



THE PROMISE OF FECAL TRANSPLANTS

A microbiome solution for veterinary practitioners

THE ANIMAL MICROBIOME

The microbiome is a community of microorganisms such as bacteria, fungi and viruses found living on and inside the body, including in the digestive system, and it affects many aspects of host physiology.^{1,2,3,4} The microbiome of the gastrointestinal tract is genetically unique and specific to each individual host. Members of this complex microbial community can be passed to offspring, and it is influenced by many medications, infectious diseases, the environment, and diet.

Diversity and certain compositions of microbes in the gut have been linked to overall health. Gut dysbiosis — a disturbance or imbalance in the gut microbiome — is increasingly shown to be associated with many health issues, including inflammatory bowel disease, liver pathologies,

metabolic disorders, skin problems, and possibly some malignancies such as colorectal cancer.^{1,2,5,6}

Thus, the microbiome is becoming well-recognized as a promising therapeutic target in both human and veterinary medicine.^{2,3} Examples include the use of healthy gut microbiomes to cure intestinal infections of *Clostridioides difficile* in humans and to improve outcomes of canine parvovirus infection in puppies.

Offspring receive their first microbes from their mother, starting at birth during passage through the vaginal canal, as well as from nursing.⁷ Cesarean section delivery may disrupt microbial transmission from mother to infant, and health conditions more frequently observed in individuals born by C-section include allergies, chronic immune disorders and metabolic disorders.^{8,9,10} In addition, imbalanced microbiomes can arise from unhealthy

diets¹¹ as well as the use of antibiotics and other medications, which can harm beneficial bacteria in healthy microbiomes in companion animals as well as humans.^{1,3,5,12}

Therapies to balance and strengthen the gut microbiome include diet, prebiotics, probiotics and fecal microbiota transplantation.¹ Prebiotics are indigestible by the host, though digestible by the microbiome, and include carbohydrates and fermented saccharides such as inulin, oligofructose, galactofructose and galacto-oligosaccharides. Beneficial gut microbes feed on prebiotics and multiply, while a poor-quality diet may allow less beneficial microbes to dominate. Probiotics are beneficial microorganisms that theoretically outcompete pathogenic microorganisms and typically include strains of *Bifidobacterium* and *Lactobacillus*. Most probiotics are of moderate benefit at best because they are not a part of the core healthy microbiome or host species-appropriate.^{13,14}

Fecal microbiota transplantation is a promising emerging therapy that involves the transfer of gut bacteria, derived from fecal samples, from a healthy donor to improve microbial balance and diversity in the recipient.¹⁵

FECAL MICROBIOTA TRANSPLANTATION

Fecal microbiota may be transferred to an ailing individual through a feeding tube, capsules or enema.¹⁶ Though FMT has only gained traction in mainstream medicine in the past 20 years, the therapy dates back to fourth-century China, where “yellow soup” was used to treat food poisoning and diarrhea.^{5,17} FMT has also been used in veterinary medicine since the 16th century, and some animals naturally practice FMT in the form of coprophagia.^{13,17}

There is a need for stool banks containing rigorously screened fecal material for FMT in veterinary medicine. Veterinarians performing FMT only occasionally may find it hard to justify the expense of rigorous testing of donated material, even though this is essential to protect the health of the recipient.



A Clinical Perspective

Veterinarian Arielle Herndobler previously used live-donor FMT in her patients and switched to oral delivery with enteric-coated capsules

in AnimalBiome's Gut Restoration Supplement (GRS), using it to treat about 50 dogs and cats since last year.



Dr. Herndobler reports success in using the GRS to treat allergies, gastrointestinal problems and autoimmune disorders. Cases include a dog with chronic drainage after ear canal ablation and a cat that was overgrooming.

Unlike probiotics, which must be given daily, the GRS is discontinued once the gut microbiome is balanced, Dr. Herndobler says. The dosage is much easier to manage than live-donor FMT, and thorough screening of donor samples promotes confidence.

Dr. Herndobler prescribes the GRS in addition to dietary modification and other therapies, and she says she considers the GRS an essential part of any veterinarian's toolkit. “I can't imagine practicing without it,” she says. ■

MicrobiomeTherapy In Practice

Microbiome research has only recently begun to blossom. Much remains to be discovered, but all signs point to the gut microbiome as both a target and a source of treatments for gastrointestinal disorders and possibly other chronic diseases.

FMT has been done for centuries, but its use was limited primarily to alternative medicine providers. With the rise of antibiotic-resistant *C. difficile* infections, FMT has come into mainstream use in human medicine since studies showing high efficacy have reached the meta-analysis stage (currently >18,000 peer-reviewed publications).

In addition to *C. difficile* infections in companion animals, there is growing interest in the application of FMT for IBD, canine parvovirus infections and atopic dermatitis in veterinary medicine. Careful transplant preparation, thorough screening

of both donor and recipient health and careful screening of fecal matter are crucial to ensure efficacy and safety.

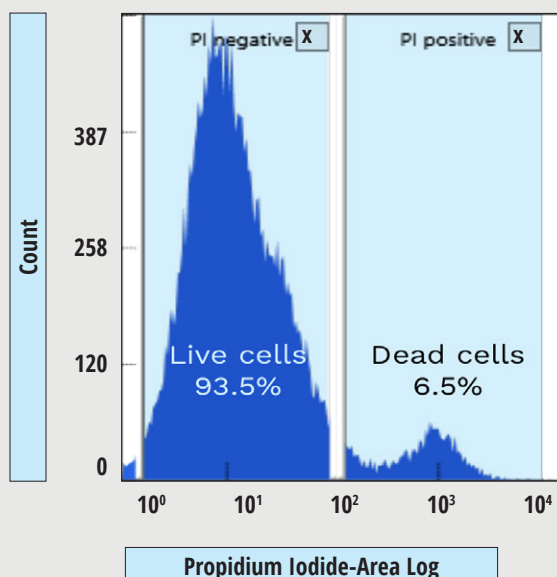
Cell viability study of freeze-dried canine and feline fecal matter

METHODS

Counts of live and dead bacterial cells in the 0.2 to 2 μm size range were obtained using a Bio-Rad ZE5 Cell Analyzer Flow Cytometer (Bio-Rad, Hercules, CA). Percentages of viable and non-viable cells were assessed with dye exclusion using the dye Propidium Iodide, which cannot pass through the cell membrane of viable bacteria (approach reviewed in Emerson et al. 2017). Cells that fluoresce brightly (PI positive) are damaged or dead. In addition, we estimated the total counts of live cells per gram using the viability stain 7-AAD.

RESULTS

The process developed for stabilizing bacteria in freeze-dried fecal material used in the AnimalBiome Gut Restoration Supplement maintains high bacterial cell viability: 93.5% for canine feces (Figure) and 90% for feline feces. The number of live bacterial cells in the Gut Restoration Supplement is 20×10^9 cells per gram for canine feces and 19×10^9 for feline feces. Weese and Martin (2011) assessed viable cell growth in 25 commercial veterinary probiotics and found that actual counts of bacteria ranged from 0 to 2×10^9 colony forming units (CFU) per g. The number of live bacterial cells in 1 g of freeze-dried canine feces was 20 billion. For reference, the estimated number of bacterial cells per ml of fresh material in the human colon is 100 billion (Sender et al. 2016). ■



FIGURE

Bacterial viability in freeze dried canine fecal matter in AnimalBiome's Gut Restoration Supplement.

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“ Screening both donors and recipients improves the safety and efficacy of FMT. ”

Safety and efficacy concerns of ad hoc approaches

Donated materials used for FMT need to be carefully screened for parasites and pathogens prior to transplantation, which may not occur when FMT is performed on an ad hoc basis.

Parasites of veterinary relevance that can be transmitted fecal-orally include *Giardia*, *Cryptosporidium*, hookworms, whipworms and roundworms, and testing should look for both ova and adult forms.

Other microorganisms of veterinary relevance are *Clostridium perfringens*, *Clostridiodes difficile*, *Campylobacter coli*, *Campylobacter jejuni*, *Salmonella spp.*, canine distemper virus, canine enteric coronavirus, canine parvovirus 2 and canine circovirus.

Screening for risk factors associated with antibiotic resistance, including recent hospitalization and antibiotic exposure in donors, is also advisable.

Microbiome screening for key gut bacteria is also important, including screening for over-representations of certain groups of bacteria, including *Escherichia coli*, *C. perfringens*, and under-representation of key bacterial groups associated with healthy microbiomes in cats and dogs, such as *Bacteroides* and *Fusobacterium*. The donor material in AnimalBiome's Gut Restoration Supplements is screened for parasites, pathogens, and a healthy microbiome composition.

MICROBIOME RESTORATION

Oral delivery of freeze-dried fecal material is the latest advancement in veterinary FMT. Establishing a bank of safe, effective fecal matter requires thorough screening of donor material for pathogens and parasites, as well as the screening of donors themselves to ensure they are healthy, have no recent history of systemic antibiotic administration and no behavioral issues, and have a sufficiently healthy and balanced microbiome.¹⁸

The recipient should also undergo a health and gut microbiome assessment. Screening both donors and recipients improves the safety and efficacy of FMT. Furthermore, thorough screening contributes to the body of knowledge about the efficacy of microbiome restoration and improves

future therapies. Clinical signs are measured in follow-up assessments, enabling further refinement of treatments.^{10,18}

EVIDENCE OF EFFICACY

In companion animals, FMT may be administered orally, nasoesophageal, or rectally (via retention enema or colonoscopic administration). Oral FMT is easier to administer, but the transplant does not reach the large intestine quickly. Therefore, survival of the microorganisms through the stomach and small intestine may be of concern in cases where FMT is administered by mouth if enteric-coated capsules are not used.

Rectal administration may be indicated for severe cases and for cases with clinical signs of distal small intestinal or colonic involvement.¹⁰ Precise delivery location will depend on the size of the patient, and a colonoscope will allow veterinarians to administer the fecal suspension in the ascending colon, ileum and cecum. It can also be used for colonic biopsies prior to an FMT procedure.¹⁰ The ileocecal brake prevents aboral migration of colonic bacteria into the ileum, and the only way to ensure ileal delivery is retrograde ileoscopy. Both enteric-coated Gut Restoration Supplement capsules as well as material for delivery via enema are available from AnimalBiome.

While few veterinary clinical trials of FMT have been conducted, the procedure has shown promise in the trials that have been run to date. As one commentary notes, “[I]n dogs and cats, FMT may have the potential to improve health in any disease associated with an alteration or dysbiosis of intestinal microbial ecology such as acute and chronic GI inflammation and idiopathic diarrhea as well as IBD.”¹⁹

For example, a study assessing the efficacy of orally or enema-administered FMT for fecal microbiome recovery after tylosin treatment in healthy dogs suggested “FMT holds promise in correcting acute intestinal dysbiosis.”

Researchers found a significant increase in dysbiosis index in all groups at day eight, although by day 15, dogs receiving oral FMT had a lower DI than control dogs. Normalization of *Clostridium hiranonis* and *Faecalibacterium*



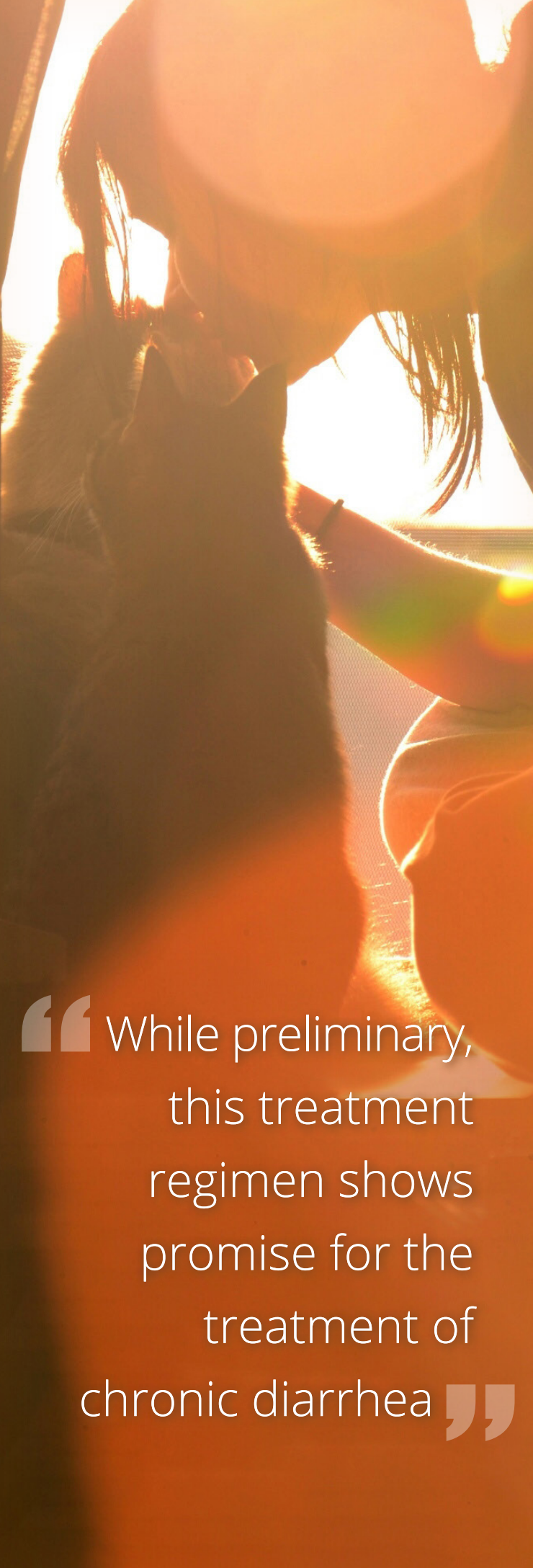
Emmy's Story

When she was less than 2 years old, Sandy Shipp's dog Emmy developed chronic stool problems and became severely underweight, frail and listless.

She was diagnosed with inflammatory bowel disease based on a tissue biopsy sample. Dietary modification and medications did not help, and she continued losing weight. Her veterinarian suggested trying FMT, but it wasn't offered through her practice.

Shipp submitted a fecal sample for microbiome testing, which revealed that Emmy's gut microbiome was imbalanced and had an overgrowth of *Escherichia*, likely *E. coli*. Shipp started Emmy on two capsules daily of the Gut Restoration Supplement.

Emmy responded within a few days. Her weight stabilized and within a few weeks, her energy levels improved. Today, she is a happy, healthy and active dog. ■



“While preliminary,
this treatment
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chronic diarrhea”

was significantly delayed — until day 22 —
in control dogs but not in FMT dogs.²⁰

In a randomized trial of eight dogs with clinical signs
of hemorrhagic diarrhea syndrome, results showed
FMT administered via colonoscopy transiently
altered the dogs' bacterial diversity.²¹

Another randomized trial found FMT plus standard
care was associated with faster resolution of diarrhea
in puppies with canine parvovirus-related acute
hemorrhagic diarrhea syndrome, compared with
puppies that received only IV fluids and antimicrobials.²²

In one study of whether FMT increased
transmission of a stable maternal microbiota to
pups and decreased the incidence of postweaning
diarrhea, no diarrhea was seen in either group,
complicating comparison. However, the researchers
observed substantial variability in microbiomes
among subjects, which were housed in a research
setting, and the study may not have been long
enough to demonstrate an effect. Therefore, they
noted that “use of oral FMT may be a pragmatic and
convenient therapy for the treatment of postweaning
diarrhea, but larger studies are needed.”²³

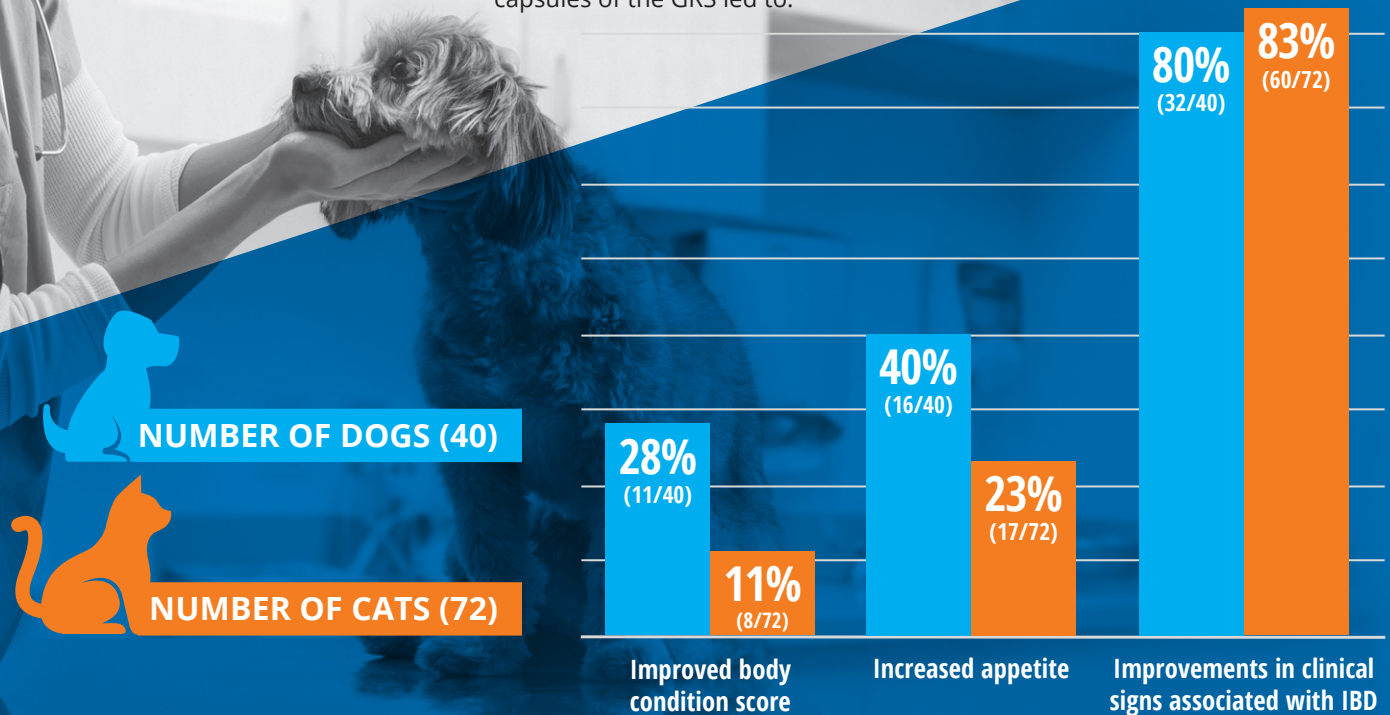
Another study demonstrated improvements in both
a 3-year-old dog that had suffered for two years
from eosinophilic inflammatory bowel and a 6-year-
old cat that had presented with vomiting and
diarrhea for 16 months. The dog's fecal consistency
improved within 24 hours after enema-administered
FMT and remained clinically normal for three
months post-treatment, other than diarrhea
following enrofloxacin administration that was
responsive to metronidazole.²⁴ Similar results were
seen in the cat, which received the same treatment,
as well as 3 ml fecal suspension into the duodenum
via endoscope. The cat demonstrated rapid
improvements in fecal consistency, attitude and
appetite as well as an absence of vomiting and
diarrhea for three months.

The researchers commented that “while preliminary,
this treatment regimen shows promise for the
treatment of chronic diarrhea,” and “the clinical
response is consistent with the marked shift in the
fecal microbiome following treatment.”

FMT enema was also successful in a 10-year-old
cat with ulcerative colitis that had not responded
to other therapies. FMT was tried as a last resort

A pilot study of AnimalBiome's Gut Restoration Supplement (GRS) involving 72 cats and 40 dogs

with diagnosed (via ultrasound, endoscopy or biopsy) or suspected inflammatory bowel disease (IBD) demonstrated positive results.²⁸ A single 25-day course of 50 capsules of the GRS led to:



before euthanasia, and fecal consistency, odor and color improved immediately. The cat's stools became normal after a second procedure.²⁵

In another study, stool consistency and frequency, as well as fecal blood and mucus, normalized in a dog diagnosed with *C. difficile*-associated colitis within three days after treatment with orally administered FMT. *C. difficile* antigen and toxin A&B genes and proteins were negative in real-time PCR analysis and immunochromatography.²⁶

An additional study aimed to determine if fecal transplantation via enema could cure *Clostridium perfringens* infections that did not respond to treatment with metronidazole and amoxicillin trihydrate/clavulanate potassium. All of the eight dogs in the study saw immediate resolution of diarrhea, and six tested negative for *C. perfringens* alpha toxin gene expression. The researchers noted that, to their knowledge, "this is the first report of fecal flora transplantation in the dog for refractory *Clostridium perfringens* infections,"

and "this treatment plan is an option for dogs failing antibiotic therapy and also reduces antibiotic use."²⁷

Finally, a pilot study of AnimalBiome's Gut Restoration Supplement (GRS) involving 72 cats and 40 dogs with diagnosed (via ultrasound, endoscopy or biopsy) or suspected inflammatory bowel disease (IBD) demonstrated positive results.²⁸ A single 25-day course of 50 capsules of the GRS led to:

- Improvements in clinical signs associated with IBD
- Increased appetite
- Improved body condition score

Meanwhile, fecal samples became better formed and less moist, with mean fecal consistency changing from 4.6 to 3.4 in dogs and from 5 to 4 in cats (based on the Purina Fecal Score Chart). Adverse responses were low in comparison, with frequency of diarrhea increasing in 5% (2/40) of dogs and 9.7% (7/72) of cats and appetite decreasing in 2.5% (1/40) of dogs and 7% (5/72) of cats. ■

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- AnimalBiome pilot study



ABOUT ANIMALBIOME

AnimalBiome provides diagnostics and therapeutics to help veterinarians address chronic health conditions related to imbalances in the gut microbiome. We have created the world's first stool bank for dogs and cats that is rigorously screened for pathogens, parasites, and microbiome composition. Using a proprietary process, we have developed a convenient and safe approach to fecal transplantation using enteric-coated capsules that are stable at room temperature and maintain high viability of bacterial cells. AnimalBiome also provides microbiome testing using genetic sequencing to characterize the microbiome of individual pets, allowing veterinarians and pet owners to make personalized changes in diet, including prebiotics and macronutrient ratios, to promote a more balanced composition of gut bacteria. AnimalBiome is a privately-held company based in Oakland, CA that was started in 2016 by researchers at the University of California, Davis.

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