

**MORE THAN A MEAL:**

PUTTING PURINA'S MICROBIOME  
SCIENCE INTO PRACTICE



Golden Retriever, Russian Blue, and *Bifidobacterium*

At Purina, we are strongly committed to advancing nutritional science to benefit pet health. Our team of more than 500 scientists – including nutritionists, veterinarians, behaviorists, molecular nutritionists, biochemists, microbiologists, and more – have a proven track record of making nutritional discoveries that improve the lives of pets around the globe.

The **microbiota** encompasses all of the bacteria, fungi, viruses or protozoa that reside within a given space (e.g., gut, skin, mouth), whereas the **microbiome** is the combined genetic material of the organisms in the gut. 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. Listed below are some of the clinically relevant learnings from decades of Purina research on the microbiome.

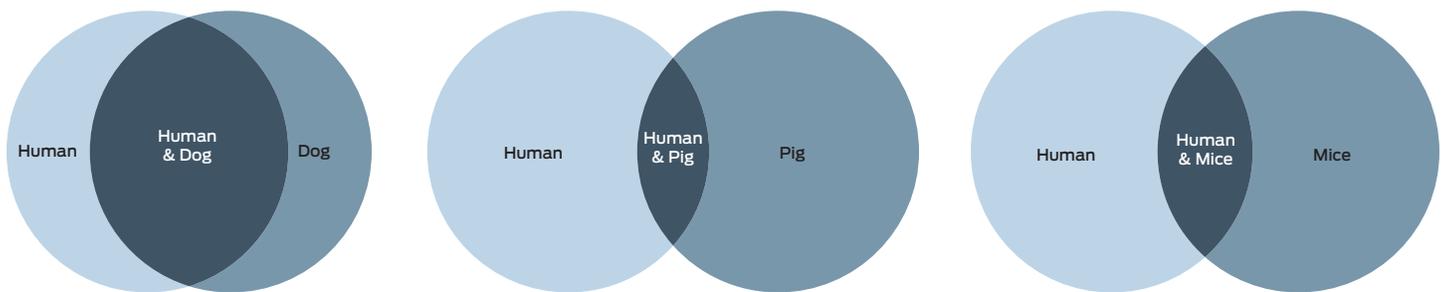


Mixed Bacterial Species

# DOGS SHARE MORE THAN OUR LIVES

The Purina “catalog” – the collection of genetic sequencing data – of dog genes provides over one million annotated genes.

- The dog microbiome is closer to the human microbiome than the pig or the mouse. The overall 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%). (*Coelho et al, 2017*)
- 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. (*Coelho et al, 2017*)



Venn diagrams show the overlap of the human microbiome with the microbiomes of dogs (left), pigs (middle) and mice (right).

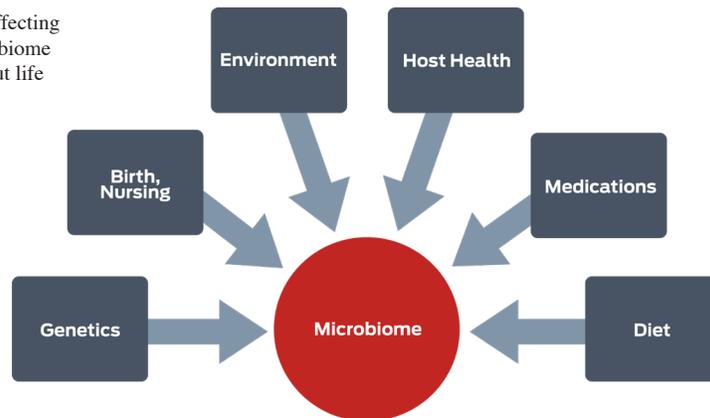
Coelho, L. P., Kultima, J. R., Costea, P. I., Fournier, C., Pan, Y., Czarnecki-Maulden, ...Bork, P. (2018). Similarity of the dog and human gut microbiomes in gene content and response to diet. *Microbiome*, 6(72). doi: 10.1186/s40168-018-0450-3

# A PET'S MICROBIOME IS SHAPED BY MANY FACTORS

The microbiomes of dogs<sup>1</sup> and cats<sup>2,3</sup> change with age, which can lead to dysbiosis (an imbalance of beneficial and potentially harmful bacteria). But age is just one factor that shapes the microbiome.

- 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; this may be due to exposure to a wider range of microbes in larger cities. (Vilson *et al*, 2018)
- The bitch's microbiome changes after whelping and during lactation, and in puppies as they mature. (Vilson *et al*, 2018)
- 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. (Vilson *et al*, 2018)
- Antibiotics such as metronidazole<sup>4</sup> and amoxicillin-clavulanate alter the microbiome, and these changes may persist after discontinuation of the antibiotics. (Torres-Henderson *et al*, 2017)

Factors affecting  
the microbiome  
throughout life



## CONVERSATION STARTER:

*“Like us, our pets have a complex and diverse community of organisms – including bacteria – living in their gut. The health of that community, called the microbiome, is strongly linked to our pets’ overall health. There are many external factors, such as environment, diet and medications, that can affect the health of the microbiome. Some diseases can be caused by or can contribute to imbalances in the community. We need to consider that when we make decisions on your pet’s treatment.”*

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](https://doi.org/10.1371/journal.pone.0193507)

# DIET CAN HAVE A SIGNIFICANT IMPACT ON THE COMPOSITION OF MICROBIOME

Diet is one of the most accessible factors that influences the microbiome, and gives owners an opportunity to contribute to microbiome and gut health through the simple act of feeding their pet.

- Even the formulation – wet diet vs dry diet, for example – can impact the microbial community and its metabolism. (Martineau & Laflamme, 2002)
- Diet has a large and reproducible effect on the dog’s microbiome, and these effects are similar to what has been observed in human microbiome studies. (Li et al, 2017)
- The microbiome of obese dogs fed a high protein, low carbohydrate diet more closely resembled the microbiome of lean dogs. (Li et al, 2017)

Prebiotics, such as inulin and mannan-oligosaccharide (MOS), are non-digestible food ingredients that selectively stimulate growth and/or activity of potentially beneficial bacteria.

- Supplementing aged dogs’ diets with MOS or inulin (from chicory) increased the amount of beneficial *Bifidobacteria*, and MOS supplementation decreased *E. coli*. (Grieshop et al, 2004)
- Prebiotics do not significantly alter the food’s digestibility. (Grieshop et al, 2004)

## CONVERSATION STARTER:

“Your pet’s diet can have a profound impact on [his/her] microbiome, which is the community of organisms that lives in your pet’s gut. Including a prebiotic in your pet’s diet, which is basically food provided specifically for the organisms in the gut, can help increase the amounts of beneficial bacteria.”

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.

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.

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

# OBESITY HAS A SIGNIFICANT IMPACT ON THE MICROBIOME

In addition to the known health risks associated with overweight and obesity, these conditions also impact the microbiome.

- The microbiomes of lean and obese dogs are different. *(Li et al, 2017)*
- Obese dogs experience larger shifts in the microbiome in response to dietary changes. This may indicate that their microbiome is less stable than that of healthy dogs, but it could also present opportunities for nutritional intervention to improve weight management. *(Li et al, 2017)*

## CONVERSATION STARTER:

*“Research has shown that the gut microbiome, which is the community of organisms that lives in our pet’s gut, is different in overweight pets when compared to pets with healthy body weights. Overweight pets’ microbiomes may be less stable, putting them at higher risk of imbalance – called dysbiosis – that could lead to health issues. We’ll need to keep that in mind and make all dietary changes slowly.”*

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.

## IMMUNOMODULATION THROUGH THE GUT AND THE MICROBIOME

The gut-associated lymphoid tissue (GALT) of the gastrointestinal tract represents almost 70% of the body's entire immune system;<sup>5</sup> this underscores the critical roles of the gastrointestinal tract and the microbiome in host immunity and defense. The GALT produces IgA, which has protective effects for the mucosa and microbiome.

- Supplementing adult dogs' diets with bovine colostrum led to greater microbial species diversity, indicating the dogs had stable microbiomes that were more resistant to challenge. (*Satyraj et al, 2013*)
- Supplementation with bovine colostrum also resulted in a significant increase in production of antigen-specific IgG in response to canine distemper vaccination, indicating an improved immune response to vaccination. (*Satyraj et al, 2013*)

Probiotics may enhance immunity and defense through direct (via cytokines) or indirect (via balancing the microbiome and mitigating dysbiosis) mechanisms.

- Feeding a dry dog food supplemented with the probiotic *Enterococcus faecium* SF68 enhanced long-term immune functions in growing dogs. (*Benyacoub et al, 2003*)
- The probiotic strain *E. faecium* SF68 may increase priming of naïve B cells in response to initial canine distemper virus vaccination, which may enhance the effectiveness of the vaccine in preventing infection. (*Benyacoub et al, 2003*)
- Kittens supplemented with *E. faecium* SF68 showed increased CD4+ cell counts compared to those supplemented with a placebo at 27 weeks of age, suggesting immunomodulatory benefits. (*Veir et al, 2007*)
- Cats with latent herpesvirus infections showed significantly fewer observation points with conjunctivitis when supplemented with *E. faecium* SF68 as compared to cats in the placebo group. (*Lappin et al, 2009*)
- Supplementation of adult Beagles with the probiotic strain *E. faecium* SF68 can induce immunomodulation as early as four weeks in dogs. (*Lappin et al, 2017*)

## CONVERSATION STARTER:

*“Based on my findings, your pet’s condition may benefit from a [probiotic/diet containing colostrum] to boost [his/her] immune response. It’s surprising to many people to learn that your pet’s gut has a major influence on the function of your pet’s immune system. I’m recommending a [diet/probiotic] that has been shown to boost immune response and may help your pet.”*

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.

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 NMo5 2016

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/S000711451300175X

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.

## PROBIOTICS AND SYNBIOTICS

Probiotics are live organisms that, when administered in adequate amounts, confer a health benefit on the host.<sup>6</sup> Synbiotics combine a probiotic with a prebiotic 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).

- Supplementation with the probiotic strain *E. faecium* SF68 was associated with lower incidence and duration of naturally occurring diarrhea, higher serum IgA antibody levels in kittens. (Czarnecki-Maulden *et al*, 2007)
- *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 *et al*, 2007)
- In addition to the immune benefits of *E. faecium* SF68 seen in cats with latent herpesvirus infections, supplementation with the probiotic preserved gut microbial diversity when cats were exposed to minor stresses (e.g., housing changes, neutering). (Lappin *et al*, 2009)
- 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 *et al*, 2011)
- Supplementation of adult dogs' diets with the probiotic strain *E. 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 flatulence. (Waldron *et al*, 2012)
- 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 *et al*, 2016)
- The probiotic strain *E. faecium* SF68 is unaffected by metronidazole, an antibiotic commonly used to treat dogs with diarrhea. (Fenimore *et al*, 2012)
- 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. (Fenimore *et al*, 2012)
- Supplementation with *E. faecium* SF68 during the administration of amoxicillin-clavulanate to cats resulted in fewer cats developing severe diarrhea, improved fecal consistency, and lower total diarrhea score compared to cats administered a placebo. (Torres-Henderson *et al*, 2017)

## PROBIOTICS AND SYNBIOTICS (CONT.)

- Administration of the probiotic *E. faecium* SF68 was associated with non-clinically relevant changes in serum folate and cobalamin. (Lucena et al, 2018)
- 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 et al, 2019)
- Use of a 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 et al, 2013)

### CONVERSATION STARTER:

*"I understand your pets' previous episodes of diarrhea responded to antibiotics, but recent research has shown that antibiotics can have long-lasting negative effects on your pet's gut and the community of organisms that lives there. It's better for your pet's long-term health if we first try a probiotic."*

OR

*"Based on my findings, your pet's diarrhea may benefit from a [probiotic/synbiotic]. These supplements have been shown to reduce the severity and incidence of diarrhea and may help us resolve the issue sooner."*

OR

*"Based on my findings, I'm concerned that your pet's microbiome, which is the community of organisms that lives in [his/her] gut, may be out of balance or at high risk of becoming out of balance. I'm recommending a probiotic that may help restore or improve the balance and make your pet's microbiome less likely to be upset by stress."*

OR

*"I understand you're bothered by the frequency and/or smell of your dog's flatulence, and I'm going to recommend a probiotic that may improve the problem."*

OR

*"Based on my findings, I recommend a probiotic to help with your pet's condition. However, because your pet also has some reduced liver function, I'm recommending one that is safe for dogs with liver disease."*

## PROBIOTICS AND SYNBIOTICS (CONT.)

Different probiotic strains are not interchangeable; they are extremely strain-specific, and only certain strains can benefit certain health conditions. Ongoing research has begun to make connections between particular strains and health conditions and to understand their mechanisms of action.

- 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. (Simpson *et al*, 2009)
- 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. (Kathrani *et al*, 2016)
- A pilot study showed that short-term supplementation with *E. faecium* SF68 was not effective for reducing oclacitinib dosage while maintaining or reducing clinical disease score or pruritis associated with atopic dermatitis. Further studies are indicated to determine the role of probiotics in the management of atopic dermatitis. (Yamazaki *et al*, 2019)

### CONVERSATION STARTER:

“We’re still learning what probiotics are best suited for which health conditions, and we do know that they’re not ‘one-size-fits-all.’ That’s why I may recommend different probiotics for your pet at different times, based on the condition we’re trying to address.”

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.

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.

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

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

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

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

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

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

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

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.

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

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; Bydgoszcz, Poland.

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

## THE GUT-BRAIN AXIS

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

- 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 versus when they were supplemented with a placebo. (McGowan *et al*, 2018)
- In addition, the dogs exhibited less daily anxious behavior when supplemented with *B. longum* BL999 compared to when they were supplemented with placebo. (McGowan *et al*, 2018)
- Supplementation with *B. longum* BL999 had an anxiolytic effect on anxious dogs, and could serve as a useful tool in a comprehensive management plan. (McGowan *et al*, 2018)

### CONVERSATION STARTER:

*“Your dog is showing signs of anxiety, and we have a number of things we can try to reduce [his/her] clinical signs. One of these tools is a probiotic that was shown to reduce some of the day-to-day anxious behaviors you’re seeing.”*

McGowan, R. T. S., Barnett, H. R., Czarniecki-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.

## MICROBIOMES BEYOND THE GUT

Although the gut often comes to mind first when the word “microbiome” is mentioned, it’s important to remember that there are numerous other microbiomes associated with the body and these microbiomes may influence each other in manners yet to be determined. For example, there is ongoing research to study the microbiomes of the oral cavity, skin, respiratory tract, urinary tract and reproductive tract.

- The canine oral microbiome is remarkably stable 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. (Bell et al, 2020)
- Eight phyla comprise more than 99% of the canine oral microbiome and were present across all dogs and samples regardless of breed, sex, diet, treatment or other factors. (Bell et al, 2020)

### CONVERSATION STARTER:

*“We hear a lot about the microbiome, and it’s usually in reference to the community of organisms that live in the gut. But, like you, your pet has many microbiomes in their body. There are microbiomes in the skin and respiratory tract, for example, and your pet’s mouth even has a different microbiome than his/her gut. We’re still learning about these microbiomes and their influences on the body and your pet’s health. As we continue to learn, that information influences the recommendations I make for your pet.”*

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

## PURINA'S MICROBIOME LEADERSHIP

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.

### ADDITIONAL REFERENCES

1. Benno, Y., Nakao, H., Uchida, K., & Mitsuoka, T. (1992). Impact of the advances in age on the gastrointestinal microflora of beagle dogs. *Journal of Veterinary Medical Science*, 54, 703-706. doi: 10.1292/jvms.54.703
2. Masuoka, H., Shimada, K., Kiyosue-Yasuda, T., Kiyosue, M., Oishi, Y., Kimura, S.,...Hirayama, K. (2017). Transition of the intestinal microbiota of cats with age. *PLoS ONE*, 12(8), e0181739. doi: 10.1371/journal.pone.0181739
3. Bermingham, E. N., Young, W., Butowski, C. F., Moon, C. D., Maclean, P. H., Rosendale, D.,...Thomas, D. G. (2018). The fecal microbiota in the domestic cat (*Felis catus*) is influenced by interactions between age and diet; A five year longitudinal study. *Frontiers in Microbiology*, 9, 1231. doi: 10.3389/fmicb.2018.01231
4. Pilla, R., Gaschen, F. P., Barr, J. W., Olson, E., Honneffer, J., Guard, B. C.,...Suchodolski, J. S. (2020). Effects of metronidazole on the fecal microbiome and metabolome of healthy dogs. *Journal of Veterinary Internal Medicine*, 34(5), 1853-1866. doi: 10.1111/jvim.15871
5. Vighi, G., Marcucci, F., Sensi, L., Di Cara, G., & Frati, F. (2008). Allergy and the gastrointestinal system. *Clinical & Experimental Allergy*, 153 (Suppl 1), 3-6. doi: 10.1111/j.1365-2249.2008.03713.X
6. 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



Advancing Science for Pet Health

Learn more at  
**[PurinaInstitute.com](https://PurinaInstitute.com)**

---

PURINA TRADEMARKS ARE OWNED BY SOCIÉTÉ DES PRODUITS NESTLÉ S.A.  
ANY OTHER MARKS ARE PROPERTY OF THEIR RESPECTIVE OWNERS. EC/CRCR