group) of *E. faecium* SF68 in the feces of the groups as well as to determine whether *Clostridium perfringens*, *Clostridium difficile* toxin A or B, *Salmonella* or *Campylobacter* were present.

Results: Eighteen kittens completed the study; one kitten in each group was removed for reasons unrelated to the study. Body weight and fecal scores were not statistically different between the two groups. Supplementation with *E. faecium* SF68 did not affect developmental parameters. The feces of all kittens were normal at the beginning of the study. Complete blood count and biochemical profiles were within normal limits for all cats at all time points. Feces from 7 of 9 kittens in the probiotic group were positive for *E. faecium* SF68 at least once during the study, whereas the entire placebo group was negative for *E. faecium* SF68 at all time points. *E. faecium* SF68 DNA was not detected in any feces from the probiotic group one week after it was discontinued. At 21 and 27 weeks of age, the mean levels of FHV-1-specific IgA in serum and saliva were greater in the probiotic group compared to the placebo group, but the difference was not statistically significant. Similarly, the mean FHV-1-specific serum IgG levels were numerically higher for the probiotic group at 15, 21 and 27 weeks of age and FPV-specific serum IgG were higher in the probiotic group at 15 weeks of age. Total IgG and IgA in serum and feces, as well as total IgA in the saliva, were similar between the groups. The percentage of CD4+ lymphocytes was significantly higher in the probiotic group at Day 27. There were no statistical differences in measurements of any other nonspecific or specific immune parameters between groups.

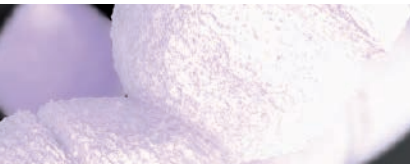


ENTEROCOCCUS FAECIUM *SF68 HAS IMMUNOMODULATORY EFFECTS IN KITTENS (CONT.)*

Discussion: *E. faecium* SF68 administration was safe for the kittens, did not interfere with normal development, and did not induce shedding of *Salmonella* or *Campylobacter*. After vaccination, each of the kittens developed specific serum antibodies against feline herpesvirus-1, feline calicivirus, and feline panleukopenia virus. The increase in CD4+ cell counts in the probiotic group compared to the placebo group at 27 weeks of age suggests systemic immune-modulating effects of the probiotic.

Veir, J. K., Knorr, R., Cavadini, C., Sherrill, S. J., Benyacoub, J., Satyaraj, E., & Lappin, M. R. (2007). Effect of supplementation with *Enterococcus faecium* (SF68) on immune function in cats. *Veterinary Therapeutics, 8*(4), 229-238.

1. Benyacoub, J., Czarnecki-Maulden, G. L., Cavadini, C., Sauthier, T., Anderson, R. E., Schiffrin, E. J., & von der Weid, T. (2003). Supplementation of food with *Enterococcus faecium* (SF68) stimulates immune function in young dogs. *Journal of Nutrition, 133*, 1158-1162.



ENTEROCOCCUS FAECIUM *SF68 PRESERVES MICROBIAL DIVERSITY DURING MINOR STRESS*

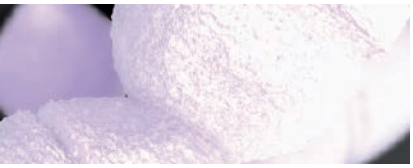
Feline herpesvirus 1 (FHV-1) infection is common in cats and causes fever, eye infections, and potentially fatal respiratory infections. Infected cats can develop chronic latent infections that result in the recurrence of clinical signs of illness when the cats are stressed by their environment or other diseases; affected cats can infect other cats. This study was conducted to evaluate the effects of the probiotic strain *Enterococcus faecium* SF68 on clinical disease as well as the frequency and severity of FHV-1 shedding episodes.

Study Design: Twelve (12) one-year-old cats with chronic FHV-1 infection and intermittent clinical illness were enrolled in the study. Following a two-week adjustment period (to allow the cats to adapt to their housing and diet), the cats were evenly divided into groups. One group of cats was administered *E. faecium* SF68 mixed with a palatability enhancer (test group) and the other group (placebo group) was fed the palatability enhancer without the probiotic. The cats were group housed for 28 days, individually housed for 28 days, and group housed for an additional 84 days. The presence or absence of sneezing, nasal discharge or conjunctivitis was recorded daily by observers blinded to the cats' treatment condition (test vs placebo). Body weight was measured weekly and blood samples were collected twice during the adjustment period and then approximately every 14 days for the duration of the study. Throat swabs were obtained weekly and feces were collected prior to supplementation and throughout the trial phases.

Results: All cats readily ate the palatability enhancer with or without the *E. faecium* SF68. Cats in the placebo group had decreased fecal microbial diversity compared to the test group ($p < 0.05$). Although sneezing or nasal discharge were uncommon in the cats during the study, there was a trend toward less conjunctivitis in test group cats during the last group housing period. During the supplementation period, the cats receiving *E. faecium* SF68 showed significantly fewer observation points with conjunctivitis compared to the placebo group (16.3% vs 29.3%, respectively; $p < 0.001$). No statistical differences were detected between groups or between periods for FHV-1 DNA detection on throat swabs, FHV-1 antibody values, or lymphocyte response.

Discussion: Administration of *E. faecium* SF68 preserved gut microbial diversity when cats were exposed to minor stresses (housing changes, neutering). The improvement in conjunctivitis observation points with *E. faecium* SF68 compared to placebo suggests a treatment effect, and further studies are indicated. However, the study did not determine the mechanism by which the effect may occur.

Lappin, M. R., Veir, J. K., Satyaraj, E., & Czarnecki-Maulden, G. (2009). Pilot study to evaluate the effect of oral supplementation of *Enterococcus faecium* SF68 on cats with latent herpesvirus 1. *Journal of Feline Medicine and Surgery, 11*(8), 650-654. doi: 10.1016/j.jfms.2008.12.006



ENTEROCOCCUS FAECIUM *SF68 DOES NOT ALTER GIARDIAL CYST SHEDDING*

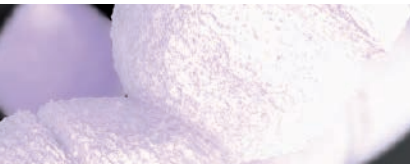
Infection with the protozoan parasite *Giardia* is fairly common, and clinically normal but infected dogs may be infectious for other dogs and possibly for humans. Previous research showed that the probiotic strain *Enterococcus faecium* SF68 reduced *Giardia* load and shedding in mice. This study was performed to evaluate the effect of six weeks of administration of *E. faecium* SF68 on dogs' immunity and shedding of giardial fecal cysts.

Study Design: Twenty (20) adult dogs with naturally acquired, chronic, subclinical *Giardia* infections were enrolled in the study. Following a 6-week dietary adjustment period, the dogs were divided into two equal groups for the (blinded?) crossover study. All dogs were maintained on the same diet, but one group received *E. faecium* SF68 while the other group received a placebo containing the same base ingredients but without the probiotic strain for 6 weeks. Each group then received the opposite condition (the group that initially received *E. faecium* SF68 received the placebo, and vice versa) for the next 6 weeks. Fecal samples were collected on 3 consecutive days each week for fecal scoring and analysis of giardial fecal cyst and antigen load. Fecal secretory IgA was measured on weeks 3, 9 and 15. Blood was collected at weeks 3, 9 and 15 to evaluate the leukocyte phagocytic ability.

Results: No significant differences were observed between the placebo or *E. faecium* SF68 treatment group for giardial cyst shedding, fecal antigen shedding, fecal IgA concentration, or leukocyte phagocytic activity. There was no evidence of upregulated adaptive immunity in response to *E. faecium* SF68.

Discussion: Six-week administration of *E. faecium* SF68 to dogs with chronic, naturally acquired, subclinical giardiasis did not alter giardial cyst shedding or antigen content and did not alter innate or adaptive immune responses. Additional studies are indicated to determine if longer-term supplementation with *E. faecium* SF68 would affect cyst shedding or decrease reinfection, or if pretreatment with *E. faecium* SF68 offers preventive benefit or decreases colonization by *Giardia*.

Simpson, K. W., Rishniw, M., Bellosa, M., Liotta, J., Lucio, A., Baumgart, M.,...Bowman, D. (2009). Influence of *Enterococcus faecium* SF68 probiotic on giardiasis in dogs. *Journal of Veterinary Internal Medicine, 23*, 476-481.



ENTEROCOCCUS FAECIUM *SF68 REDUCES PREVALENCE OF DIARRHEA IN SHELTER CATS*

Diarrhea is common in pets living in shelters. It may result from a variety of causes including stress, diet change, and pathogens (such as viruses, bacteria and parasites). Diarrhea can delay adoption and strain shelters' financial resources (due to the cost of treatment), and sufficiently severe or chronic cases may result in euthanasia. This study was performed to evaluate the efficacy of a specific probiotic strain of *Enterococcus faecium*, SF68, for the prevention of diarrhea in shelter dogs and cats.

Study Design: This double-blinded, placebo-controlled study evaluated dogs and cats housed in an animal shelter. The dogs and cats in the study were separated by species and each species was divided into two groups that were separately housed. Following a 4-week period to assess the baseline prevalence of diarrhea in the housing area, each group was fed either the *E. faecium* SF68 in a palatability enhancer or the placebo (only the palatability enhancer) for 4 weeks as a supplement to a complete and balanced diet. This was followed by a 1-week washout period during which they received neither supplement, then a 4-week period of the opposite condition (those receiving placebo in the first period received SF68 in the second, and vice versa). Daily fecal scoring was performed by individuals blinded to whether the dogs/cats were receiving the placebo or test condition, and those individuals dosing the dogs/cats were also blinded to the condition. Feces with a score of 4 or higher were considered diarrhea.

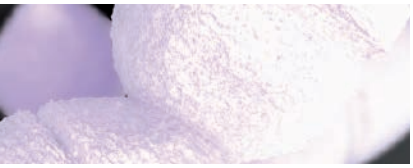
Results: 130 cats and 102 dogs were included in data analysis. Although the full study period was 13 weeks, some of the cats and dogs were placed in homes during the study. As a result not all of the dogs or cats were housed in the shelter for the full study period. Therefore, the diarrhea prevalence rates were reported for each housing area instead of consistent groups of the same animals.

Cats: The percentage of cats with diarrhea was significantly lower in the SF68 group compared to the placebo group (7.7% vs 20.7%, respectively; $p = 0.0297$). There were no significant differences between the groups for the number of episodes of diarrhea.

Dogs: No significant differences were observed between the groups, but the prevalence of diarrhea was too low to provide sufficient analysis: only one dog in each group had diarrhea for two or more days.

Discussion: Shelter cats supplemented with *E. faecium* SF68 had fewer episodes of diarrhea of 2 or more days' duration when compared to placebo-fed cats. Decreasing the prevalence of diarrhea could indirectly save shelters time and money, as well as improve animal welfare and likelihood of finding a home.

Bybee, S. N., Scorza, A. V., & Lappin, M. R. (2011). Effect of the probiotic *Enterococcus faecium* SF68 on presence of diarrhea in cats and dogs housed in an animal shelter. *Journal of Veterinary Internal Medicine, 25*, 856-860.



ENTEROCOCCUS FAECIUM *SF68 REDUCES FLATULENCE IN DOGS*

The purpose of this study was to determine the effect of supplementation with the probiotic strain *Enterococcus faecium* SF68 for 14 days on flatulence in dogs.

Study Design: Ten (10) adult dogs were enrolled in the study. All of the dogs were fed the same complete and balanced canine diet. Following baseline sample collection on Day 0, the dogs' diets were supplemented with *Enterococcus faecium* SF68 daily for 14 days. All dogs were fitted with a device that non-invasively sampled rectal gas, the number of flatus emissions, and hydrogen sulfide concentrations every 4 seconds for a period of four hours; sampling was performed on Day 0 and Day 14.

Results: Supplementation with *E. faecium* SF68 significantly reduced the number of flatus events/emissions and the maximum amount of hydrogen sulfide released.

Discussion: Supplementation of adult dogs' diets with the probiotic strain *Enterococcus faecium* SF68 for two weeks resulted in a reduction in the total number of flatulence events and in the maximum amount of hydrogen sulfide released during flatus.

Waldron, M., Kerr, W., Czarnecki-Maulden, G., & Davis, J. Supplementation with *Enterococcus faecium* SF68 reduces flatulence in dogs. Presented at: International Scientific Congress of the European Society of Veterinary and Comparative Nutrition; September 2012; Bydgosczc, Poland

ENTEROCOCCUS FAECIUM *SF68 DOES NOT ALTER FOOD INTAKE, BODY WEIGHT, BODY COMPOSITION OR METABOLIC PARAMETERS IN OVERWEIGHT OR OBESE CATS*

There is increasing evidence to support the role of the microbiome in the management of overweight and obesity. This study was performed to determine the effects of the probiotic strain *Enterococcus faecium* SF68 on food intake, body weight and composition, and metabolic profile of overweight and obese cats without comorbidities.

Study Design: Twenty (20) overweight (Body Condition Score 5.5-7/9) and obese (BCS 8-9/9), specific pathogen-free cats without comorbidities were divided into two groups of 10 cats each based on body weight and BCS. Following a 4-week acclimation and baseline period, cats in the probiotic group were given *E. faecium* SF68 mixed in their morning meal for eight weeks. A six-week washout period followed the intervention period, for a total study length of 18 weeks (4-week acclimation, 8-week intervention, 6-week washout). Food intake was measured daily and body weight and BCS were assessed weekly during each period. At the end of each of the three periods, body fat mass was calculated and blood was collected for analysis of triglyceride, cholesterol, fructosamine, insulin, leptin and adiponectin concentrations.

Results: Sixteen cats completed the study and were included in the analyzed data. Two cats were eliminated from each group for various reasons. All cats in the probiotic group accepted and consumed their daily amount of *E. faecium* SF68. Insulin levels were significantly higher and body weight was higher in the probiotic group at the end of the acclimatization period (before administration of the probiotic), and these differences carried over into the test period. There were no statistically significant differences in the other measured parameters between the two groups at any of the three time points.

Discussion: Short-term use of probiotic *E. faecium* SF68 had no effect on food intake, body weight, body composition or metabolic parameters in overweight or obese specific pathogen-free cats without comorbidities. However, the differences in the groups before the test period served as a confounder. Further studies are indicated to determine if longer duration of administration, a larger sample size, concurrent caloric restriction or use in client-owned cats affect the response. Feces were not collected for microbiome analysis in this study but may have provided valuable insight into the cats' microbiomes in response to the probiotic.

Kathrani, A., Larsen, J. A., Kass, P. H., & Fascetti, A. J. (2016). Effect of short-term probiotic *Enterococcus faecium* SF68 dietary supplementation in overweight and obese cats without comorbidities. *Veterinary Record Open, 3*, e000164. doi: 10.1136/vetreco-2015-000164

ENTEROCOCCUS FAECIUM *SF68 IMPROVES DIARRHEA IN DOGS WITH INTESTINAL OVERGROWTH OR ANTIBIOTIC-RESPONSIVE DIARRHEA*

Small intestinal diarrhea in dogs can be a diagnostic challenge for veterinarians. This study assessed whether dogs suffering from small intestinal diarrhea due to small intestinal bacterial overgrowth or antibiotic-responsive diarrhea would benefit from a combination of a therapeutic enteric diet[a] and the probiotic strain *Enterococcus faecium* SF68.

Study Design: Twenty-six (26) client-owned adult dogs with clinical signs compatible with chronic small intestinal diarrhea of at least 15 days duration that could not be attributed to a specific cause (e.g., infectious disease, neoplasia, food allergy or food intolerance, malabsorption syndrome, exocrine pancreatic insufficiency) were enrolled in the double-blinded, placebo-controlled clinical trial. The dogs were randomly divided into two groups: both groups were fed the therapeutic enteric diet, but only the test group was supplemented with *E. faecium* SF68; the placebo group received the same ingredients of the *E. faecium* SF68 supplement, but without the *E. faecium* SF68 probiotic itself. The dogs were clinically reevaluated every 7 days over a 20-day period, including complete physical examination, body weight, fecal score and microscopic fecal examination, urinalysis, hematology and serum biochemistry.

Results: Hematology and serum biochemistry values remained within normal limits for all dogs throughout the study period. During the first week of the study, no significant differences were found between the groups for diarrhea/fecal score. However, diarrhea had completely resolved in 6/16 (37.5%) of the dogs in the group receiving *E. faecium* SF68, compared to none of the dogs in the placebo group. After Day 14, there was a statistically significant difference between the groups, with the *E. faecium* SF68-supplemented group showing resolution of diarrhea ($p = 0.0002$) which continued during the third week of administration ($p = 0.0001$).

Discussion: The administration of the probiotic strain *E. faecium* SF68 in combination with a therapeutic diet specifically formulated for intestinal disorders improved diarrhea after 14 days in dogs with suspected short intestinal bacterial overgrowth or antibiotic-responsive diarrhea compared to the therapeutic diet alone.

Rallis, T. S., Pardali, D., Adamama-Moraitou, K. K., & Kavarnos, I. (2016). Effect of *Enterococcus faecium* SF68® (FortifFlora®) administration in dogs with antibiotic responsive or small intestinal bacterial overgrowth diarrhoea. *Hellenic Journal of Companion Animal Medicine, 5*(2), 13-16.

a: Purina® Pro Plan® Veterinary Diets EN Gastroenteric® Canine Formula

ENTEROCOCCUS FAECIUM *SF68 MAY IMPROVE CLINICAL OUTCOMES IN DOGS TREATED WITH METRONIDAZOLE FOR DIARRHEA*

This study was performed to determine whether the administration of a specific strain of *Enterococcus faecium* (SF68) in addition to metronidazole would improve clinical outcomes compared to metronidazole alone for dogs with diarrhea.

Study Design: Forty-eight (48) shelter dogs were enrolled in the study. All of the dogs were fed a standardized diet and were administered metronidazole (25 mg/kg, PO, q12h) for 7 days per shelter protocols. Twenty-four (24) dogs were also administered *E. faeciu*m SF68 with a palatability enhancer (treatment group), while the other 24 dogs received a placebo (only the palatability enhancer) during the time they were treated with metronidazole. Standardized fecal scoring system was used for all samples daily by an individual blinded to whether or not the dogs were receiving *E. faecium* SF68 or the placebo. The dogs were also assessed daily for their attitude (e.g., alertness or depression) and food consumption. The study did not determine the specific cause of the dogs' diarrhea, but was meant to replicate a typical shelter environment in which a short course of metronidazole treatment is often the first course of treatment for diarrhea.

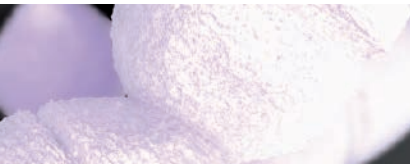
Results: All of the dogs readily ate the diet, the placebo and the *E. faecium* SF68. No side effects were observed from metronidazole treatment or *E. faecium* SF68 administration. Sixteen dogs in each group completed the study and were included in the statistical analysis. For dogs with fecal scores less than 5 on day 7, the median time to the initial normal stool was 2.5 days for the *E. faecium* SF68 group and 3 days for the placebo group; this difference was not significantly different. On Day 7, more dogs (11/16; 68.5%) in the *E. faecium* SF68 group had fecal scores less than 5 compared to the placebo group (6/16; 37.5%), but the difference was not statistically different. The mean daily diarrhea scores were greater (more diarrhea) for placebo group dogs on Days 5, 6 and 7, but the results were not significantly different. The percentage of fecal samples with a score of <5 was significantly higher for dogs in the *E. faecium* SF68 group compared to the placebo group on Days 4-7 (approximately 60% vs approximately 40%, respectively; $p = 0.0496$). Parasites detected in the dogs' feces included *Giardia*, *Cryptosporidium*, and *Cystoisospora*, and several dogs were coinfected with two or more of these parasites. Although both *Giardia*-infected dogs in the *E. faecium* SF68 group responded to treatment and cleared their infections while the majority (4/7) of placebo-treated dogs were still *Giardia* positive and had diarrhea on Day 7, the small number of dogs prevented statistical comparison.

Discussion: The probiotic strain *Enterococcus faecium* SF68 is unaffected by metronidazole, an antibiotic commonly used to treat dogs with diarrhea. The administration of *E. faecium* SF68 suggested an enhanced treatment effect compared to metronidazole alone. Dogs on dual therapy had significantly greater percentages of days with normal stool; a numerically higher percentage of dogs with normal stool on Day 7; and numerically lower diarrhea severity scores on Days 5-7.

ENTEROCOCCUS FAECIUM *SF68 MAY IMPROVE CLINICAL OUTCOMES IN DOGS TREATED WITH METRONIDAZOLE FOR DIARRHEA (CONT.)*

Discussion (cont.): Because the study did not determine the cause of the specific dogs' diarrhea, the investigators could not rule out the possibility of chronic enteropathy, food intolerance/food allergy or antimicrobial resistance as causes of persistent diarrhea in 15 of the dogs at the end of the study. Six dogs in the placebo group were persistently infected with *Giardia* despite treatment with metronidazole, suggesting the possibility of resistant strains. A larger study of *Giardia*-infected dogs would be necessary to determine if the combination of metronidazole and *E. faecium* SF68 is superior to metronidazole alone for treatment of *Giardia* infection. Diarrhea is a deterrent to adoption, causes discomfort for the dog, and drains shelter resources. Administration of *E. faecium* SF68 in combination with metronidazole appears to have a clinical benefit.

Fenimore, A., Groshong, L., Scorza, V., & Lappin, M. R. (2017). Evaluation of metronidazole with and without *Enterococcus faecium* SF68 in shelter dogs with diarrhea. *Topics in Companion Animal Medicine, 32*, 100-103. doi: 10.1053/j.tcam.2017.11.001



ENTEROCOCCUS FAECIUM *SF68 INDUCES IMMUNOMODULATION IN PUPPIES*

A previous study demonstrated the probiotic strain *Enterococcus faecium* SF68 improved immune responses to vaccination when fed to puppies from weaning to one year of age.[1] This study was performed to evaluate the effect of *E. faecium* SF68 on select immune responses in adult dogs.

Study Design: Seven (7) age-matched, clinically healthy Beagles were chosen for the study. The dogs were divided into two groups: one group received *E. faecium* SF68 while the other did not. Blood was collected from each dog prior to supplementation and then monthly for 12 weeks for B cell, T cell and cytokine analysis.

Results: Supplementation with *E. faecium* SF68 resulted in significantly greater T cell proliferative responses to stimulation ($p = 0.03$). Supplementation significantly increased the number of B cells expressing surface-bound IgG or MHCII as well as MHCII GMFI on Week 4 compared to Week 0; the increased levels were maintained through the completion of the study at Week 12. Compared to Week 0, dogs supplemented with *E. faecium* SF68 showed significantly greater T cell CD8+ CD11a+ and T cell CD8+ and CD11a GMFI but the effect was not apparent in Week 12. There were no significant differences detected in cytokine levels between the groups.

Discussion: Supplementation of adult Beagles with the probiotic strain *E. faecium* SF68 can induce immunomodulation as early as four weeks in dogs.

Lappin, M. R., Coy, J., Hawley, J., & Dow, S. Effect of a commercially available probiotic on immune responses in healthy dogs. Presented at: American College of Veterinary Internal Medicine; June 8-10, 2017; National Harbor, Maryland. Abstract NM05 2016

1. Benyacoub, J., Czarnecki-Maulden, G. L., Cavadini, C., Sauthier, T., Anderson, R. E., Schiffrin, E. J., & von der Weid, T. (2003). Supplementation of food with *Enterococcus faecium* (SF68) stimulates immune function in young dogs. *Journal of Nutrition, 133*, 1158-1162.

ENTEROCOCCUS FAECIUM *SF68 MAY REDUCE THE NEGATIVE IMPACTS OF AMOXICILLIN-CLAVULANATE IN CATS*

Antibiotics serve critical purposes in animal health, but can produce undesirable and debilitating side effects (e.g., diarrhea, vomiting, loss of appetite) in some animals. This study was performed to describe the clinical and microbiome impacts of amoxicillin-clavulanate (a broad spectrum antibiotic) administration in cats and to evaluate whether the administration of the probiotic strain *Enterococcus faecium* SF68 would mitigate these effects.

Study Design: This was a blinded, placebo-controlled study. Thirty-four (34) domestic cats were randomly allotted into 2 groups by body condition and housed by group in two separate rooms. All cats were fed the same diet throughout the study. After a 10-day equilibration period, all of the cats were administered canned food mixed with either the placebo or the *E. faecium* SF68 (depending on the group to which the cat was assigned) as its morning meal. Amoxicillin-clavulanate (62.5 mg/kg by mouth) was administered two hours later and again in the evening for 7 days. Administration of the placebo or *E. faecium* SF68 was continued for an additional 7 days after stopping the antibiotic. Fecal consistency was scored using a standardized system by observers blinded to the condition. Hydration, attitude and appetite scores were determined daily, and vomiting was recorded if noted in the cages. Blood samples were collected to evaluate serum cyanocobalamin and folate concentrations, and feces were collected for microbiome analysis prior to starting the 14-day supplementation period, the first morning following the last day of antibiotic administration, and 7 days after the last day of antibiotic administration.

Results: Thirteen cats in the probiotic group and 14 cats in the placebo group completed the trial and were included in the data analysis. The remaining cats were excluded for being prone to stress diarrhea. At least one episode of vomiting was noted during the antibiotic administration period in 53.8% of the probiotic group cats and 35.7% of the placebo group cats; however, the total number of vomiting episodes during the antibiotic administration period were not significantly different between the two groups. During antibiotic administration and for 4 days afterward, the number of cats with diarrhea did not differ between the groups but the placebo group cats showed more severe diarrhea. Fewer cats in the probiotic group developed fecal scores >5 than cats in the placebo group (69.2% vs 85.7%, respectively), but the difference was not statistically significant. When the severity of the diarrhea was considered, none of the cats in the probiotic group developed a fecal score of >6 whereas 3 of the cats in the placebo group had fecal scores >6 on at least several days. The total diarrhea score for Days 1-11 were significantly lower in the cats in the probiotic group compared to the placebo group. There were significant changes in the microbiome of both groups on Day 7 of antibiotic administration, and these changes persisted 7 days after stopping the antibiotic. The administration of the antibiotic altered the microbiome by significantly reducing the number of species observed in the microbiome analysis. *Bifidobacterium*, *Dialister* and *Peptococcus* species reduced in abundance during antibiotic administration, but did not rebound after the antibiotic was stopped. Enterobacteriaceae significantly increased during antibiotic administration but returned to baseline after antibiotics were discontinued. There were no differences between the placebo and the probiotic groups in any of the periods measured.

ENTEROCOCCUS FAECIUM *SF68 MAY REDUCE THE NEGATIVE IMPACTS OF AMOXICILLIN-CLAVULANATE IN CATS (CONT.)*

Discussion: Although analysis did not detect statistically significant differences between the probiotic and placebo groups, clinically relevant findings suggest that the administration of *E. faecium* SF68 was beneficial:

- Fewer cats in the probiotic group developed severe diarrhea: none of the cats in the probiotic group developed a fecal score >6, whereas 3 cats in the placebo group had fecal scores >6 on at least several days
- The total diarrhea scores for Days 1-11 were significantly lower for cats in the probiotic group

The administration of amoxicillin-clavulanate alters the microbiome, and the microbiome is not restored to baseline for at least 7 days after stopping the antibiotic. This antibiotic is known to inactivate *E. faecium* SF68; this may explain the reduced benefits of the probiotic in combination with this antibiotic when compared to the significant benefits observed with co-administration with metronidazole, which does not inactivate *E. faecium* SF68.

Torres-Henderson, C., Summers, S., Suchodolski, J., & Lappin, M. R. (2017). Effect of *Enterococcus faecium* SF68 on gastrointestinal signs and fecal microbiome in cats administered amoxicillin-clavulanate. *Topics in Companion Animal Medicine, 32*, 104-108. doi: 10.1053/j.tcam.2017.11.002

ENTEROCOCCUS FAECIUM *SF68 PRODUCES NON-CLINICALLY RELEVANT CHANGES IN SERUM COBALAMIN AND FOLATE*

Cobalamin and folate are water-soluble B vitamins that are plentiful in the normal diet of dogs and cats. Both vitamins are considered markers of intestinal absorption because they are absorbed in the small intestine. This study was performed to determine the effect of a 14-day administration of the probiotic strain *Enterococcus faeciu*m SF68 on serum concentrations of cobalamin and folate in healthy dogs.

Study Design: Thirty-six (36) healthy, privately-owned adult dogs (2-5 years of age) were enrolled in the study. The dogs were divided by sex and randomly assigned to either a control group or the probiotic group, with 18 dogs in each group and approximately equal male:female distribution. All dogs were fed the same balanced diet for two weeks prior to the initiation of the study and for the duration of the study to minimize dietary influence on the vitamin levels; the cobalamin and folate levels of the diet were not measured. The dogs in the probiotic group received *E. faecium* SF68 once daily, sprinkled over the dogs' morning meal, for 14 days. Blood samples were obtained on Day 1 (baseline; at the start of probiotic administration), Day 14 (when the probiotic administration ended) and Day 28 (two weeks after probiotic administration was ended) following 18-hour food withdrawal to determine serum cobalamin and folate concentrations. Canine inflammatory bowel disease activity index (CIBDAI) scores were also determined based on the dogs' general attitude, appetite, weight, fecal consistency, defecation frequency, and vomiting.

Results: The probiotic was accepted and well tolerated by all dogs.

Cobalamin

The probiotic group demonstrated significantly lower serum cobalamin concentrations at Day 28 (two weeks after the probiotic was discontinued) compared to Day 1; on the last day of probiotic administration (Day 14), there was no significant difference in mean serum cobalamin concentrations. Hypocobalinemia (defined as serum cobalamin levels below normal reference range) was observed in four dogs at Day 14 and in eight dogs at Day 28; this included one dog that had baseline levels below the normal reference range. Serum cobalamin levels for the remaining dogs remained within the normal reference range. In the control group, serum cobalamin concentrations were not significantly different between baseline and Day 14 or Day 28. Two dogs in the control group had baseline serum cobalamin levels below the normal reference range: one of these dogs remained low at Day 14 but was normal at Day 28. Three control dogs at Day 14 and one control dog at Day 28 had serum cobalamin levels below normal reference range. At Day 28 (14 days after the probiotic was discontinued), serum cobalamin concentrations were reduced in 16/18 (89%) and 4/18 (22%) of dogs in the probiotic vs control groups, respectively. Serum cobalamin levels reduced by more than 20% in 9/18 (50%) dogs in the probiotic group and 1/18 (5.6%) dogs in the control group. Although mean serum cobalamin concentrations decreased in the probiotic group at Day 14 and 28 compared to baseline, comparisons between the probiotic and control groups were non-significant.

ENTEROCOCCUS FAECIUM *SF68 PRODUCES NON-CLINICALLY RELEVANT CHANGES IN SERUM COBALAMIN AND FOLATE (CONT.)*

Results (cont.):

Folate

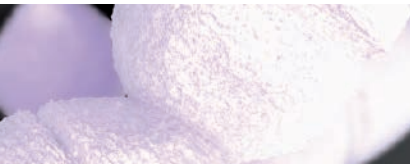
Mean serum folate concentrations in the probiotic group were not significantly different when comparing Day 1 with Day 14 (last day of probiotic administration) and Day 28 (14 days after the probiotic was discontinued). However, the mean serum folate at Day 28 was significantly lower than at Day 14. No significant differences were observed in the control group when comparing folate concentrations at any time point.

CIBDAI score

The CIBDAI remained unaltered at a score of 0 throughout the study.

Discussion: Short-term administration of the probiotic strain *Enterococcus faecium* SF68 was associated with a significant reduction of mean cobalamin concentration at Day 28 (two weeks after the probiotic was discontinued) compared to baseline. Despite the reduction, no significant differences were found between the probiotic and control groups and all of the dogs remained clinically normal. Administration of the probiotic was associated with a non-significant increase in serum folate and a significant decrease in serum folate after administration was discontinued. *E. faecium* SF68 produces folate, which can explain the folate increase during its administration and the reduction in folate associated with discontinuation of the probiotic.

Lucena, R., Olmedilla, A. B., Blanco, B., Novales, M., & Ginel, P. J. (2018). Effect of *Enterococcus faecium* SF68 on serum cobalamin and folate concentrations in healthy dogs. *Journal of Small Animal Practice, 59*, 438-443. doi: 10.1111/jsap.12845



ENTEROCOCCUS FAECIUM *SF68 DOES NOT ALTER LIVER ENZYMES IN DOGS*

Probiotics have been associated with alterations in some serum biochemistry test results. This study was performed to evaluate the effect of the probiotic strain *Enterococcus faecium* SF68 on the levels of several liver enzymes as well as cholesterol and triglyceride concentrations in healthy dogs, and was part of the same study for which some results (the effects of the probiotic on serum cobalamin and folate levels) have previously been published.[1]

Study Design: In this blinded, controlled study, thirty-six (36) healthy, privately-owned adult dogs (2-5 years of age) were enrolled in the study. The dogs were divided by sex and randomly assigned to either a control group or the probiotic group, with 18 dogs in each group and approximately equal male:female distribution. All dogs were fed the same balanced diet for two weeks prior to the initiation of the study and for the duration of the study to minimize dietary influence on the vitamin levels; the cobalamin and folate levels of the diet were not measured. The dogs in the probiotic group received *E. faecium* SF68 once daily, sprinkled over the dogs' morning meal, for 14 days. Blood samples were obtained on Day 1 (baseline; at the start of probiotic administration), Day 14 (when the probiotic administration ended) and Day 28 (two weeks after probiotic administration was ended) following 18-hour food withdrawal to determine serum alanine transferase (ALT) and alkaline phosphatase (ALP) activity, total cholesterol, and triglyceride concentrations.

Results: The probiotic did not induce any significant changes in mean ALT and ALP levels. Two dogs in the probiotic group showed concentrations above reference range on Day 14, but both dogs remained clinically normal and their levels returned to normal. Mean serum cholesterol concentration did not change during the 14-day probiotic administration period, but a significant decrease in cholesterol was seen on Day 28 (14 days after the probiotic was discontinued). However, this change was not clinically relevant because all values remained within the reference range. Mean triglyceride concentration increased progressively, with a significant increase observed at Day 28. However, these changes were not clinically relevant because the values remained within the reference range. One dog in the probiotic group developed hypertriglyceridemia (defined as triglyceride levels above normal reference range) on Day 28 (two weeks after probiotic administration was discontinued).

Discussion: Short-term administration of the probiotic strain *E. faecium* SF68 did not alter the levels of two primary liver enzymes and did not produce clinically relevant changes in cholesterol or triglyceride concentrations. This indicates the probiotic would not affect levels of these enzymes when administered to dogs with liver disease, and may be the probiotic of choice in dogs with liver disease.

Lucena, R., Novales, M., Blanco, B., Hernández, E., & Ginel, P. J. (2019). Effect of probiotic *Enterococcus faecium* SF68 on liver function in healthy dogs. *Journal of Veterinary Internal Medicine, 33*, 2628-2634. doi: 10.1111/jvim.15625

1. Lucena, R., Olmedilla, A. B., Blanco, B., Novales, M., & Ginel, P. J. (2018). Effect of *Enterococcus faecium* SF68 on serum cobalamin and folate concentrations in healthy dogs. *Journal of Small Animal Practice, 59*, 438-443. doi: 10.1111/jsap.12845

ENTEROCOCCUS FAECIUM *SF68 DOES NOT FACILITATE REDUCTION OF OCLACITINIB DOSAGE IN DOGS WITH ATOPIC DERMATITIS*

Previous studies have suggested an immunomodulatory role of the probiotic strain *Enterococcus faecium* SF68.[1] A limited number of studies have suggested that increasing lactic acid-producing species in the gut, which has been shown to occur with *E. faecium* SF68 supplementation, may prevent or alleviate the signs of atopic dermatitis. This study was performed to evaluate whether the addition of *E. faecium* SF68 to the management regimen for chronic atopic dermatitis would impact the clinical disease scores.

Study Design: This was a blinded, placebo controlled trial. Twenty-one (21) client-owned dogs with controlled environmental atopic dermatitis while on oclacitinib were enrolled. The dogs were blindly and randomly allocated to two groups; one group received twice daily administration 1x108 CFU/g of *E. faecium* SF68 for 12 weeks, while the other received a placebo. The dogs were maintained on oclacitinib throughout the study. Scores based on the Canine Atopic Dermatitis Extent and Severity Index (CADESI-4) were assessed by the investigators at enrollment (Day 0), Day 56, Day 70 and Day 84 of the study. Clients were asked to record scores based on the Pruritis Visual Analog Score (PVAS) three times weekly for the first 6 weeks, then daily for the remaining 6 weeks of the study. Skin and ear cytology were evaluated if applicable based on these scores and treated appropriately with topical medications if infection was identified. Disease "flares" (increases in either or both scores) were addressed as needed. The dose of oclacitinib was reduced by approximately 25% at Day 56 or Day 70 if the scores were at or below baseline. If a disease flare occurred, the dog's dose was returned to baseline.

Results: Nineteen dogs (10 in the probiotic group and 9 in the placebo group; one dog was removed from the probiotic group at Day 56 and one dog was removed from the placebo group at Day 70) completed the study. Five (5) of the 11 dogs originally in the probiotic group qualified for oclacitinib reduction and three of the five remained controlled at the lower dose. Eight (8) of 10 dogs in the placebo group qualified for dose reduction, and seven of the eight remained controlled at the lower dose. There were no statistical differences in the effects of oclacitinib reduction in the probiotic versus placebo groups. Seven dogs (4 in probiotic group, 3 in placebo group) experienced disease flares before oclacitinib dose reduction. Of these seven dogs, one dog in each group qualified for oclacitinib dose reduction and both remained controlled at the reduced dose. Two dogs (one in each group) experienced a disease flare at Day 56 in response to oclacitinib dose reduction; the dogs improved when the oclacitinib dose was returned to baseline. The CADESI-4 scores were significantly lower in the placebo group compared to the probiotic group at Day 56 and the scores in the placebo group were significantly decreased from baseline at Day 84. However, there were no statistical differences in the clinical disease scores at study completion. The PVAS scores did not significantly change in either group at any time point compared to baseline.

ENTEROCOCCUS FAECIUM *SF68 DOES NOT FACILITATE REDUCTION OF OCLACITINIB DOSAGE IN DOGS WITH ATOPIC DERMATITIS (CONT.)*

Discussion: Based on the results of this pilot study, *E. faecium* SF68 was not effective for reducing oclacitinib dosage while maintaining or reducing clinical disease score or pruritis. This may have been due to a number of factors including small sample size; suboptimal probiotic dosing or insufficient duration of administration; choice of probiotic strain; patient variability, including the cyclical nature of atopic dermatitis; variation in systemic and topical treatments during the study; and client compliance. In addition, the immunomodulatory properties of *E. faecium* SF68 are believed to be due to anti-pathogenic activity in the gastrointestinal tract, which would be expected to impact its efficacy for atopic dermatitis. Further large-scale studies are indicated to determine the role of probiotics in the management of atopic dermatitis in dogs.

Yamazaki, C., Rosenkrantz, W., & Griffin, C. (2019). Pilot evaluation of *Enterococcus faecium* SF68 as adjunctive therapy for oclacitinib-responsive adult atopic dermatitis in dogs. *Journal of Small Animal Practice, 60*(8), 499-506. doi: 10.1111/jsap.13042

1. Benyacoub, J., Czarnecki-Maulden, G. L., Cavadini, C., Sauthier, T., Anderson, R. E., Schiffrin, E. J., & von der Weid, T. (2003). Supplementation of food with *Enterococcus faecium* (SF68) stimulates immune function in young dogs. *Journal of Nutrition, 133*, 1158-1162.

THE CANINE ORAL MICROBIOME IS STABLE OVER TIME AND NOT AFFECTED BY SUPPLEMENTATION WITH ENTEROCOCCUS FAECIUM *SF68*

The administration of an oral probiotic impacts the oral microbiome of humans, but has not been evaluated in dogs. The objective of this study was to determine the core organisms of the normal canine oral microbiome and to evaluate the effects of administration of the probiotic strain *Enterococcus faecium* SF68 on the oral microbiome in healthy dogs.

Study Design: Working dogs in training were enrolled in the study. The dogs were assigned to test or control group and acclimated to one of three commercial diets for a minimum of 30 days prior to the study. Following acclimation, dogs in the test group received daily supplementation with *E. faecium* SF68 while control dogs received no supplement for 7 weeks. Oral swabs were used to obtain weekly samples for microbiome analysis. The dog handlers and sample collectors were blinded to each dog's group assignment.

Results: Thirteen (13) working dogs in training were enrolled in the study. The dogs' mean age was 13.5 months old and their average weight was 26 kg (57.2 lbs). Breeds in the study included German Shepherds, Belgian Malinois, Dutch Shepherds and Labrador Retrievers. All of the dogs were free of evidence of oral disease. The dogs were individually housed in kennels during the day for 5 days a week, and in foster homes in the evenings and on weekends. Six (6) dogs were placed in the test group and seven (7) dogs were placed in the control group. Once the dogs were acclimated to their diet, no dietary changes were made during the duration of the study.
The oral microbiome was dominated by eight phyla representing more than 99% of the relative abundance:

- Proteobacteria (43.8%)
- Bacteroidetes (22.5%)
- Firmicutes (18.9%)
- Actinobacteria (6.1%)
- Fusobacteria (3.6%)
- Gracilibacteria (2.1%)
- SR1 Absconditabacteria (1.5%)
- Saccharibacteria (1.3%)

There was no effect of diet or probiotic administration on the relative abundance of the microbial composition of the oral microbiome. The data showed there are ubiquitous taxa that were present across all dogs and all samples regardless of breed, sex, diet, treatment or other factors. These genera included *Actinomyces*, *Corynebacterium*, *Capnocytophaga*, *Flavobacterium*, *Gemella*, *Abiotrophia*, *Streptococcus* and *Frederiksenia*.

Discussion: Based on the results of this study, the canine oral microbiome is remarkably stable (highly conserved) over time and the oral microbiome of normal, healthy working dogs was not affected by diet, time or the administration of the probiotic strain *E. faecium* SF68. This study identified the core organisms, eight phyla that represent more than 99% of the oral microbiome.

Bell, S. E., Nash, A. K., Zanghi, B. M., Otto, C. M., & Perry, E. B. (2020). An assessment of the stability of the oral canine microbiota after probiotic administration in healthy dogs over time. *Frontiers in Veterinary Science, 7*, 616. doi: 10.3389/fvets.2020.00616

4. PROBIOTICS AND THE GUT-BRAIN AXIS

The gastrointestinal microbiota affects brain function and behavior, and the brain, in turn, influences the microbiota through bidirectional intercommunication. Altering the gut microbiota via nutritional interventions has the potential to facilitate cross-talk between the gut and brain, and influence behavior and mood.

Weimaraner

PROBIOTIC STRAIN OF BIFIDOBACTERIUM LONGUM *HAS ANXIOLYTIC EFFECTS*

Pet anxiety is a major concern for pet owners, and chronic anxiety and behavioral issues can negatively impact animal welfare and the pet-owner bond. There is growing evidence that the gut microbiome can influence behavior through the gut-brain axis. The purpose of this study was to evaluate the effects of supplementation with the probiotic strain *Bifidobacterium longum* BL999 on anxious behavior in dogs.

Study Design: This was a blinded, placebo-controlled, crossover study. Twenty-four (24) anxious Labrador Retrievers were enrolled in the study. All of the dogs were acclimated to a complete and balanced diet. One group of dogs was supplemented with the probiotic strain *B. longum* BL999 and the other group was supplemented with a placebo for six (6) weeks. Following a 3-week washout period, the group that previously received the probiotic received the placebo, and vice versa, for an additional 6 weeks. During each phase, the dogs' typical behavior in response to day-to-day stimuli was observed to look for a defined set of anxious behaviors. All dogs also underwent a formal anxiety test at the end of each phase. Cardiac activity was recorded through the entirety of the anxiety test, and salivary cortisol concentrations were assessed prior to and following the anxiety test.

Results: The dogs supplemented with *B. longum* BL999 demonstrated significant improvement in four day-to-day anxious behaviors (barking, jumping, spinning, pacing) compared to when they were supplemented with the placebo. During the formal anxiety test, *B. longum* BL999-supplemented dogs showed increased exploratory behavior in a novel environment, a decrease in mean heart rate and an increase in heart rate variability compared to when they were supplemented with the placebo. The dogs also showed significantly reduced salivary cortisol concentrations in response to exercise and to anxiety-inducing stimuli when supplemented with *B. longum* BL999.

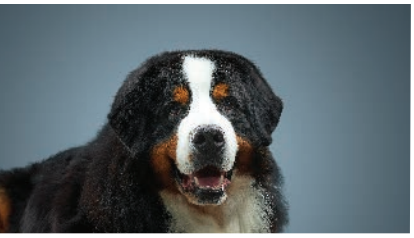
Discussion: Dogs supplemented with *B. longum* BL999 were less reactive (as indicated by lower cortisol levels), more calm (as indicated by lower mean heart rates), and potentially in a better emotional state (as indicated by increased heart rate variability) when experiencing anxiety-provoking stimuli than when they were supplemented with a placebo. In addition, the dogs exhibited less daily anxious behavior when supplemented with *B. longum* BL999 compared to when they were supplemented with placebo. Supplementation with *B. longum* BL999 had an anxiolytic effect on anxious dogs, and could serve as a useful tool in a comprehensive anxiety management plan.

McGowan, R. T. S., Barnett, H. R., Czarnecki-Maulden, G., Si, X., Perez-Camargo, G., & Martin, F. Tapping into those 'gut feelings': Impact of BL999 (*Bifidobacterium longum*) on anxiety in dogs. ACVB Veterinary Behavior Symposium; July 12, 2018; Denver, Colorado.

5. SYNBIOTICS

Synbiotics are combinations of prebiotics and probiotics and are intended to introduce beneficial bacterial populations (as probiotics) as well as promote proliferation of beneficial bacterial species by providing preferred energy and food sources for the bacteria (in the form of prebiotics).

Bernese Mountain Dog and *Enterococcus faecium*



SYNBIOTIC PRODUCES BENEFICIAL EFFECT ON THE DOGS' MICROBIOME AND DECREASES PREVALENCE OF DIARRHEA IN TRAINING SLED DOGS

Synbiotics are combinations of prebiotics and probiotics and are intended to introduce beneficial bacterial populations (as probiotics) as well as promote proliferation of beneficial bacterial species by providing preferred energy and food sources for the bacteria (in the form of prebiotics). The goal of this study was to examine alterations in fecal quality, short-chain fatty acids (SCFA), and the fecal microbiome in two groups of training sled dogs fed a synbiotic or a placebo. Sled dogs were evaluated in the study because they exhibit a high prevalence of diarrhea during athletic events.

Study Design: This was a prospective, double-blinded, placebo-controlled study. Twenty clinically healthy training sled dogs 2-6 years of age were gender matched and randomly allocated to two groups. Dogs in the test group were fed a diet supplemented with a synbiotic[a] while dogs in the control group were fed the same diet supplemented with a placebo for 6 weeks. The dogs were kenneled individually, but were not segregated to treatment groups during training or travel. Fecal scores were assessed at baseline (10 days prior to the test period) and every day during the test period. Fecal pH and fecal SCFA (acetate, propionate and butyrate) concentrations were measured and the dogs' microbiomes were analyzed at baseline and after two weeks of treatment with either the synbiotic or the placebo.

Results: Seventeen (17) dogs completed the study: 9 dogs in the synbiotic group and 8 dogs in the control group. Three dogs were excluded from the study due to acute injuries during training. An initial microbial analysis of the synbiotic confirmed the presence of viable organisms. At the end of the trial, only *E. faecium* SF68 could be cultured from the synbiotic supplement. Dogs in the synbiotic group showed a significant rise in Lactobacillaceae after 2 weeks of treatment. This increase correlated with an overall increase in butyrate concentration. *Lactobacillus* and *Bifidobacteria* species were significantly increased in synbiotic-fed dogs after two weeks of supplementation; these changes were not observed in the placebo group. There was no significant difference in fecal pH between the two groups. Between Weeks 4 and 5 of administration, the dogs in the synbiotic group showed an improved fecal score and fewer days of diarrhea. This coincided with an outbreak of presumed contagious diarrhea in the kennel. When examined as total days of diarrhea between the two groups, the synbiotic group showed significantly fewer days of diarrhea than the placebo group.

Discussion: Based on the results of this study, use of this synbiotic resulted in a presumed beneficial effect on the dogs' microbiome and a decrease in the prevalence of diarrhea in training sled dogs. Fewer days of diarrhea were observed when a presumed contagious outbreak of diarrhea was observed in the dogs supplemented with the synbiotic during Week 5 of treatment, further suggesting a beneficial effect.

Gagné, J. W., Wakshlag, J. J., Simpson, K. W., Dowd, S. E., Latchman, S., Brown, D. A.,...Fahey, G. D. (2013). Effects of a synbiotic on fecal quality, short-chain fatty acid concentrations, and the microbiome of healthy sled dogs. *BMC Veterinary Research, 9*, 246. doi: 10.1186/1746-6148-9-246

a: The synbiotic contained the following probiotic bacterial strains: *Enterococcus faecium* SF68, *Bacillus coagulans*, and *Lactobacillus acidophilus*. The synbiotic also contained fructooligosaccharides and mannanoligosaccharides as prebiotics. See the full manuscript for a complete description of the ingredients.

Purina was the first to offer a shelf-stable probiotic supplement proven to promote a healthy immune system and provide dietary management of dogs or cats with diarrhea. This probiotic, a specific strain of *Enterococcus faecium* we call *E. faecium* SF68 (NCIMB 10415 4b1705), remains the most studied probiotic in veterinary medicine based on publications to date. Purina was also the first to offer a shelf-stable probiotic (*Bifidobacterium longum* BL999, NCC 3001) proven to help dogs maintain calm behavior. In addition to product development-focused research, Purina performs and funds research that advances scientific knowledge of the microbiome health and the impact of nutrition.

As part of Nestlé, Purina draws on the unequaled culture collection and decades of microbiome and probiotic research (reflected by more than 300 peer-reviewed publications to date) to evaluate potential probiotic strains of value to veterinary medicine and pet health.

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