Hill's™
Transforming Lives™

HILL'S GLOBAL SYMPOSIUM

2019 TORONTO

Harnessing the Power of the Microbiome

14-15 July 2019

SCIENTIFIC PROCEEDINGS
Toronto, 14th - 15th July 2019
www.hillsglobalsymposium.com

CONTENTS

Defining the gut microbiota

Social media

Modifying the gut microbiota

New targets for intervention

Communication

Click on any title to access the article

Editors:
Dr Maureen Revington BVSc, MSc, PhD
S Dru Forrester DVM, MS, DACVIM
Iveta Bečvářová DVM, MS, DACVN

 ISSN 1479 - 8999

Opportunities from the Human Microbiome and Human Microbiome Project in Veterinary Science

Curtis Huttenhower, PhD
Professor of Computational Biology and Bioinformatics
Departments of Biostatistics and Immunology and Infectious Disease, Harvard T.H. Chan School of Public Health Associate Member, Broad Institute of MIT and Harvard
Boston, MA, USA

Introduction

Molecular methods for population-scale microbiome studies have had a tremendous impact on human basic and translational biology over the past decade.[1-6] Many of the same opportunities present equal or greater potential in animal science, including disease management, novel therapeutics, and (especially important in veterinary applications) diagnostic and prognostic biomarkers.[8] The NIH Human Microbiome Project (HMP)[9] has been one of the main platforms for development of data, resources, communities, and basic biological knowledge regarding the microbiome. To provide one in-depth example, during the second phase of the HMP (the Integrative HMP, iHMP or HMP2)[10], we investigated the inflammatory bowel disease microbiome by following 132 Crohn's disease (CD), ulcerative colitis (UC) patients, and control subjects for one year each to analyze 2,965 stool, biopsy, and blood specimens using shotgun metagenomics, metatranscriptomics, metabolomic, proteomic, transcriptional, genetic, and epigenetic profiling, among others (**Fig. 1**).[7] Together, the resulting data and methodology provide both novel insights into the functional dysbiosis of the gut microbiome in IBD (**Fig. 2**), as well as a generalizable platform for multi'omic microbiome research.

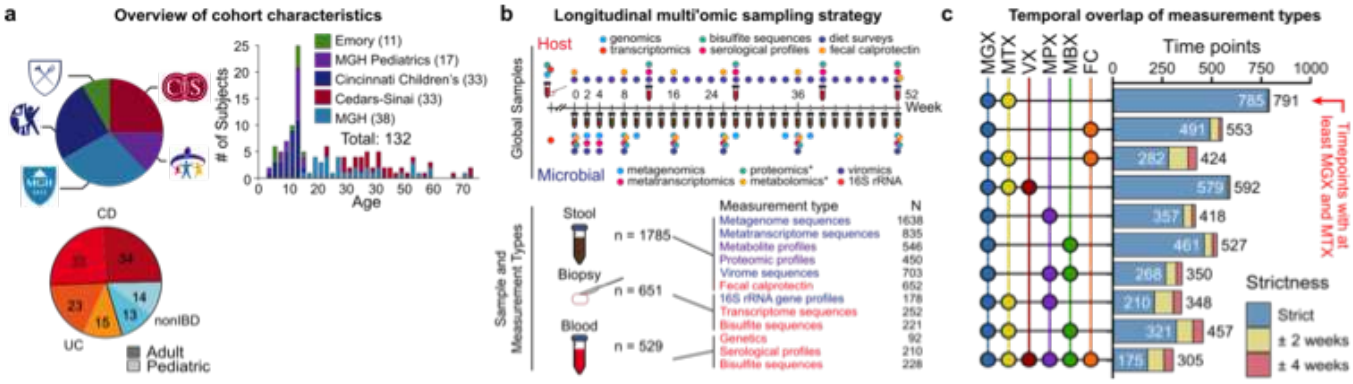


Figure 1: Multi'omics of the IBD microbiome in the IBDMDB study. A) As part of the HMP2, the Inflammatory Bowel Disease Multi'omics Database study recruited 132 CD, UC, and non-IBD control subjects from five clinical centers, from a range of ages and clinical phenotypes.[7] **B)** Subjects were followed for one year each, providing stool (biweekly), blood (~quarterly), and colon biopsies (at baseline). Data were generated from a subset of global time points' samples for all subjects, with dense time courses profiled for a subset. Numbers indicate raw, non-quality-controlled sample counts. **C)** The study design typically yielded many different host and microbiome measurements from the same samples and time points, either identically (strict) or near-concordant (with up to 2 or 4 weeks' time difference). Figure components reproduced from.[7]

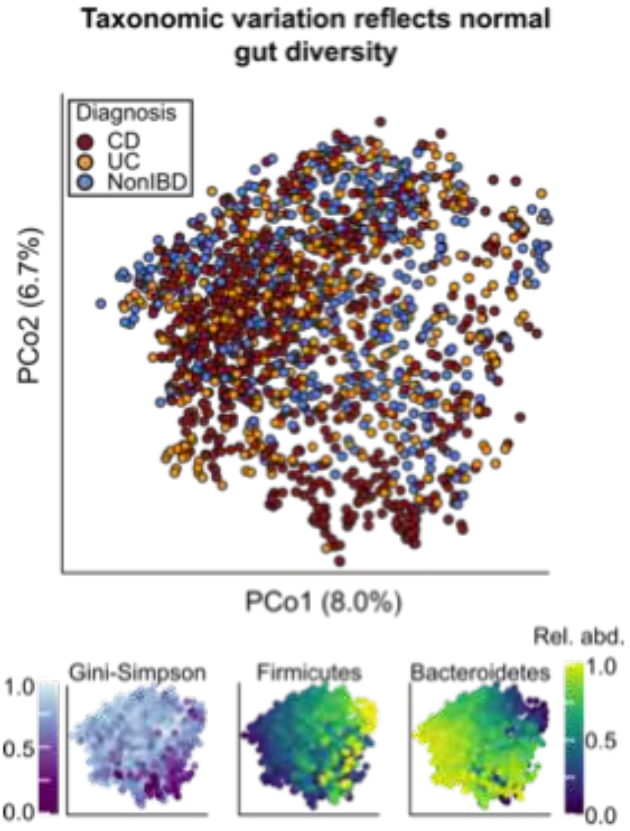


Figure 2: Taxonomic profiles of the longitudinal IBD gut microbiome. In a principal coordinates analysis (PCoA) based on species-level Bray-Curtis dissimilarity, the population is not strikingly different from typical gut microbial profiles, with a gradient between phylum Bacteroidetes vs. Firmicutes among individuals. IBD samples (CD in particular) had weakly lower Gini-Simpson alpha diversity (Wald test p-values 0.26 and 0.014 for UC and CD vs non-IBD, respectively). Figure components reproduced fro.[7]

Results

Briefly, we defined a dysbiosis score for each sample based on microbial ecological excursions (increases in Bray-Curtis beta-diversity) relative to the 90th percentile of distances to non-IBD control samples (**Fig. 3A**). This identified a subset of time points within CD and UC patients at which greater disease activity was suggested. Statistical tests for differential multi'omic features (microbial taxa, metagenomic functions, metatranscriptomic elements, metabolites, and others) during these periods identified a characteristic increase in facultative anaerobes at the expense of obligate anaerobes (**Fig. 3B**), as well as molecular disruptions in microbial transcription (e.g. among clostridia), metabolite pools (acylcarnitines, bile acids, and short-chain fatty acids), and host serum antibody levels.

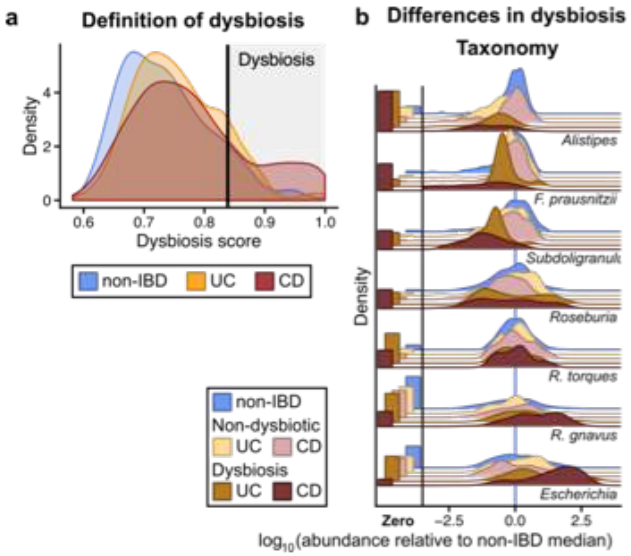


Figure 3: Functional and structural dysbiosis of the gut microbiome during IBD. A) A microbial dysbiosis score was defined to capture periods of putative disease activity based on the 90th percentile of median Bray-Curtis dissimilarity between a sample and non-IBD samples. **B)** Many gut microbiome feature types were differential based on multivariate linear modeling[7] in samples meeting the resulting criteria, including metagenomic species (shown here, N=1,595 samples from 130 subjects), metabolites (N=546 samples from 106 subjects), and microbial transcribers (N=818 samples from 106 subjects; Wald test; all FDR p < 0.05). Figure components reproduced fro.[7]

Disease was also marked by greater temporal variability, i.e. reduced longitudinal stability of the microbiome within subjects over time.[7] This included taxa that tended to be rapidly lost (e.g. *Faecalibacterium prausnitzii, Roseburia intestinalis*) or gained (e.g. *Escherichia coli*) during periods of disease activity, as well as organisms that were uniquely unstable in non-IBD subjects (*Prevotella copri*). Taxonomic shifts were again accompanied by functional consequences such as metabolite pool changes (e.g. in urate, urobilin, and others). Finally, integrative analysis identified microbial, biochemical, and host factors central to the dysregulation (Fig. 4). The study's infrastructure resources, results, and data, available through the Inflammatory Bowel Disease Multi'omics Database (http://ibdmdb.org), provide the most comprehensive description to date of host and microbial activities in IBD. We anticipate extending these discoveries both to the contribution of the gut microbiome in animal IBD specifically, and as a platform for livestock and companion animal microbiome research generally in the future.

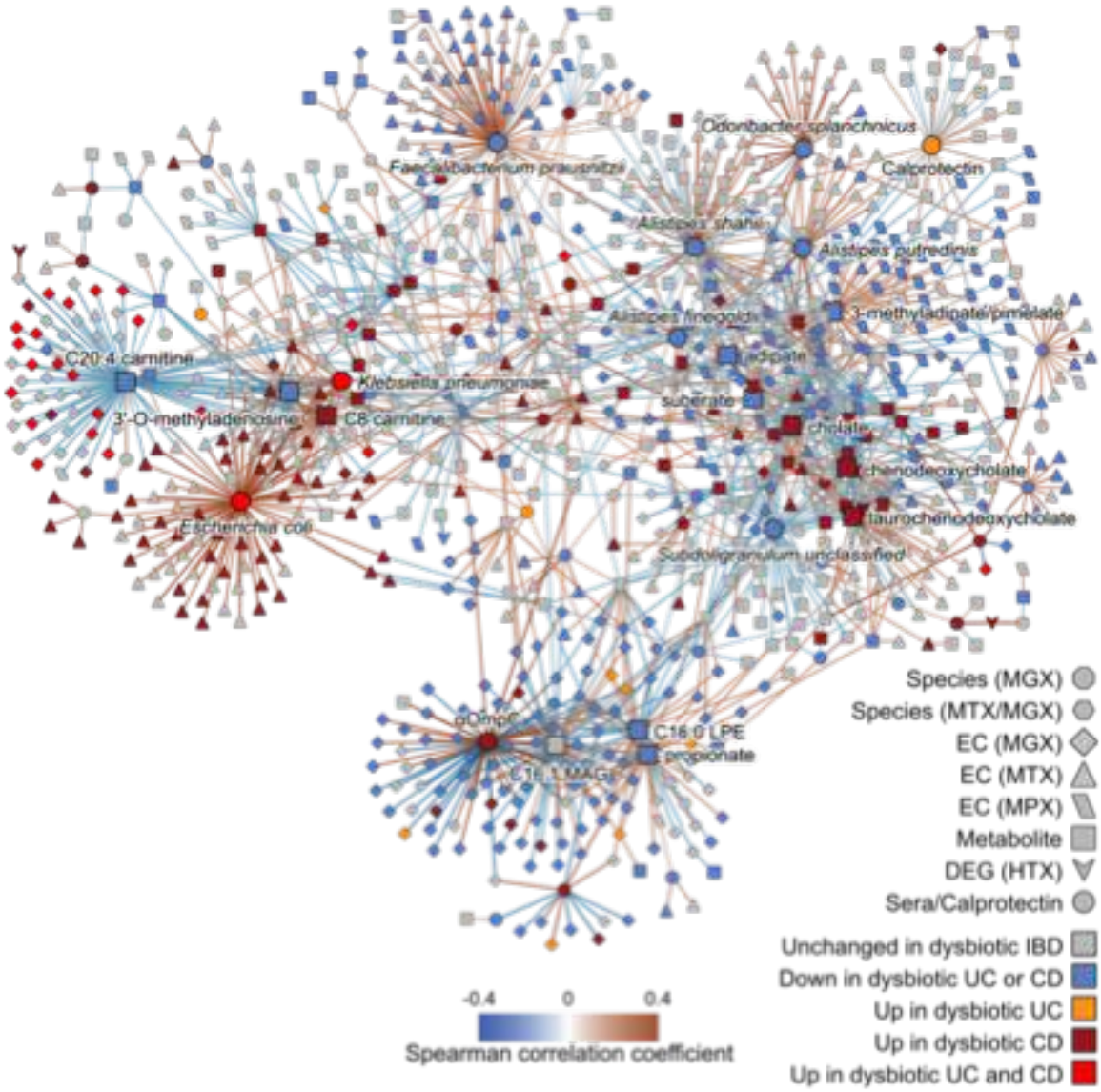


Figure 4: Host and microbial multi'omic interactions in the inflamed gut. Significant associations among 10 different aspects of host-microbiome interactions: metagenomic species, species-level transcription ratios, functional profiles as EC gene families (MGX, MTX and MPX), metabolites, host transcription (rectum and ileum), serology, and calprotectin. Network shows top 300 significant correlations (FDR p<0.05) between each pair of measurement types (for serology, FDR p<0.25). Nodes colored by the disease group they are "high" in, edges by sign and strength of association. Spearman correlations use residuals of a mixed-effects model with subjects as random effects (or a simple linear model when only baseline samples were used, i.e. biopsies) after covariate adjustment. Time points approximately matched with maximum separation 4 weeks. Singletons pruned for visualization. Hubs (nodes with ≥20 connections) emphasized. Figure components reproduced fro.[7]

Conclusions and future work

These and related advances in human microbiome research are critical to translate into companion animal veterinary applications, in addition to their broader agricultural relevance.[11-13] A key understudied aspect of the companion animal microbiome is its potential as a diagnostic or prognostic biomarker. While better or more efficient diagnostics than microbiome profiling often exist for human disease, animals obviously possess a much narrower range of options for communicating distress or symptoms, and early detection of veterinary disease is severely limited. Conversely, stool samples are easier to collect longitudinally and frequently from companion animals than they are from humans. Further, companion animals (and livestock) are subject to a substantially monotonous diet, raising opportunities for health improvement or maintenance that do not exist in humans undergoing regular dietary and environmental microbial perturbations.[14, 15] This is particularly true in early and late life, where healthy aging over the time scale of companion animals presents yet another unique opportunity relative to human biology.[16-18] Finally, human microbiome research itself has advanced to the point where specific translationally-relevant pharmaceutical[4, 19] and immunological[5, 7, 20] interventions are possible in conditions as diverse as IBD and graft-versus-host disease, among many others, raising the possibility of translating these advances into veterinary medicine. Overall, the technical, methodological, and analytical tools for microbiome studies are now robust and cost-effective, and they present an exciting and complementary opportunity for continued advances in animal science.

Acknowledgments

The authors of the IBDMDB study[7] are Jason Lloyd-Price, Cesar Arze, Ashwin N. Ananthakrishnan, Melanie Schirmer, Julian Avila-Pacheco, Tiffany W. Poon, Elizabeth Andrews, Nadim J. Ajami, Kevin S. Bonham, Colin J. Brislawn, David Casero, Holly Courtney, Antonio Gonzalez, Thomas G. Graeber, A. Brantley Hall, Kathleen Lake, Carol J. Landers, Himel Mallick, Damian R. Plichta, Mahadev Prasad, Gholamali Rahnavard, Jenny Sauk, Dmitry Shungin, Yoshiki Vázquez-Baeza1, Richard A. White III, the IBDMDB Investigators, Jonathan Braun, Lee A. Denson, Janet K. Jansson, Rob Knight, Subra Kugathasan, Dermot P. B. McGovern, Joseph F. Petrosino, Thaddeus S. Stappenbeck, Harland S. Winter, Clary B. Clish, Eric A. Franzosa, Hera Vlamakis, Ramnik J. Xavier, and Curtis Huttenhower. We are especially grateful to the IBDMDB participants who made the project possible.

References

1. Eckburg, P.B. et al. Diversity of the human intestinal microbial flora. *Science* **308**, 1635-1638 (2005).
2. Structure, function and diversity of the healthy human microbiome. *Nature* **486**, 207-214 (2012).
3. Routy, B. et al. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. *Science* **359**, 91-97 (2018).
4. Haiser, H.J. et al. Predicting and manipulating cardiac drug inactivation by the human gut bacterium Eggerthella lenta. *Science* **341**, 295-298 (2013).
5. Taur, Y. et al. The effects of intestinal tract bacterial diversity on mortality following allogeneic hematopoietic stem cell transplantation. *Blood* **124**, 1174-1182 (2014).
6. Lloyd-Price, J. et al. Strains, functions and dynamics in the expanded Human Microbiome Project. *Nature* **550**, 61-66 (2017).
7. Lloyd-Price, J. et al. Multi-omics of the gut microbial ecosystem in inflammatory bowel diseases. *Nature* **569**, 655-662 (2019).
8. Barko, P.C., McMichael, M.A., Swanson, K.S. & Williams, D.A. The Gastrointestinal Microbiome: A Review. *J Vet Intern Med* **32**, 9-25 (2018).
9. Turnbaugh, P.J. et al. The human microbiome project. *Nature* **449**, 804-810 (2007).
10. Integrative, H.M.P.R.N.C. The Integrative Human Microbiome Project: dynamic analysis of microbiome-host omics profiles during periods of human health and disease. *Cell Host Microbe* **16**, 276-289 (2014).
11. Mizrahi, I. & Jami, E. Review: The compositional variation of the rumen microbiome and its effect on host performance and methane emission. *Animal* **12**, s220-s232 (2018).
12. McCormack, U.M. et al. Exploring a Possible Link between the Intestinal Microbiota and Feed Efficiency in Pigs. *Appl Environ Microbiol* **83** (2017).
13. Relman, D.A. & Lipsitch, M. Microbiome as a tool and a target in the effort to address antimicrobial resistance. *Proc Natl Acad Sci U S A* **115**, 12902-12910 (2018).
14. Deusch, O. et al. Deep Illumina-based shotgun sequencing reveals dietary effects on the structure and function of the fecal microbiome of growing kittens. *PLoS One* **9**, e101021 (2014).
15. Young, W., Moon, C.D., Thomas, D.G., Cave, N.J. & Bermingham, E.N. Pre- and post-weaning diet alters the faecal metagenome in the cat with differences in vitamin and carbohydrate metabolism gene abundances. *Sci Rep* **6**, 34668 (2016).
16. Masuoka, H. et al. Transition of the intestinal microbiota of cats with age. *PLoS One* **12**, e0181739 (2017).
17. Bermingham, E.N. et al. 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 (2018).
18. Masuoka, H. et al. Transition of the intestinal microbiota of dogs with age. *Biosci Microbiota Food Health* **36**, 27-31 (2017).
19. Wallace, B.D. et al. Alleviating cancer drug toxicity by inhibiting a bacterial enzyme. *Science* **330**, 831-835 (2010).
20. Narula, N. et al. Systematic Review and Meta-analysis: Fecal Microbiota Transplantation for Treatment of Active Ulcerative Colitis. *Inflamm Bowel Dis* **23**, 1702-1709 (2017).

Relevance of Carbohydrate and Protein Fermentation to Gut Health

Megan Shepherd, DVM, PhD, DACVN
Clinical Assistant Professor, Nutrition
Virginia Maryland College of Veterinary Medicine
Blacksburg, VA, USA

What is the gut microbiome?

The gastrointestinal (GI, gut) microbiota is a collection of microorganisms (microbes) that reside within the GI tract, from mouth to anus; the microbiome is the term used for the collective microbial genome.[1] The make-up of the gut microbiota differs by region of the GI tract (e.g. stomach vs. small intestine vs. colon). The majority of microbes are bacteria that live symbiotically with the host. While archaea, yeast and fungi also reside in the GI tract, less is known about these populations. Until recently, it was thought that establishment of the gut microbiota began at birth; now, establishment of the gut microbiota appears to begin in utero.[2] The gut microbiota is influenced by many host factors, including diet, environment, and health (e.g. age[3], obesity[4,5], GI disease[6], use of antibiotics). Some gut microbes are capable of changing jobs and using different substrates, when substrate availability changes (e.g. change in host diet), or becoming dormant. Knowledge of the gut microbiome has expanded dramatically, yet the investigation of this complex population continues.

What does the gut microbiome do?

Gut microbes interact directly and indirectly with each other and the host. The gut microbiome is associated with health of GI tract and other organ systems, even the brain.[7,8] Gut microbes can make use of dietary substrates that the host cannot assimilate, such as fiber. Gut microbes produce a variety of compounds, recently coined "postbiotics", that include volatile fatty acids (VFAs, aka. short chain fatty acids), lactic acid, gases (e.g. carbon dioxide, methane, hydrogen), ammonia and biogenic amines (e.g. histamine).[8]

Carbohydrate Fermentation

Microbial fermentation of carbohydrates has been studied for decades. Carbohydrate terminology is tricky; understanding of carbohydrate classification is important in interpreting results from reports of microbial fermentation of carbohydrates. Carbohydrates can be classified various ways, including structure (monosaccharides, disaccharides, oligosaccharides and polysaccharides), bond between monosaccharide subunits (alpha, beta), digestibility (digestible, non-digestible), speed by which they are generally fermented (rapidly, moderately, slowly), viscosity and solubility (soluble vs. insoluble). Non-fiber carbohydrates, such as starch, are generally digested in the small intestine. However, in the presence of gut microbes (e.g. resistant starch that escapes digestion and travels to the large intestine) non-fiber carbohydrates are rapidly fermented. Fibers consist of monosaccharides linked by beta-glycosidic bonds, which cannot be broken by mammalian enzymes in the small intestine. Gut microbes, primarily in the large intestine, break the beta glycosidic bond and turn fiber into usable products for the host. Fiber fermentation differs between soluble fiber (rapidly fermented; e.g. fructooligosaccharides) and insoluble fibers (slowly fermented; e.g. cellulose). Prebiotics are fibers, typically oligosaccharides, thought to support beneficial microbes in the GI tract. Sources of prebiotics are broad and include whole grains, vegetables, fruits, refined fiber supplements (e.g. psyllium husk, cellulose) and yeast (e.g. cell wall of *Saccharomyces cerevisiae*).[9]

Benefits of dietary fiber on the host health, specifically around metabolism and gut health, are numerous and yet fiber is not recognized as an essential nutrient. Fiber solubility or fermentability influences the postprandial glucose/insulin response in that mixed fiber sources generally appear to be superior.[10–13] The most studied carbohydrate fermentation products are VFAs, specifically acetate, propionate and butyrate. Carbohydrates generally enhance VFA production. Volatile fatty promote gut motility, promote water absorption, generally negatively influence luminal pH and support gut barrier function by stimulating tight junctions and mucous production.[14,15] Furthermore, VFAs have immune-regulatory function and may negatively influence inflammation.

A variety of studies have highlighted the in vivo effects of carbohydrates in complex food (e.g. kibble) on gut health. These studies expand our knowledge from in vivo studies (e.g. application of a single fiber source to fecal microbes). Diets enhanced in insoluble fiber (e.g. sugarcane fiber) increases fecal dry matter and stool bulk, and reduce diet digestibility.[3,16] However, diets enhanced in soluble (e.g. guar gum) and moderately soluble fiber (e.g. beet pulp) may positively influence fecal water and can have a positive influence on digestibility, as compared to diets enhanced in

insoluble fibers (e.g. soybean hulls, cellulose).[16] Furthermore, soluble fiber (e.g. guar gum) and moderately soluble fiber (e.g. beet pulp, yeast cell wall) positively influence total VFA production, including butyrate, and stimulate gut immunity (i.e. GALT), enhancing colonocyte health.[3,17–20]

Protein Fermentation

Products of protein fermentation include VFAs, branch-chain fatty acids (BCFAs), ammonia and biogenic amines (e.g. indole, putrescine, cadaverine, spermidine and spermine).[5,19,21] Dietary protein concentration appears to positively influence butyrate production and/or the abundance of microbial groups in which butyrate producers are represented.[5] Indole is the product of microbial metabolism of the amino acid tryptophan and is metabolized to indoxyl sulfate in the liver. Blood indoxyl sulfate concentration has been associated with disease (e.g. chronic kidney disease in cats); however, association is not causation.[22] The role of indole on host health not clear and current studies are limited to work in other species. Indole may have anti-inflammatory affects (e.g. reduce TNF-alpha) and strengthen gut barrier function and thus may offer direct benefit to the gut.[23] However, indole may have a negative impact on beneficial microbes and favor colonization of pathogenic bacteria (e.g. *Clostridium difficile*).[24] The role of other products of microbial fermentation (e.g. BCFAs) are less clear.[25]

Intersection of Carbohydrate and Protein Fermentation

Nutrients are rarely fed in isolation. For example, when dietary protein is increased, dietary carbohydrates generally decrease. Furthermore, dietary carbohydrates influence protein fermentation and vice versa. Feeding a high protein, low starch diet to adult Beagles was associated with diarrhea, lower total fecal VFAs and higher fecal valeric acid, pH, ammonia and calprotectin (negative marker of intestinal inflammation).[26] Inclusion of soybean meal (source of oligosaccharides and protein), as compared to beet pulp an sugarcane fiber, has a positive influence on diet digestibility and a negative impact on fecal spermidine.[3] Inclusion of guar gum, as compared to cellulose, reduces protein digestion and increases protein fermentation and fecal indole[17] Conversely, inclusion of fructooligosaccharides (FOS) attenuates biogenic amine production associated with a high protein diet, thus reducing fecal indole, tyramine and histamine.[19,27] Yeast (*Saccharomyces cerevisiae*) negatively influences fecal phenol.[7] Therefore, dietary carbohydrate and protein, in both quantity and source, influence host health in a variety of ways.

Interpreting the Science

Methods for studying carbohydrate and protein fermentation often have challenges and/or limitations. Many studies have employed Beagles, while adorable, Beagles do not represent all dog breeds. The definition of high vs. low carbohydrate and protein vary across studies. Furthermore, carbohydrates are not created equal, neither are proteins, as the molecular structure (e.g. amino acid profile) and bioavailability (e.g. resistant starch) influence presentation to and fermentation by microbes.[26]

Additionally, how we characterize dietary carbohydrates has limitations. Proximate analysis is a quantitative analysis of macronutrients where digestible carbohydrates (NFE, nitrogen-free extract) are determined by difference. Specifically, NFE is calculated by subtracting crude protein (measures nitrogen), crude fat (ether extract), crude fiber and ash from diet dry matter.[28] Crude fiber does not represent total dietary fiber (TDF) as it only includes a insoluble fiber and excludes soluble fiber. Therefore, when using proximate analysis to present diet macronutrient profile, NFE is likely overestimated.[29]

Apparent digestibility collectively captures both host assimilation and microbial fermentation. Therefore, a reduction in apparent digestibility of protein could be because less protein is assimilated, or because there is increased microbial protein production. Furthermore, feces does not completely mirror what is occurring in the GI tract; feces represents what has passed through the GI tract. For example, VFAs measured in feces does not accurately reflect microbial VFA production because VFA are rapidly absorbed through the GI mucosa. Furthermore, plasma propionate and butyrate do not accurately represent VFA absorption due to hepatic and colonocyte metabolism.[17] Therefore, when interpreting the science, consider the challenges and limitations.

Disclosures

This review weighs the scientific evidence more heavily toward dogs vs. cats and does not include other companion animals. This review also does not draw attention to the roles of specific microbes in carbohydrate and protein fermentation. Due to the complexity of the gut microbiome, this review focused on microbial fermentation as a collective. This review does not address the impact of dietary lipid/fat[30], vitamins, minerals and non-essential compounds (e.g. polyphenols, keratin, cartilage) on microbial fermentation.[31] The role of raw meat-based diets, which are often high in protein, were not included in this review. Feeding of raw meat-based diets does impact the gut microbiota[32]; however, the raw (vs. heat processed) nature cannot be separated from the macronutrient profile. Therefore, the discussion about carbohydrate and protein fermentation above is quite simple in its current state.

Conclusion

Diet, host and gut microbes are intimately involved in a complex way. While cats and dogs are not herbivores and do not harbor large fermentation chambers (e.g. rumen, cecum), gut microbial fermentation still has a great impact on host health. Cat and dog gut microbes ferment carbohydrates and protein. While products of carbohydrate and protein fermentation generally differ (e.g. indole), there is overlap in some products (e.g. butyrate).

References

1. Barko PC, McMichael MA, Swanson KS, et al. The Gastrointestinal Microbiome: A Review. J Vet Intern Med 2018;32:9–25.

2. Lyman CC, Holyoak GR, Meinkoth K, et al. Canine endometrial and vaginal microbiomes reveal distinct and complex ecosystems. PloS One 2019;14:e0210157.

3. Maria APJ, Ayane L, Putarov TC, et al. The effect of age and carbohydrate and protein sources on digestibility, fecal microbiota, fermentation products, fecal IgA, and immunological blood parameters in dogs. J Anim Sci 2017;95:2452–2466.

4. Li Q, Lauber CL, Czarnecki-Maulden G, et al. Effects of the Dietary Protein and Carbohydrate Ratio on Gut Microbiomes in Dogs of Different Body Conditions. mBio 2017;8.

5. Xu J, Verbrugghe A, Lourenço M, et al. The response of canine faecal microbiota to increased dietary protein is influenced by body condition. BMC Vet Res 2017;13:374.

6. Hullar MAJ, Lampe JW, Torok-Storb BJ, et al. The canine gut microbiome is associated with higher risk of gastric dilatation-volvulus and high risk genetic variants of the immune system. PloS One 2018;13:e0197686.

7. Wang L-J, Yang C-Y, Chou W-J, et al. Gut microbiota and dietary patterns in children with attention-deficit/hyperactivity disorder. Eur Child Adolesc Psychiatry 2019.

8. Abdul Rahim MBH, Chilloux J, Martinez-Gili L, et al. Diet-induced metabolic changes of the human gut microbiome: importance of short-chain fatty acids, methylamines and indoles. Acta Diabetol 2019;56:493–500.

9. Kim D-H, Jeong D, Kang I-B, et al. Modulation of the intestinal microbiota of dogs by kefir as a functional dairy product. J Dairy Sci 2019;102:3903–3911.

10. Deng P, Beloshapka AN, Vester Boler BM, et al. Dietary fibre fermentability but not viscosity elicited the "second-meal effect" in healthy adult dogs. Br J Nutr 2013;110:960–968.

11. Nelson RW, Duesberg CA, Ford SL, et al. Effect of dietary insoluble fiber on control of glycemia in dogs with naturally acquired diabetes mellitus. J Am Vet Med Assoc 1998;212:380–386.

12. Kimmel SE, Michel KE, Hess RS, et al. Effects of insoluble and soluble dietary fiber on glycemic control in dogs with naturally occurring insulin-dependent diabetes mellitus. J Am Vet Med Assoc 2000;216:1076–1081.

13. Massimino SP, McBurney MI, Field CJ, et al. Fermentable dietary fiber increases GLP-1 secretion and improves glucose homeostasis despite increased intestinal glucose transport capacity in healthy dogs. J Nutr 1998;128:1786–1793.

14. Zheng L, Kelly CJ, Battista KD, et al. Microbial-Derived Butyrate Promotes Epithelial Barrier Function through IL-10 Receptor-Dependent Repression of Claudin-2. J Immunol Baltim Md 1950 2017;199:2976–2984.

15. Hung TV, Suzuki T. Dietary Fermentable Fibers Attenuate Chronic Kidney Disease in Mice by Protecting the Intestinal Barrier. J Nutr 2018;148:552–561.

16. Detweiler KB, He F, Mangian HF, et al. Extruded feline diets formulated with high inclusion of soybean hulls: effects on apparent total tract macronutrient digestibility, and fecal quality and metabolites. J Anim Sci 2019;97:1042–1051.

17. Rochus K, Janssens GPJ, Van de Velde H, et al. Highly viscous guar gum shifts dietary amino acids from metabolic use to fermentation substrate in domestic cats. Br J Nutr 2013;109:1022–1030.

18. Bueno AR, Cappel TG, Sunvold GD, et al. Feline colonic microbes and fatty acid transport: Effects of feeding cellulose, beet pulp and pectin/gum arabic fibers. Nutr Res 2000;20:1319–1328.

19. Pinna C, Vecchiato CG, Bolduan C, et al. Influence of dietary protein and fructooligosaccharides on fecal fermentative end-products, fecal bacterial populations and apparent total tract digestibility in dogs. BMC Vet Res 2018;14:106.

20. Lin C-Y, Alexander C, Steelman AJ, et al. Effects of a Saccharomyces cerevisiae fermentation product on fecal characteristics, nutrient digestibility, fecal fermentative end-products, fecal microbial populations, immune function, and diet palatability in adult dogs1. J Anim Sci 2019;97:1586–1599.

21. Nery J, Goudez R, Biourge V, et al. Influence of dietary protein content and source on colonic fermentative activity in dogs differing in body size and digestive tolerance. J Anim Sci 2012;90:2570–2580.

22. Summers SC, Quimby JM, Isaiah A, et al. The fecal microbiome and serum concentrations of indoxyl sulfate and p-cresol sulfate in cats with chronic kidney disease. J Vet Intern Med 2019;33:662–669.

23. Bansal T, Alaniz RC, Wood TK, et al. The bacterial signal indole increases epithelial-cell tight-junction resistance and attenuates indicators of inflammation. Proc Natl Acad Sci U S A 2010;107:228–233.

24. Darkoh C, Plants-Paris K, Bishoff D, et al. Clostridium difficile Modulates the Gut Microbiota by Inducing the Production of Indole, an Interkingdom Signaling and Antimicrobial Molecule. mSystems 2019;4.

25. Ashaolu TJ, Saibandith B, Yupanqui CT, et al. Human colonic microbiota modulation and branched chain fatty acids production affected by soy protein hydrolysate. Int J Food Sci Technol 2019;54:141–148.

26. Hang I, Heilmann RM, Grützner N, et al. Impact of diets with a high content of greaves-meal protein or carbohydrates on faecal characteristics, volatile fatty acids and faecal calprotectin concentrations in healthy dogs. BMC Vet Res 2013;9:201.

27. Barry KA, Hernot DC, Van Loo J, et al. Fructan supplementation of senior cats affects stool metabolite concentrations and fecal microbiota concentrations, but not nitrogen partitioning in excreta. J Anim Sci 2014;92:4964–4971.

28. Urrego MIG, Matheus LF de O, de Melo Santos K, et al. Effects of different protein sources on fermentation metabolites and nutrient digestibility of brachycephalic dogs. J Nutr Sci 2017;6:e43.

29. Farcas AK, Larsen JA, Owens TJ, et al. Evaluation of total dietary fiber concentration and composition of commercial diets used for management of diabetes mellitus, obesity, and dietary fat-responsive disease in dogs. J Am Vet Med Assoc 2015;247:501–507.

30. Schauf S, de la Fuente G, Newbold CJ, et al. Effect of dietary fat to starch content on fecal microbiota composition and activity in dogs. J Anim Sci 2018.

31. Deb-Choudhury S, Bermingham EN, Young W, et al. The effects of a wool hydrolysate on short-chain fatty acid production and fecal microbial composition in the domestic cat (Felis catus). Food Funct 2018;9:4107–4121.

32. Schmidt M, Unterer S, Suchodolski JS, et al. The fecal microbiome and metabolome differs between dogs fed Bones and Raw Food (BARF) diets and dogs fed commercial diets. PloS One 2018;13:e0201279.

Assessment of the Intestinal Microbiome

Jan Suchodolski, MedVet, DrVetMed, PhD, AGAF
Diplomate ACVM (Immunology)
Associate Professor, Small Animal Medicine
Associate Director for Research, Head of Microbiome Sciences
Gastrointestinal Laboratory
Texas A&M University, College of Veterinary Medicine, USA

INTRODUCTION

The intestinal microbiota is defined as the collection of all living microorganisms (i.e., bacteria, fungi, protozoa, and viruses) that inhabit the gastrointestinal tract. With the development of novel molecular analysis tools (based most commonly on sequencing of the bacterial 16S rRNA gene), it is now appreciated that the gastrointestinal microbiota of mammals is highly diverse, comprising several hundred to over a thousand bacterial phylotypes.[1] Gut microbes are useful to the host by acting as a defending barrier against transient pathogens, they aid in digestion and help to harvest energy from the diet, they provide nutrition for enterocytes, and play a very important role in the development and regulation of the host immune system. However, the intestinal microbiota can also have a detrimental influence on gastrointestinal health, as in the last few years convincing evidence has been gathered associating alterations in the composition of the intestinal microbiota with chronic enteropathies of humans, dogs, and cats.[2,3]

For proper assessment of the contributions of the microbiota to health and disease, it is important to recognize which bacterial groups are present in the gastrointestinal tract, as well as to study the functional properties of the resident microbiota and their impact on the host. This can be achieved through shot-gun sequencing of microbial DNA, and assessment of microbial derived metabolites (e.g., short-chain fatty acids, indoles, and secondary bile acids).[4]

Characterization of gastrointestinal microbiota

Until recently, traditional bacterial culture was the most commonly used method for describing the bacterial groups present in the gastrointestinal (GI) tract of dogs and cats. Bacterial culture can be a useful technique for the detection of specific intestinal pathogens *(e.g., Salmonella, Campylobacter jejuni)* of interest, however, it is now well recognized that bacterial culture is not well suited for in-depth characterization of complex environments such as the mammalian gastrointestinal tract. Because the majority of intestinal bacteria cannot be cultured, a culture based method underestimates total bacterial numbers, and does not allow identification of the majority of bacterial groups present in the GI tract. Some reasons for our inability to culture most intestinal bacteria include our lack of knowledge regarding their optimal growth requirements and the fact that the canine and feline gastrointestinal tract harbors predominantly anaerobic bacteria, which are prone to sampling and handling damage. Furthermore, many selective culture media lack sufficient specificity and often other organisms than the targets are enumerated.

Molecular characterization of the intestinal microbiota

Molecular tools allow the identification of previously uncharacterized intestinal microbes and these techniques are also able to provide information about the functionality of the microbiome by means of metagenomics and transcriptomics. Several methods are available and all of these approaches are ideally used in a complementary fashion. A brief overview of these methods is provided in Table 1.

Gastrointestinal microbiota of healthy dogs and cats

Due to differences in anatomical and physiological properties along the gastrointestinal tract (i.e., differences in pH, bile concentrations, intestinal motility), the microbial composition differs among the segments of the GI tract. Furthermore, differences are observed between luminal and mucosa-adherent microbial populations. Of special note is that each dog and cat harbors a very unique and individual microbial profile.[5] These differences in bacterial composition between individual animals may explain, in part, why there is a highly individualized response to therapeutic approaches that are designed to modulate intestinal microbiota: not every animal will respond similarly to dietary changes, or administration of antibiotics or nutraceuticals (i.e., probiotics).

Differences also exist in the number of total bacteria in the different compartments of the GI tract. Bacterial counts in the duodenum and jejunum of dogs and cats can range from 10^2 to 10^9 cfu/ mL of contents. The distal small intestine (i.e., ileum) contains a more diverse microbiota and higher bacterial numbers (10^7 cfu/ mL of contents) than the proximal small intestine. Bacterial counts in the colon range from 10^9 and 10^{11} cfu/ml of intestinal content.[6]

The phyla Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, and Fusobacteria constitute almost 99% of all gut microbiota in dogs and cats. Aerobic bacteria occur in higher abundance in the small intestine, whereas anaerobic bacteria predominate in the large intestine. In the stomach, mucosa-adherent Helicobacter spp. are the major group, followed by various lactic acid bacteria (i.e., *Lactobacillus and Streptococcus* spp.) and Clostridia spp. The most abundant groups in the small intestine are Clostridia, Lactobacillales, and Proteobacteria, whereas Firmicutes, Bacteroidetes, and Fusobacteria are the predominant bacterial phyla in the large intestine.[7] The phylum Firmicutes comprises many phylogenetically distinct bacterial groups, the so called Clostridium clusters. Clusters XIVa and IV encompass many important short-chain fatty acid producing bacteria (i.e., *Ruminococcus* spp., *Faecalibacterium* spp., *Dorea* spp.) and are the major groups in the ileum and colon of cats and dogs.

More recent studies are attempting to study the functional properties of the intestinal microbiota. This is important because it remains challenging to correlate the presence of specific bacterial groups with gastrointestinal health and disease. For example, administration of antibiotics to healthy animals leads to decreases in some of the beneficial bacterial groups, but this change does not lead to obvious gastrointestinal problems.[8] It is believed that a functional redundancy exists in the GI tract, with several members of the bacterial community performing similar functions, and if one group is displaced because of perturbations (e.g., antibiotic therapy), other members of the community appear to maintain a stable ecosystem function. Therefore, it is crucial to evaluate the intestinal microbiome as an entity, including phylogenetic relationships and metabolic functions (i.e., metagenome, transcriptome, and metabolome).

Fungi, archaea, and viruses

In addition to bacteria, the mammalian GI tract harbors various fungi, archaea, protozoa, and viruses. Recent molecular studies have provided more in depth analysis about the diversity of the fungal microbiota and the virome in dogs, but their role in disease remain unclear.[9,10]

Assessment of intestinal dysbiosis

Microbiota dysbiosis is defined as an altered composition of the bacterial microbiota. It should be noted that some disease processes may be associated with changes in microbiota function (e.g. reduced production of SCFA and other metabolites) rather than shifts in microbiota composition. Therefore, the definition of dysbiosis is an evolving concept. These alterations are often not readily detected due to the current lack of assays to comprehensively capture the microbiota along the entire GI tract. Furthermore, it is currently not possible to assess interactions between microbiota and the host immune system. Because all these inaccessible factors play a crucial role in the intricate communication between bacteria and the host immune system, crude assessment of bacterial changes in intestinal samples often does not explain the entire disease process. Nevertheless, much progress has been made in characterizing intestinal dysbiosis in GI diseases, and recent metabolomics studies have also provided initial insights into the functional consequences of dysbiosis, and its role in the pathophysiology of some GI disorders in dogs.[11]

There is currently no single best method for assessing GI microbiota and dysbiosis. Because the gut is a complex ecosystem, the best diagnostic approach for dysbiosis would be a combination of molecular tools that include next generation (NGS) sequencing, direct quantification of specific bacterial taxa by quantitative PCR (qPCR), and FISH to visualize the translocation of bacteria into the mucosal epithelium. However, such an extensive approach is currently available only in research settings. More recent studies are attempting to study the functional properties of the intestinal microbiota. Novel metabolomics approaches using multiple mass spectrometry platforms allow assessing changes in metabolite profiles, either produced by the host or by the microbiota, with both together yielding a better understanding of the pathophysiology of GI disease. In human medicine such assays are becoming increasingly available. For example, measurement of fecal bile acids can help diagnosing dysbiosis associated with bile acid diarrhea, and treatment with bile acid sequestrants (e.g., cholestyramine) may lead to improvement of diarrhea.[4,12]

Studies using sequencing of 16S rRNA genes have described dysbiosis in dogs and cats with chronic enteropathies (CE) and acute diarrhea.[2] In CE there is an increase in proportions of bacterial genera belonging to Proteobacteria (e.g. *E. coli*) and decreases in Fusobacteria, Bacteroidetes, and members of Firmicutes (i.e. Faecalibacterium, Ruminococcaceae, Turicibacter, Blautia). In fecal samples of dogs with acute hemorrhagic diarrhea syndrome (AHDS) an increase in *C. perfringens* expressing the gene for *netF*-toxin has been reported which is highly associated with AHDS.13 However, the dysbiosis pattern improves and C. perfringens harboring the gene decrease very rapidly in these dogs within 3-7 days, even without the use of antibiotics.[13]

The canine microbiota dysbiosis index (DI) is a recently developed rapid PCR based assay that quantifies the abundances of 8 bacterial groups. These bacterial groups (e.g., *Faecalibacterium, E. coli, Blautia, Streptococcus, Turicibacter*) have been reported to be commonly altered in dogs with chronic enteropathies.3 The advantage of the dysbiosis index is that it summarizes the results of these 8 bacteria taxa in one single number, allowing us to define a reference interval

for healthy animals. A DI below 0 indicates normal fecal microbiota, while a DI of 0 or above indicates fecal dysbiosis. An increase in the dysbiosis index is observed in dogs with chronic enteropathy (food-responsive and antibiotic-responsive diarrhea, idiopathic IBD), dogs with exocrine pancreatic insufficiency, and also dogs with acute diarrhea. Of importance is that dysbiosis is a now recognized to be a component of chronic enteropathies, and the presence of dysbiosis does not predict which therapy a dog will respond best to (i.e., also dogs with food-responsive diarrhea may have an increased dysbiosis index). The dysbiosis patterns in CE do not resolve for at least several months, even when dogs improve clinically. This is likely due to the remaining underlying inflammation due to residual histological inflammation. The use of the dysbiosis index has been reported in a recent study evaluating the impact of fecal microbiota transplantation (FMT) on the microbiota in dogs with chronic enteropathies.[14] Weekly measurements of the dysbiosis index allowed monitoring the improvement of dysbiosis one week after FMT, but the dysbiosis index increased in one dog approximately 3 weeks after FMT. The dysbiosis index is currently available for dogs through the Gastrointestinal Laboratory at Texas A&M University.

As mentioned above, in dogs with chronic enteropathies, dysbiosis can be a component of the multi-factorial disease process. While in some dogs dysbiosis is the main driver of clinical signs, in other dogs dysbiosis is more of an effect of the underlying histological inflammatory process. Therefore, fecal dysbiosis is not a predictor of whether a dog will benefit from antibiotics. There are, however, conditions were antibiotics are warranted to clear an infection or bacterial translocation. Fluorescence in-situ hybridization (FISH) allows visualizing the translocation of bacteria into the mucosal epithelium, and can be a particular useful assay for diseases like granulomatous colitis of Boxer dogs, which is one specific form of CE that responds to antibiotics. It has been associated with mucosal infiltration of invasive and adherent *E. coli* in the colon.[15]

A subset of animals with CE has a dysbiosis in the small intestine (SID), and these dogs typically respond well to antibiotic administration. This disease syndrome is called antibiotic-responsive diarrhea (ARD) by some authors. It is difficult to definitively diagnose SID/ARD, as there is no routinely available duodenal culture or molecular assay that would allow diagnosing small intestinal dysbiosis or a bacterial overgrowth. A tentative diagnosis can be made by evaluating clinical signs, together with findings of altered serum cobalamin and folate concentrations, and a positive response to an antibiotic therapeutic trial. However, since diseases due to undetected intestinal pathogens may also respond to antibiotic therapy, a positive response to therapy does not necessarily confirm the presence of small intestinal dysbiosis. Serum cobalamin concentrations may be decreased and serum folate concentrations may be increased in dogs with SID/ARD. Changes in small intestinal microbiota may lead to an increased bacterial utilization of cobalamin, resulting in decreased absorption of cobalamin in the ileum. Bacteria in the distal small and large intestine produce folic acid, but folate absorption via carriers takes place in the proximal small intestine. When folate producing bacteria accumulate in the proximal small intestine, an increased amount of bacterial folate will be absorbed by the host, resulting in increased serum folate concentration. However, cobalamin and folate uptake from the small intestine is highly complex and can be affected by several mechanisms and it is, therefore, not highly specific for SID/ARD. A diet high in folate may lead to falsely increased serum folate concentrations. Inflammation of the ileum may damage cobalamin receptors and thus may lead to cobalamin malabsorption. Dogs with exocrine pancreatic insufficiency (EPI) have a decreased secretion of antibacterial products with subsequent small intestinal bacterial overgrowth. As a consequence, dogs with EPI often have increased serum folate concentrations. Thus, in dogs with an abnormal serum concentration of cobalamin and/or folate, serum trypsin-like immunoreactivity (TLI) should be evaluated to rule out EPI. It has been demonstrated that administration of tylosin does not lead, as would be expected, to a decrease in serum folate and an increase in serum cobalamin concentration. Therefore, serum folate concentrations may not reflect therapeutic success and serum folate concentrations should always be evaluated together with the clinical picture. When both serum cobalamin and folate concentrations are altered, this is highly suggestive of SID. However, both have a rather poor sensitivity and specificity for the diagnosis of SID.[16]

Conclusions

Recent advances in molecular diagnostics have allowed us to gain a better overview of the microbes present in the GI tract, however, our understanding of the complex interactions between microorganism and the host are still very rudimentary. Future studies will need to encompass metagenomics, transcriptomics, and metabolomics to understand the crosstalk between microbes and the host. These may allow us better to diagnose dysbiosis on a functional level, and to develop treatment modalities targeted at modulating the intestinal microbiota.

Table 1. Commonly used methods for characterization of the intestinal microbiota

Method	Purpose	Description	Advantages / Disadvantages
Fluorescence in situ hybridization (FISH)	identification, quantification, visualization of bacterial cells	fluorescent dye-labeled oligonucleotide probes are hybridized to ribosomal RNA sequence in bacterial cells	useful method for quantifying bacteria, allows visualization of bacteria in tissue / labor intense, FISH probes need to be developed for groups of interest
Quantitative real-time PCR (qPCR)	quantification of bacterial groups	target organisms are detected in real-time using fluorescent dye-labeled primers and/or probes	rapid, inexpensive, quantitative / primer/probes need to be designed for groups of interest
16S rRNA gene sequencing	identification of bacteria in a sample	bacteria in a sample are amplified using universal primers, PCR amplicons are separated and sequenced using a high-throughput sequencer	rapid, relative inexpensive, allows identification of bacteria, semi-quantitative, allows to describe changes within a community / requires advanced bioinformatics
Metagenomics (shotgun sequencing of genomic DNA)	identification of microbial genes present in sample	genomic DNA is fragmented and then randomly sequenced (without PCR amplification) on a high-throughput sequencer	provides not only phylogenetic information but also what functional genes are present in sample / expensive, requires advanced bioinformatics

References

1. Handl S, Dowd SE, Garcia-Mazcorro JF, et al. Massive parallel 16S rRNA gene pyrosequencing reveals highly diverse fecal bacterial and fungal communities in healthy dogs and cats. *FEMS Microbiol Ecol* 2011;76:301-310.
2. Honneffer JB, Minamoto Y, Suchodolski JS. Microbiota alterations in acute and chronic gastrointestinal inflammation of cats and dogs. *World J Gastroenterol* 2014;20:16489-16497.
3. AlShawaqfeh MK, Wajid B, Minamoto Y, et al. A dysbiosis index to assess microbial changes in fecal samples of dogs with chronic inflammatory enteropathy. *FEMS Microbiol Ecol* 2017; 93: doi: 10.1093/femsec/fix136.
4. Giaretta PR, Rech RR, Guard BC, et al. Comparison of intestinal expression of the apical sodium-dependent bile acid transporter between dogs with and without chronic inflammatory enteropathy. *J Vet Intern Med* 2018;32:1918-1926.
5. Guard BC, Suchodolski JS. Canine intestinal microbiology and metagenomics: From phylogeny to function. *J Anim Sci* 2016;94:2247-2261.
6. Mentula S, Harmoinen J, Heikkila M, et al. Comparison between Cultured Small-Intestinal and Fecal Microbiotas in Beagle Dogs. *Applied and Environmental Microbiology* 2005;71:4169-4175.
7. Honneffer JB, Guard B, Suchodolski JS. Variation of the microbiota and metabolome along the canine gastrointestinal tract. *Metabolomics* 2017;13:doi:10.1007/s11306-11017-11165-11303.
8. Suchodolski JS, Dowd SE, Westermarck E, et al. The effect of the macrolide antibiotic tylosin on microbial diversity in the canine small intestine as demonstrated by massive parallel 16S rRNA gene sequencing. *BMC Microbiol* 2009;9:210.
9. Foster ML, Dowd SE, Stephenson C, et al. Characterization of the fungal microbiome (mycobiome) in fecal samples from dogs. *Vet Med Int* 2013;2013:658373.
10. Moreno PS, Wagner J, Mansfield CS, et al. Characterisation of the canine faecal virome in healthy dogs and dogs with acute diarrhoea using shotgun metagenomics. *PLoS One* 2017;12:e0178433.
11. Guard BC, Barr JW, Reddivari L, et al. Characterization of Microbial Dysbiosis and Metabolomic Changes in Dogs with Acute Diarrhea. *PLoS ONE* 2015;10:e0127259.
12. Guard BC, Honneffer JB, Jergens AE, et al. Longitudinal assessment of microbial dysbiosis, fecal unconjugated bile acid concentrations, and disease activity in dogs with steroid-responsive chronic inflammatory enteropathy. *J Vet Intern Med* 2019; doi: 10.1111/jvim.15493.
13. Ziese AL, Suchodolski JS, Hartmann K, et al. Effect of probiotic treatment on the clinical course, intestinal microbiome, and toxigenic Clostridium perfringens in dogs with acute hemorrhagic diarrhea. *PLoS One* 2018;13:e0204691.
14. Gerbec Z. Evaluation of therapeutic potential of restoring gastrointestinal homeostasis by a fecal microbiota transplant in dogs. *Pharmacology*: University of Ljubljana, Slovenia, 2016.
15. Manchester AC, Hill S, Sabatino B, et al. Association between Granulomatous Colitis in French Bulldogs and Invasive Escherichia coli and Response to Fluoroquinolone Antimicrobials. *J Vet Intern Med* 2013;27:56-61.
16. German AJ, Day MJ, Ruaux CG, et al. Comparison of direct and indirect tests for small intestinal bacterial overgrowth and antibiotic-responsive diarrhea in dogs. *J Vet Intern Med* 2003;17:33-43.

Microbiome Research at Hill's Pet Nutrition: Past, Present and Future

Jennifer Radosevich, Ph.D.
Worldwide Director, Research
Hill's Pet Nutrition Center
Topeka, KS, USA

Hill's Past

In 2012, Hill's Pet Nutrition embarked on an exciting initiative to research and understand the gut microbiome of cats and dogs, and how nutrition can shape their microbiomes. It all started with a new laboratory and a vision to become the global leader in providing optimal nutrition not only for the pet but also for the pet's microbiome. Hill's efforts in the microbiome field started well before this; however, it is just now because of the availability of state of the art tools in the early 2010's that we can delve into much more biology than we ever thought possible.

Hill's initial microbiome research sought to understand how certain disease conditions could influence the preponderance of particular taxa in the microbiome such as Lactobacillus and Bifidobacterium[1]. Our knowledge of the microbiome was limited to hybridization and cultural techniques that detected large abundance species and we were missing many of the lesser abundance microbes and so-called "un-culturables". The state of these technologies was such that we could only demonstrate the presence and quantity of limited numbers of bacteria, and we missed many microbes that held critical roles in determining gut health.

Despite limitations of initial techniques, we were able to learn about the gut microbiome of pets and what we could feed the pet to shift it to encourage a healthier microbiome. In our research we evaluated probiotics (live organisms to benefit host health)[2] and prebiotics (non-digestible food ingredients that beneficially stimulate bacteria in the colon to improve host health)[3]. Very quickly we determined that feeding prebiotics to the host's microbes is a better strategy to impact a pet's microbiome than administering probiotics. In part, probiotics exert their beneficial effects by attaching to the host gut via specific proteins, and their efficacy is profoundly linked to their specific recognition of the host through co-evolution[4]. Therefore, the bacterial species of the probiotic should originate from the gut of the specific host species; however, this is not true for most probiotics currently marketed for companion animals. In contrast, prebiotics feed the existing microbiome rather than adding a proportionately small amount of probiotic organism not specifically linked to the host. The first prebiotics tested were fiber-based because it was well known that many fibers escape digestion and absorption in the stomach and small intestine and reach the colon where they are available for use by the pet's microbiome. Through our research, we began to understand that not all fiber benefits pet health in the same way. In fact, if inappropriate types and/or ratios of fibers are included in the food, the pets' gastrointestinal system may react with undesirable results for both the pet and pet caretaker. Understanding the differences between soluble versus insoluble fiber (i.e. whether the fiber is soluble in water) and fermentable versus non-fermentable fiber (i.e. whether the fiber can be fermented by the microbiome) are key in formulating a pet food that strikes the right balance for the pet's gut microbiome and health condition.

Hill's Present

Our capabilities have continued to grow and have enabled us to develop specific nutrition to positively affect fiber-responsive diseases and gut health[5]. This has been facilitated by next generation sequencing (NGS) technologies, which allow us to target the 16S ribosomal DNA of an organism (the "fingerprint" that identifies each bacterium). Through this technology we not only can identify largely abundant and culturable bacteria, we can now also identify and quantify bacteria that are less abundant and unculturable. These "keystone"

bacteria may be present at low levels, but they fulfill a critical role in enabling the rest of the microbiome to metabolize fibers that benefit pet health.

Additionally, we can now evaluate the functional capacity of the microbiome to determine what bacteria are doing in terms of their metabolism. Two approaches are currently employed to determine microbiome function. First is to infer microbial community functions by predicting metabolic pathways and functions of the bacterial population using the gene sequences of the bacteria in the sample. The second and more reliable approach for determining the impact of prebiotics on microbiome function is to measure the postbiotics of the microbial population in response to a prebiotic ingredient. Postbiotics are metabolites produced by the gut microbiome[6,7]. Postbiotics include not only metabolites derived from undigested carbohydrates, fat and protein, but also microbial derivatives of other compounds, such as plant secondary metabolites; these metabolites can have a beneficial or detrimental effect on the host, depending upon the nature of the compound. By carefully choosing a balanced mix of prebiotic fiber sources, the desired outcomes can be designed into the food for optimized gastrointestinal health, as evidenced by Hill's new Prescription Diet Gastrointestinal Biome food. This food has been shown to nourish and activate pet's gut microbiome to promote digestive health and well-being.

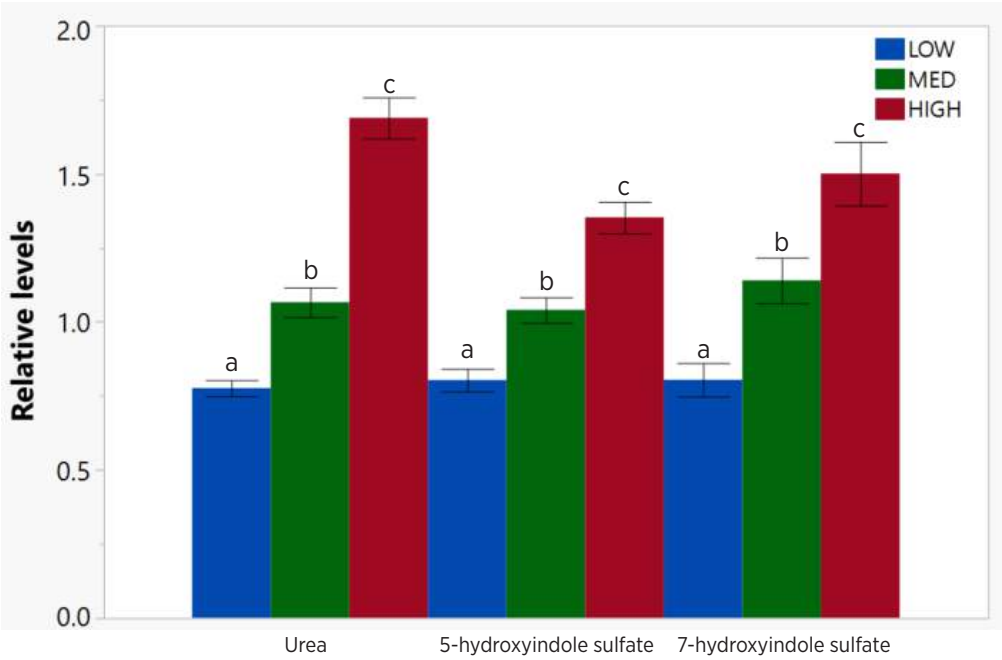
Hill's Future

Most recently, our understanding of "prebiotic" has extended beyond just fiber to macronutrients including protein and fat. The macronutrient ratios of the foods ingested by individuals can have profound effects on their gut microbiota and the resulting postbiotics[8,9].

Undigested fibers that enter the colon undergo saccharolytic fermentation to produce beneficial postbiotics such as short chain fatty acids, including butyrate. On the other hand, high protein foods have been shown to cause gut microbes to process protein during fermentation, resulting in a reduction in the number of microbes that produce beneficial postbiotics such as butyrate. This happens when the dietary protein load cannot be fully digested by the pet in the small intestine and is released to the colon where the microbiome ferments protein. Protein fermentation can result in putrefactive products such as H_2S, indoles, phenols, and branched chain fatty acids[10]. To avoid this issue, it is important to consider the appropriate protein to carbohydrate ratio of a food, to provide substrates to the microbiome for saccharolytic fermentation. Furthermore, high quality protein that is highly digestible by the pet keeps protein nutrition directed to the pet rather than allowing it to bypass and result in putrefactive end products.

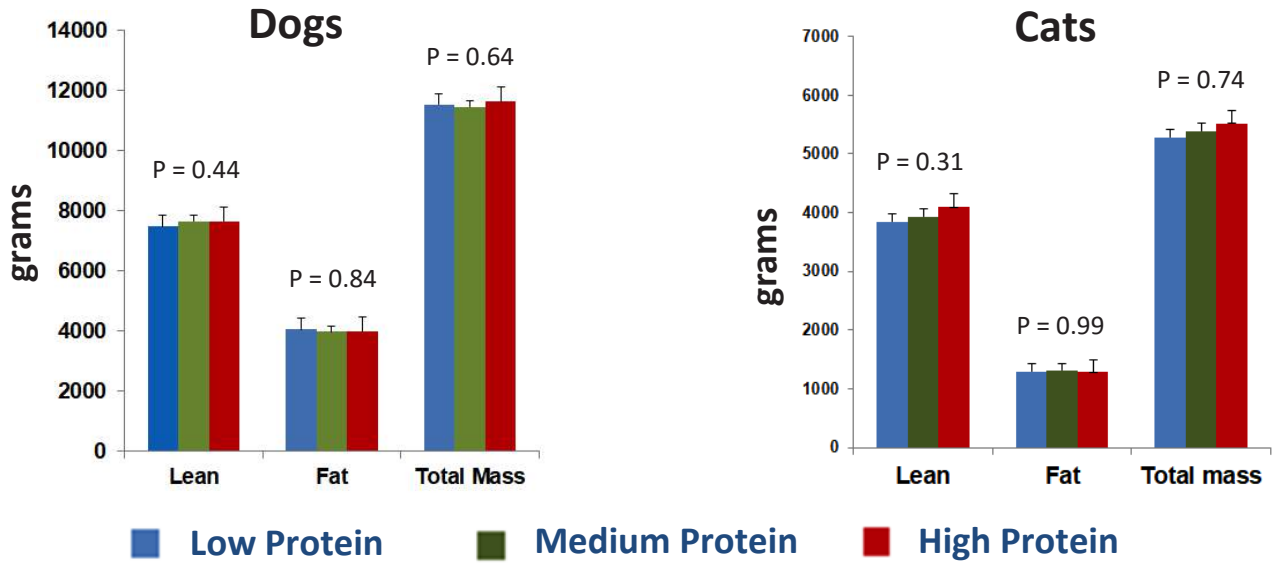
In a recent study, we demonstrated that the ratio of protein to carbohydrate in a pet's food is paramount to maintain the balance of postbiotics for pet health. For example, feeding dogs increased dietary protein and reduced dietary carbohydrate resulted in a significant decrease of beneficial short chain fatty acids (SCFA) in feces of canine, and significantly increased circulating levels of uremic toxins and indole sulfates in canine (**Figure 1**).[8] It is important to note that lean body and total mass of the pets did not vary between high, medium, and low levels of protein at the end of the 90-day feeding period (**Figure 2**).[8,9] Therefore, we might expect to see optimized levels of protein and other non-fiber prebiotics in Hill's foods to ensure the gut microbiome health of the pet.

Figure 1. Increased protein consumption led to high blood levels of uremic toxins in dogs at the end of the 90-day feeding period



Columns with different subscripts are significantly different at the $p < 0.01$ level

Figure 2. Varying protein consumption did not change body composition at the end of the 90-day feeding period



Future research will enable us to develop nutritional solutions that work through the gut microbiome to enhance more than gut health. It is clear that the gut microbiome affects other organ systems such as the kidney and the brain. For example, the postbiotic indoxyl sulfate, resulting from bacterial metabolism of protein, has been correlated with decreased renal function of cats[11]. Additionally, the postbiotic 4-ethyl phenyl sulfate has been associated with anxiety-like behaviors in mice.[12,13] Understanding how to formulate foods that result in optimal levels of postbiotics has the potential to enhance pet health in ways not thought possible even a decade ago.

Summary

Hill's has built a state of the art research program that has evolved over time to enhance our knowledge about the pet's gut microbiome. Understanding how dietary components can positively modulate the gut microbial profile and functions will allow further enhancement of companion animal health through nutrition. It is also clear that feeding both the pet and their microbiome are critical to pet health, and future nutritional solutions must address both to support overall well being of pets. Furthermore, the gastrointestinal system is only one aspect of a pet's health that can be affected by the gut microbiome; further research regarding the gut-kidney and gut-brain axes likely will result in foods that enhance pet health in those areas as well.

References

1. Inness, V. L., McCartney, A. L., Khoo, C., Gross, K. L. and Gibson G. R. (2007) Molecular characterisation of the gut microflora of healthy and inflammatory bowel disease cats using fluorescence in situ hybridisation with special reference to Desulfovibrio spp. J. Animal Physiol. and Animal Nutr. 91, 48–53
2. Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO). Guidelines for the Evaluation of Probiotics in Food (2002)
3. Gibson, G.R., and Roberfroid, M.B. (1995). Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J. Nutr. 125, 1401-1412. Epub 1995/06/01.
4. McFarland, L.V., Evans, C.T., and Goldstein, E.J.C (2018). Strain-Specificity and Disease Specificity of Probiotic Efficacy: A Systematic Review and Meta-Analysis. Front. Med. 5: 124
5. Jackson, M.I and Jewell, D.E. (2018). Balance of saccharolysis and proteolysis underpins improvements in stool quality induced by adding a fiber bundle containing bound polyphenols to either hydrolyzed meat or grain-rich foods, Gut Microbes, DOI:10.1080/19490976.2018.1526580
6. Tsilingiri K, Barbosa T, Penna G, Caprioli F, Sonzogni A, Viale G, et al. Probiotic and postbiotic activity in health and disease: comparison on a novel polarised ex-vivo organ culture model. Gut. 2012 Jul;61(7):1007-15. doi: 10.1136/gutjnl-2011-300971.
7. Ojeda P, Bobe A, Dolan K, Leone V, Martinez K. Nutritional modulation of gut microbiota - the impact on metabolic disease pathophysiology. J Nutr Biochem. 2016 Feb;28:191-200. doi: 10.1016/j.jnutbio.2015.08.013.
8. Ephraim, E. and Jewell, D. (2019) Long term consumption of high protein disrupts dog gut microbiome and metabolites. Microbiome & Probiotics Series: Europe, Rotterdam, May 20-22
9. Badri, D.V. and Jewell, D.E. (2019) Dietary protein levels show no effect on lean body mass but impacts blood, urine and fecal clinical parameters in cats. ASM Microbe, San Francisco, June 20-24
10. de Godoy M.R.C., Kerr K.R. and Fahey, G.C. (2013) Alternative dietary fiber sources in companion animal nutrition, Nutrients. 5: 3099-3117
11. Summers, S.C., Quimby, J.M., Isaiah, A., Suchodolski, J.S. Lunghofer, P.J., Gustafson, D.L. (2018) The fecal microbiome and serum concentrations of indoxylsulfate and p-cresol sulfate in cats with chronic kidney disease. J. Vet. Intern. Med. 1–8.
12. Hsiao E.Y., McBride S.W., Hsien S., Sharon G., Hyde E.R., McCue T., Codelli J.A., Chow J., Reisman S.E., Petrosino J.F., Patterson P.H. and Mazmanian S.K. The microbiota modulates gut physiology and behavioral abnormalities associated with autism. (2013) Cell 155(7):1451–1463
13. Dorrestein P.C., Mazmanian S.K. and Knight R. (2014) Finding the missing links among metabolites, microbes, and the host. Immunity 40: 824-832.

The Power of Microbiome: Feline Constipation

Susan Little, DVM, DABVP (Feline)
Bytown Cat Hospital
Ottawa, Ontario, Canada

Constipation is the infrequent and difficult evacuation of feces with retention of feces within the colon and rectum; intractable constipation is called obstipation. The typical feline patient with constipation is middle-aged and male **(Image 1)**. Many cats have one or two episodes of constipation without further problems; however, some cats with chronic constipation and obstipation develop megacolon with a dilated large bowel that is poorly responsive to therapy. Cats with idiopathic megacolon have generalized dysfunction of colonic smooth muscle.[1] Some of the more common underlying causes of constipation include certain drugs, stressors and litter box aversion, difficulty in defecating (pain, neurologic problems), excessive fecal bulk, dehydration (e.g., associated with chronic kidney disease), intra- or extra-luminal colon masses, narrowed pelvic canal, and idiopathic megacolon. Whenever possible, the underlying cause should be identified and corrected.

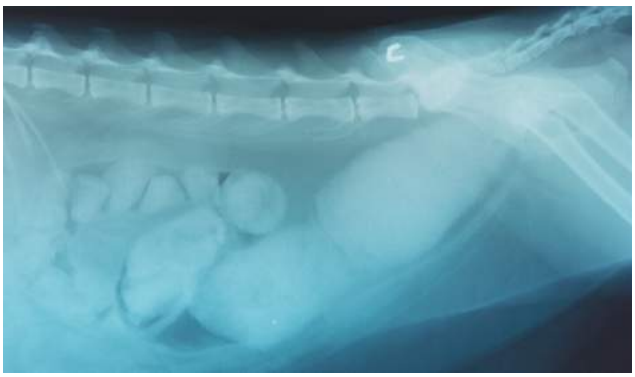


Image 1. A lateral x-ray of a severely constipated 6 yr old neutered male cat

Clinical signs of constipation are typically obvious to owners and include tenesmus and scant hard dry feces, sometimes with blood. Cats will also strain in the litter box due to lower urinary tract obstruction and owners may misinterpret this as due to constipation. Occasionally, constipated cats will have intermittent diarrhea as the colon is irritated due to hard, dry fecal matter. Other clinical signs are non-specific, such as vomiting, inappetence and lethargy.

Physical examination confirms the presence of large amounts of feces in the colon sometimes accompanied by abdominal pain. During abdominal palpation, the colon may be identified as a long, firm tube or discrete concretions of feces. A careful evaluation (e.g., musculoskeletal system, caudal spinal cord function, anorectal area) should be made for underlying causes. Rectal exam should be performed, under sedation if necessary, for masses, pelvic fracture malunion and anal gland abnormalities especially in middle-aged and older cats with chronic clinical signs. A minimum database (complete blood count, serum chemistries/electrolytes, urinalysis, +/- total T4) should be assessed, especially to determine hydration and electrolyte status and identify underlying diseases such as chronic kidney disease. Survey radiographs are useful to confirm the diagnosis and assess severity as well as to evaluate for potential underlying causes, such as previous pelvic trauma and arthritis. Enlargement of the colon beyond 1.5 times the length of the body of the 5th lumbar vertebra has been proposed as indicating chronic dysfunction and megacolon.[2]

The first step in acute management of cats with constipation is correction of dehydration with intravenous fluid therapy followed by removal of obstructing feces. Pre-treatment with an anti-emetic such as maropitant is recommended before attempts to remove feces. One or two doses of a 5 mL microenema containing sodium lauryl sulfoacetate (e.g., MicroLax) is easily administered and will usually produce results within 20-30 minutes in mildly constipated cats. Obstipated cats will require warm water or isotonic saline enemas (5-10 mL/kg); safe additions to the water include mineral oil (5-10 mL/cat), or docusate (5-10 mL/cat), but do not administer the two together. Soaps or detergents may be irritating to an already compromised colonic mucosa. Lactulose solution

can also be administered as an enema (5-10 mL/cat). Sodium phosphate containing enemas must not be used as they can induce life-threatening hypernatremia, hyperphosphatemia and hypocalcemia in cats. Enemas are administered slowly with a lubricated 10-12 French feeding tube. In severe cases, manual manipulation of the feces via abdominal palpation or per rectum (manual disimpaction) under general anesthesia with endotracheal intubation (in case of vomiting) is also required. In these cases, opioids (e.g., buprenorphine, 0.01-0.03 mg/kg IM, IV, or transmucosal every 6-8 hours) should be administered short-term for pain relief. Long term use of opioids may cause constipation as an adverse effect.

An alternative to enemas is administration of an oral polyethylene glycol (PEG 3350) solution (e.g., CoLyte, GoLytely). A nasoesophageal tube is placed and the solution is given as a slow trickle (6-10 mL/kg/hour) over 4-18 hours. Defecation usually results in 6-12 hours. In a retrospective study of 9 cats, median time to defecation was 8 hours and the median total dose of PEG 3350 was 80 mL/kg.[3] No adverse effects were noted.

In addition to managing underlying conditions, long-term treatment may be accomplished with dietary therapy alone in many cats. Others may also need a prokinetic agent and/or a laxative. Dietary therapy may be successful with moderate fiber diets, including those with psyllium. Dietary fiber increases the production of short chain fatty acids which stimulate feline colonic smooth muscle contraction. Dietary fiber serves as a laxative by increasing fecal bulk and may not be beneficial for all patients.

A promising approach for managing cats with constipation includes dietary pre- and probiotics. An imbalance in colonic microflora is thought to contribute to some chronic gastrointestinal disease in people. Some probiotics lower colonic pH which increases peristalsis and decreases transit time. One pilot study of a probiotic containing *Lactobacillus* spp. and *Bifidobacterium* spp. was conducted in cats with chronic constipation refractory to traditional therapy.[4] Treated cats showed significant clinical improvement and biopsies of the colonic mucosa showed an anti-inflammatory effect.

If a cat's current diet cannot be changed for some reason, psyllium powder can be mixed with canned food at 1-4 teaspoons daily with meals.[5] A certain amount of trial and error is necessary to determine the best diet type for an individual patient.

It is also important to ensure adequate water intake by various methods, such as feeding moist diets. Using dog water bowls, which are larger than most bowls designed for cats, may help encourage drinking because cats dislike having their whiskers touch the side of containers. Other methods for increasing water intake include:

- Mix water with dry diets 1:1
- Flavor water with frozen cubes of meat or fish broth
- Try distilled or filtered water
- Ensure water is fresh every day, and provide multiple water bowls
- Ensure water bowls are kept clean
- Keep food and water bowls away from the litter box
- Feed multiple smaller meals instead of one or two larger meals

Cisapride stimulates contraction of feline colonic smooth muscle. A typical starting dose is 2.5 mg/cat BID, PO and it is better absorbed when given with food. Doses up to 7.5 mg/cat every 8 hours have been reported for large cats.[6] The drug is only available from compounding pharmacies in most countries. It has been withdrawn from the human market due to the occurrence of life-threatening arrhythmias in predisposed individuals (not known to occur in cats). It may be prudent to advise clients handling cisapride to wear gloves. Hyperosmotic laxatives include lactulose and PEG 3350; they stimulate colonic fluid secretion and propulsive motility. The dose of lactulose solution is 0.5 mL/kg, PO, BID-TID. PEG 3350 is available as a powder meant to be mixed in liquids for human use (e.g., RestoraLAX, MiraLAX). A suggested dose for cats is 1/8 to 1/4 teaspoon twice daily in food.

Litter box modification may be helpful for cats with arthritis. Most cat litter boxes are too small and have high sides. A winter boot tray or an under-the-bed type of storage box with low sides is a better alternative to make access easier. The litter box should also be in an accessible but private area, avoiding the need to navigate stairs if possible.

Subtotal colectomy (95-98% excision, with preservation of the ileocolic junction) should be considered for patients that are refractory to medical and dietary therapy. Long term outcome is considered excellent.[7] Most patients will experience transient diarrhea in the immediate post-operative period (1-6 weeks). In a small number of patients, diarrhea will persist.

References

1. Washabau RJ, Stalis IH. Alterations in colonic smooth muscle function in cats with idiopathic megacolon. *Am J Vet Res* 1996;57:580-587.
2. Trevail TIM, Gunn-Moore D, Carrera I, et al. Radiographic diameter of the colon in normal and constipated cats and in cats with megacolon. *Vet Radiol Ultrasound* 2011;52:516-520.
3. Carr AP, Gaunt MC. Constipation resolution with administration of polyethylene glycol solution in cats (abstract). *J Vet Intern Med* 2010;24:753-754.
4. Rossi, G., Jergens, A., Cerquetella, M., et al. (2018). Effects of a probiotic (SLAB51TM) on clinical and histologic variables and microbiota of cats with chronic constipation/megacolon: A pilot study. Beneficial Microbes, 9(1), 101–110.
5. Washabau RJ, Day MJ (eds). Canine & Feline Gastroenterology. St Louis: Elsevier Saunders, 2013; p. 96.
6. Plumb's Veterinary Drugs.
7. White RN. Surgical management of constipation. *J Feline Med Surg* 2002;4:129-138.

The Irresistible Magic of Story

Jessica Vogelsang, DVM, CVJ
Director, Pawcurious Media LLC
San Diego, CA, USA

When I stepped into work that fateful New Years' Eve, I had no idea that I was beginning both the best and worst day of my career.

I'm going to come back to that, but consider for a moment what you felt reading that sentence. Anticipation, wondering what happened? Dread, as your brain thought back to a horrible day you experienced? If nothing else, it's likely you at least feel some curiosity wondering how a day in the clinic can be both horrible and wonderful.

What if I had started with this sentence instead: "A recent study showed that 30% of geriatric dogs have subclinical disease that can increase the incidence of adverse events during anesthetic procedures." Would that have elicited the same curiosity to continue reading? Doubtful.

There is a reason that J.R.R. Tolkien's *Lord of the Rings* is so beloved while few people have made it through the *Silmarillion*. One is an epic tale focused on Frodo's journey to vanquish an evil ring of power, while the other one is a (fictional) history book. One centers on a single hero's story, while the other talks in more general terms about larger events and concepts. Only one of the two is optimized to bring you into the story and draw you along its path.

When it comes to communicating with our fellow man (and woman), there is no other tool as precise as that of a well-crafted story. While this has been proven over and over through the mass of scientific evidence in psychology, anthropology, and neurophysiology, none of us really need this body of proof to see its impact all around us. Without a single publication to lead the way, Neanderthals were making cave art 65,000 years ago, intuitively harnessing both visual communication and storytelling. Even back then, "Lions are dangerous" simply did not have the same impact as "our neighbor Urk was messily devoured last year when he wasn't paying attention to the bushes."

Facts are abstract concepts. When we hear facts, two relatively small parts of the brain are activated: Broca's and Wernicke's areas. These are responsible for language processing and comprehension. None of our other senses are employed, and the brain soon shelves the information. If I were to tell you, for example, that brachycephalic dogs are at increased risk for breathing problems due to elongated palates, stenotic nares, and narrow tracheas, you would think about this, and get on with your day.

Stories, on the other hand, create characters we identify with. When we are listening to a story, the experience of the character activates the parts of the brain associated with the senses involved with the experience: motor, auditory, olfactory, somatosensory, and visual. Let's try this:

"The rattling of Sam's excited breathing quieted down as the pug settled into his pre-anesthetic medications. My technicians began prepping him for his dental as I set my lunch into the microwave for a quick bite. Moses' anxious summons called me back to Sam's side before my lunch was done heating up. Hours later, long after the tears dried and the calls ended, it remained in the microwave, cold, rubbery, congealed, and forgotten."

Did you hear the dog in your mind? Hear your technician calling your name in panicked tones? See a congealed Lean Cuisine sitting alone and unloved in a microwave? The brain is lighting up all over with each sensory cue.

Emotion is itself a neural activator. Think of it like a vaccine adjuvant, stimulating the brain to better respond to the facts of the story. The stronger the emotion, the more likely we are to remember the events surrounding it.

A good story sets the stakes. Will our hero succeed or fail? What do those things look like? If our hero is created with empathy, the reader will identify with him or her and naturally root for them to succeed and mourn for them if they do not. With curiosity piqued, the reader awaits the resolution, feeling emotionally vested in the outcome. The outcome neatly outlines a motivation for action should the reader find him or herself in a similar situation.

"I knew Sam's owner was understandably devastated when our attempts to resuscitate him were unsuccessful, but I had no idea just how upset until two months later, when the news reporter showed up in the lobby to ask if I had any comment on the murder of Sam. Years later, with clinical practice long behind me, I came to identify this moment as one of the defining memories of practice. This, and sitting in a courtroom reading the accusation of elder abuse levelled at me later that year by Sam's owner."

As veterinarians, our job is to provide information to help our clients make the best decisions possible for their pet's health. Too often, we do so by dispassionately sharing the data while neglecting the emotion involved in the journey the owner is on with a sick pet. When we don't provide a story for context, they write their own.

Why is it that you are passionate about heartworm prevention? Is it because, as some people think, you're working towards a Hawaiian vacation as an incentive for prescribing a certain amount? Or is it because you'll never forget the look on Mrs. Jones's face when she saw the x-ray of Ranger's huge, heartworm infected heart? Much as we might like an owner's decision to be influenced by the logical map showing increased incidences of heartworm disease in their area, it is ultimately emotion that drives consumer decisions.

Why is it that GI health is so important? Maybe for some of us, that alone is something we care enough about to drive an emotional response. It's vital we understand our clients don't often see it the same way. Maybe the discovery that Diet A decreases GI hypermotility is cool enough to be all that we need to know, but for Mr. Smith, it's more the fact that his dog's accidents in the living room every night is causing strain in his marriage. This diet could prevent him from having to make the agonizing choice between his marital success and saying goodbye to the companion who got him through the death of his father.

The stories are there. They always are. We just need to bring them to light.

I was so devastated by the media attention and courtroom experience after Sam's untimely death that I began to feel physically ill when I showed up to the clinic every day. Maybe in today's world I would have seen this for the burnout that it was, but this was years ago, and I tried to tough it out. It didn't work. When my health began to deteriorate, I took a sabbatical to focus time on my young family.

During that period, I finally had time to go on international veterinary trips and rediscover my enthusiasm for working with animals.

I had the opportunity to focus on my writing, eventually leading to a book deal with a major publisher.

I was brought into hospice work by my good friend, which set me on the path of advocating fiercely for hands-on end-of-life care as an educational tool to help people deal with the deaths of other family members in a healthier way.

I will never be glad Sam died that day, but I will be grateful that in response to the set of events that took place after, I am here talking to you wonderful people.

Because of that path, I fulfilled a lifelong dream of seeing my book on the shelves at Barnes & Noble, my son's face lighting up with pride when he saw his mother's name on the cover.

And most importantly, when my mother was diagnosed with terminal cancer, I was able to be there emotionally and physically, and advocate for her because my path led me to a place where I was truly prepared.

We never know where these paths will lead, but I do know that every path is a story to be shared. Don't deprive the world of the wonder of your journey! You and your clients will be better for it.

The Big Benefits of a Social Media Savvy Team

Danielle K. Lambert
Founder, SnoutSchool.com
Huntington Beach, CA, USA

Have you heard the term "influencer marketing"? An online influencer is someone who has a targeted, engaged following on social media. From Grumpy Cat's 2.7 million followers to the Kardashian family's combined Instagram following of over 590 million, Instagram and YouTube are full of people who have developed entire careers around building a presence.

Why is there inherit value in having a following? It's simple: Do you ask friends for recommendations before you buy something or choose a service provider based on their suggestions? The people in our social sphere have the ability to influence our decisions, and undoubtedly this is why many companies are looking to internet influencers to market their products.

And this isn't a small phenomenon: It is estimated that influencer marketing could be a $10 billion industry by 2020 (Source: http://mediakix.com/2018/03/influencer-marketing-industry-ad-spend-chart/#gs.gllog3).

Major brands allocate huge percentages of their advertising budgets to influencer marketing programs, and it's no surprise considering the decrease in traditional television viewership (Source: https://www.washingtonpost.com/news/the-switch/wp/2015/12/21/42-percent-of-cord-cutters-dont-even-subscribe-to-home-broadband/?noredirect=on&utm_term=.26701f38ba70).

Advertising has always been about getting your message where the eyeballs are, and social media is what is currently stealing all of our attention. So what is your veterinary hospital's current strategy for advertising on social media? Do you think of social media as a place to post promos and patient pics? Do you assume pet owners only want to hear from your veterinarians or hospital account? Then you're missing big opportunities to brand out and connect with pet owners in your community.

Although big influencers with millions of followers might not have an audience relevant to the average veterinary clinic, there are potential influencers standing in front of you every single day: Your veterinary team.

Think about it: When a brand is choosing an influencer for a campaign, they're mostly evaluating the quality of the audience. The people on your veterinary team likely have the best audience a local business could need: Local people! Your team members are connecting on social media with their family, friends, and frequented businesses. Doesn't that sound like exactly the type of people you want coming through your doors with their pets?

You can leverage that influence by empowering them to act as brand ambassadors for your hospital. Many clinics fear their employees talking about work online, but this is a missed opportunity to connect with the local community. Making social media part of your team culture can help you to successfully communicate, educate, and connect online and in clinic.

Instead of teaching your team, "Don't post that!", it is critical to train on what is and isn't okay to post on social media. Overall, encourage your team members to do three things:

1) Keep all posts POSITIVE. Never shame, make fun of, or otherwise negatively comment on a case.

2) Post like you assume the pet's owner will see the post. Have tact, and avoid the gory details. No pet owner wants to log onto Instagram to see their pet's guts strewn about the surgical suite.

3) Do not give medical advice. Plain and simple.

Of course, you should have a social media consent form for patients. This way, pet owners know to expect

to see their pet's image and story online. Additionally discussing the post with the pet owner is also a smart move, both because it and have an attorney approve one for your team).

Each time a team member posts about a case on their personal social media, it's a reminder to their followers that they work at your clinic and that you are providing great care. Encourage them to take it a step further by including a call-to-action, which is simply asking your followers to do something that is relevant to your business. An example of a call-to-action on social media could be a reminder that followers can book a visit to see their favorite team member by clicking the link in their Instagram biography.

Having your team act as influencers for your brand online doesn't just end at sharing patient photos, though. Encourage team members with specific interests, like nutrition or dentistry, to show off their knowledge online. Hosting Q&As, sharing fun facts, and otherwise engaging with the community is a fun way to educate online without it seeming boring.

You might be thinking: Why would we have the team members do this on their individual profiles when we have a hospital account? If I let them build a personal brand, won't they just leave?

This is where it's time to talk about your mindset: Is it one of abundance, or is it one of scarcity? An "abundance mindset" is rooted in the concept that there is enough to go around. When we frame things like this, we are excited for others to learn and succeed. We see opportunity everywhere, and we don't fear change as much.

How does this compare to a "scarcity mindset"? When we think in scarcity, we worry about all of the potential outcomes. We worry about people stealing our ideas. We don't think there is enough opportunity to go around.

Empowering your team to succeed and grow requires a mindset of abundance and an overall positive team culture. Instead of worrying about what will happen if a team member builds a personal brand while working for you, why don't you consider what will happen if you try to control them?

Instead of saying "no", why don't you say "how?" Have conversations surrounding social media with your team, discuss what is and isn't ok to post, and help provide training that will improve their skills.

The more you empower your entire team to be involved in social media, the more opportunities they have to connect with your community, improve your online reputation, and educate your clients.

Social media hacks for the modern vet

Caitlin DeWilde, DVM
CEO, The Social DVM,
Webster Groves, MO, USA

Know Your Purpose

You cannot accurately complete the "return on investment" calculation without knowing what your product is, or the other parts of the equation, for that matter. Your "purpose" could vary wildly in our industry, and range from having a creative outlet to bringing in new clients to increasing revenue. Not one of them is wrong, but valuation of these end "products" is very different.

Just as importantly, knowing the "why" behind your social media efforts helps you maximize your efficiency. Your strategy will be more refined, your creative more on-point, and your planning much smoother.

Example "purposes" (and you can have more than one!):

- Revenue
- Loyalty
- Awareness
- Creative outlet
- Increased visits by existing clients
- New client acquisition
- Improving client service and accessibility

***Hack:** With your purpose in mind, include call to action statements and clickable links with your social whenever possible to drive viewers to that goal. For instance, if your purpose is getting more new clients, make sure your caption ends with a directive statement "we'd love to help your pet today" and a link to your appointment booking page.

Know Your Audience

Having a few clues about WHO it is you're trying to engage with will help your social media convert the casual scroller into an engaged fan. You may spend money and time creating the "perfect" video to advertise your practice, but if it doesn't resonate with your intended audience, both will be wasted. Here are some tips to get to know YOUR specific audience:

- Client registration forms should contain boxes for each social and review platform
- Access demographic data from practice management software and veterinary data companies
- Before bringing up taking photos, videos, requesting permission to do so, check your client's file to find out if they've authorized social media and their original referral source.

***Hack:** In addition to running new client referral reports in your PMS, check your Facebook Insights, Instagram Insights, Google Business Insights and Google Analytics for information on your follower's locations, age range, gender, etc.

Tracking

Just as we've discussed that knowing your objectives and your audiences is important, it's also important to track that knowledge over time. You'll have no way of knowing if you are meeting your goals without instituting some way to measure. Similarly, your audience (in person or online) deserves to be valued, so that you can be sure your message is reaching the people you care about. Your metrics to track are unique to the objective and methods used, but could include things like booked appointments, new clients, number of followers, web page views and more. Set up referral tracking in your PMS, make time to run regular reports (i.e. on new client numbers or overall revenue), monitor your social engagement, and work with your webmaster to utilize tools like Google Analytics and a Facebook

Pixel to measure traffic driven from your social media content.

Hack: Set SMART goals (Specific, Measureable, Attainable, Relevant, Timeline) for bite-sized analysis of your data. For instance, if your goal is to increase your senior dog visits, a smart goal might look like this:

- Specific: Increase existing senior canine patients (>8 years of age) visits by 15%
- Measureable: Can track/run a report on a "geriatric canine exam" service code in PMS
- Attainable: Currently not marketing to senior dog owners
- Relevant: Data from Dr. Mary Gardner (Lap of Love) shows that 34% of dogs over 10 years of age are not seen in the 18 months prior to euthanasia. Revenue per patient of those that were seen in the 12 months prior to euthanasia is $660.
- Timeline: 3 months

Equipment

Forget the fancy DSLR, the overhead lighting, the clunky full size tripod. If it's cumbersome, takes too long to setup, or is nerve-wracking for your patients (or clients), your content will suffer. The smartphone in your pocket will be more than adequate. The one "must" have for video content is a tripod.

Hack: If you have time for setup and a medium that requires a little "extra," e.g. a live video event, you can still get great photo and video within minimal equipment. Here are my favorites:

1. Tripod recommendations: Joby GripTight ONE GP Magnetic Impulse, $60
   a. This tripod folds up smaller than most smartphones, has magnetic feet (perfect for exam room sinks and wet tables), and comes with a tiny Bluetooth remote that lets you take pictures and start/stop video from across the room without that awkward finger push.
   b. https://joby.com/us-en/griptight-one-gp-magnetic-impulse-jb01494-bww/
2. Microphone recommendations: Rode VideoMicTM Me-L, $80
   a. This tiny microphone reduces the surrounding noise and focuses on what you're filming, plus fits in Apple device lightning ports without extra jacks.
   b. https://www.rode.com/microphones/videomicmel
3. Ring Light: $13
   a. Brighten up your face and focus area with a little light. This clips onto your cell phone and gives you multiple shades of brightness.
   b. https://www.amazon.com/QIAYA-Selfie-Photography-Lighting-Rechargeable/dp/B01HXTHPXU/ref=sr_1_1?qid=1558323237&refinements=p_89%3AQIAYA&s=photo&sr=1-1

Stories & Storage

Stories > words. You can accomplish much more, and your viewers will retain more of your information, if told in a story. Sometimes this means saving photos and videos of your patients, their x-rays, their foreign bodies after removal and the happy recheck visit - over a period of days, weeks and months....and then piecing it together with your personal touches and helpful information. All these photos and videos can add up, but are worth saving to create a great end-product.

Hack: Invest in automatic backup for your mobile device with tools like Google Photos and/or splurge on extra storage (Google Drive, Dropbox or iCloud) and add ongoing case photos to an individual "patient project" in Asana or folder on your phone/email to make finding them easier when the case is complete.

Don't Hide Behind the Camera

Veterinarians have the same degree, the same access to information, the same access to medicines and equipment, the same social media templated resources, regardless of where they work. The only thing that differentiates each practice is the unique personality (individual or collective). Clients WANT to know more about the people who are caring for their pets. They WANT to see behind the scenes. They WANT to stop their scroll and read about that vet they recognize in the photo. While there is admittedly some value to the cute puppy photo and the helpful informative article, getting the same information from a video with THEIR doctor or experiencing the same emotion seeing the puppy AND the favorite technician is much more impactful. Forget about the perfect hair, the lack of makeup, the coffee stain on your scrubs. If you're presentable enough to be in the exam room, you can be in front of the camera too. Relax - you know your stuff. Just tell it to the camera like you tell it to your clients every day.

Hack: if you're stumbling over words, try a cue card behind the camera with your key points. If you absolutely have to, grab a nearby tablet and run a teleprompter app like Teleprompter Lite.

https://itunes.apple.com/us/app/teleprompter-lite/id941620509?mt=8

Get Creative

Get out of your left brain and put that right hemisphere to work. Trying some new formats, creative tools to make your content stand out, and apps to help capture and tell your story makes social media more fun and effective.

Hack: Organize your apps into folders on your phone to make them easily accessible. Try folders like "social," "video," and "photo" to help keep your tools organized. Save all your clinic logos in a "logo" album on your phone so you can easily pull them into apps to brand your photos and videos.

My essential list:

Website	What It Does	Where To Get It
Canva	Graphic design (think Photoshop for dummies) and ready-made templates	www.canva.com

PicMonkey	Quick editing of photos, collages	www.picmonkey.com
Kapwing	Online video creation/editing platform. Trim, edit, subtitle, loop, filter and more.	www.kapwing.com

App	What It Does	Find It In the App Store
InShot	Video Editor: add music, emojis, text, voice-overs	
Bark Cam	Barking, squeaking toys, and whistles to get your patients to look *right* at your camera	
Boomerang	Takes a burst of photos and stitches them together into a high-quality mini video that plays forward and backward.	
Hyperlapse	Time-lapse video (perfect to show demos of long-form exams, procedures, etc)	
PicsArt	Quick photo editing and filters. Easily put your logo on photos.	
Repost	Reshare Instagram photos from other users on your own channels, giving proper credit	
Facetune 2	Photo editor that easily blurs out backgrounds/ unwanted items	
iMovie (for iPhone/Mac users)	Video editing desktop and mobile app	

Change is Inevitable

What works today won't work tomorrow. Keeping an eye on your stats, insights and tracking measures will help you spot trends before and as they emerge, so you can shift strategy quickly. Instagram has exploded in growth for businesses over the last two years, while the once common Vine video platform fell like a one-hit-wonder. Be open to new ideas and platforms, particularly if you see that your clients are engaging with them. Early adoption is key to staying ahead of the game, but "don't give up on your day job" with proven social media/marketing avenues. Continue with the tried-and-true platforms and try one new trend at a time, if your time allows.

Hack: Stay aware of new trends and ideas, in the veterinary industry and on the outside. Check out the Social Media Examiner for a daily, easy to read email of the latest social media scoop. Also check out veterinary-specific Facebook groups for fellow marketing geeks!

Repurpose Your Content

Work smarter, not harder. Data shows that the "new school pet owners" (AKA the largest group of pet-owning citizens) prefer to receive information in a variety of touchpoints. A recent study showed that 92% of client education is still done in person, despite the fact that we are only face-to-face with a pet owner less than 0.01% of their waking hours.1 Broaden your impact with content in multiple formats, from photos, videos, blogs and social media updates. More touchpoints doesn't have to mean more work, however. Whenever possible, consider how you could "double up" when creating content. Turn your client education video in for transcribing and use the text as a blog post. Have a team member take photos while recording a how-to-video, so you can promote the Facebook video with a static photo on Instagram, and recycle it onto Facebook again the following week. No time for blog writing? Dictate your content on your drive home into a talk-to-text app or website (even in Google Docs!). Convert your client education handouts into a blog post, and share those regularly on your website, social media, and e-newsletters. Remember that much of our standard medical content (e.g. how to crate train a puppy, or common cat vaccines) doesn't change - the content can be utilized time and time again. Make your content work for you!

Hack: Use a social media scheduling tool like Buffer or Meet Edgar to easily reshare content over and over again. Given that each post only reaches a small percentage of your audience, your content will likely hit a different segment the next time around.

Figure 7. Pet owner touch points for information gathering

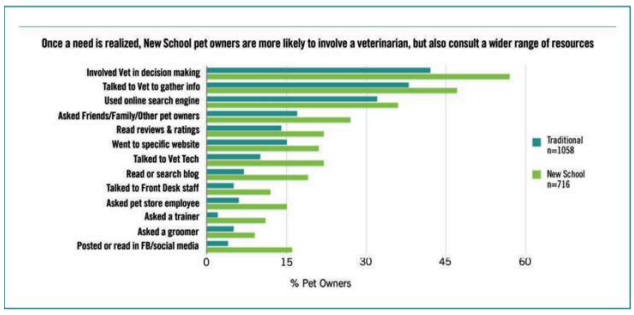


Figure 7: Merck Pet Owner Paths Study.

Make Technology Work For You

Being a vet is hard. Being a vet who also helps with social media and marketing is hard. You can do it, but you have to remember 1) your purpose and 2) that you can't do it all. Focus on what you enjoy, what helps you meet your objectives, and find solutions to help with the rest. There are apps, websites, and general support out there to help you get the job done. Don't be ashamed to ask for help or to outsource work that doesn't bring you joy (or simply can't get done).

***Hack:** Turn to these tools for help:*

Tool	What It Does	Where To Get It
Google Alerts	Sends you an email based on your frequency preferences when any of your search terms appear on the internet. Hack: set these up for your name, your staff names, your practice names, and relevant material that would make for good social content (e.g. "Dogs and YOUR CITY" or "Cats and OUR NEIGHBORHOOD").	www.google.com/alerts
Perch	Combines notifications and ratings for Yelp, Facebook, Google, all in one place. Use the app for monitoring on the go or get a daily/weekly email digest. Free.	www.perchapp.com (app available)
Rev	Captions videos, transcribes audio (like you just recording what you say to clients in the exam room so you can turn it into a blog!) for $1/minute with less than 24 hour turnaround.	www.rev.com
Upwork	Find freelancers from all over the country who specialize in that task you've been dreading. Get your podcast or video files edited, find a graphic designer, or get a ghostwriter to clean up your blogs.	www.upwork.com (app available)
Cyfe	All-in-one online business dashboard. Social media, marketing, ad spend, web analytics, and more can be monitored in one screen. Set up scheduled reports to get a snapshot of where you're going.	www.cyfe.com
Asana	Organize all your ideas, projects and tasks, and assign them with due dates to others on your teams	www.asana.com (app available)
Buffer or Hootsuite	Schedule all your content to multiple places at once, for months in advance. Make short work of a month's worth of posts to Facebook, Instagram, Twitter, LinkedIn and more.	www.buffer.com www.hootsuite.com (app available)

References:

1. Merck Pet Owner Paths Study website. Pet Owner Paths™. Available at: https://merckpetownerpaths.com. Accessed May 10 2019.

Fecal Microbiota Transplantation (FMT) in Human Patients: Where We've Been and Where We're Going

John K. DiBaise, MD
Professor of Medicine
Division of Gastroenterology and Hepatology
Mayo Clinic
Scottsdale, AZ, USA

In recent years, fecal microbiota transplantation (aka fecal transplantation, fecal bacteriotherapy, FMT) has moved to the mainstream of medical practice as a method for treating recurrent and refractory *Clostridioides difficile* infection (CDI) and is being investigated as a treatment in many other conditions. Greater understanding of the role of microbial communities and their manipulation for therapeutic purposes has led to a revolution in thought regarding the management of common and not so common gastrointestinal and non-gastrointestinal diseases. Manipulation of the gut microbiome as a mechanism to impact animal and human well-being is a centuries old concept.[1] Ancient healers often observed the patient's feces to assess the state of health, and some even prescribed preparations of feces from healthy subjects to treat gastrointestinal illnesses. In veterinary practice, transfaunation is used to impact the health of co-habitant animals.[2] Veterinary pharmaceutical companies have long recommended the use of antibiotics as growth promoters in feed animals, knowing that alteration of the animal's gut microbiota might lead to fatter and more profitable livestock.

Although the first records of fecal transplantation dates back to 4th century China, the modern-day application of FMT is credited to Eiseman, who in 1958 used it to treat antibiotic-associated colitis.[3] Two main factors have led to the revival of FMT: a pandemic of CDI, and the development of high throughput microbial sequencing techniques. *Clostridioides difficile* is the most important healthcare-acquired pathogen in the United States. The major difficulty in treating CDI is its high recurrence rate. Despite that 90% of human patients respond to initial therapy, 15% to 35% will experience symptomatic relapse within the first few weeks following treatment discontinuation.[4] Some of these patients will go on to experience multiple relapses for which optimal management has been poorly standardized. Development of rapid, high throughput microbial sequencing techniques has enabled scientists to catalog the diversity of microbes within the human gastrointestinal tract in both health and disease, and develop a better understanding of their functions. Disruptions in the gut's microbial diversity have been hypothesized to lead to altered metabolism and immune signaling, which may be signatures of human disease. Understanding these interactions and the impact of their manipulation with diet, antimicrobials, probiotics, prebiotics and FMT is now coming of age.[5]

Despite many reports suggesting excellent outcomes of FMT for treatment of recurrent CDI, there is much heterogeneity in the actual performance of FMT, making implementation challenging for most clinicians. Furthermore, logistics of performing FMT and measurement of FMT outcomes, and safety for the illness treated, remain poorly standardized.

Current FMT indications

The U.S. Food and Drug Administration (FDA) considers FMT a biological product and drug and, therefore, maintains that the FMT process (e.g., donor eligibility, screening and stool processing) falls under its jurisdiction. Although the FDA has not "approved" FMT for recurrent CDI (or any condition), it has allowed FMT to be performed for recurrent and severe and refractory CDI under 'enforcement discretion' standards since 2013. FMT for indications other than CDI, at present, can only be done after obtaining an investigational new drug (IND) application from the FDA; thus, primarily in the context of research protocols. In Europe, no such status exists, making it easier to use FMT for other indications.

The Fecal Microbiota Transplantation Workgroup[6] has proposed standard indications for FMT (**Table 1**). The FMT Workgroup guidelines suggest that FMT is relatively contraindicated in patients with immunosuppression,

although this is poorly defined and FMT has been safely performed in solid organ transplant and inflammatory bowel disease patients receiving chronic immunosuppressive medications.[7]

Table 1. Recipient Eligibility Criteria

- Recurrent *C. difficile* disease – despite standard guideline directed antimicrobial therapy
- Two or more documented episodes of severe CDI
- Recent positive *C. difficile* assay consistent with recurrence
- Presence of diarrhea - at least three unformed stools per day when not on therapy

Donor determination and screening

In the earlier days of FMT, once a candidate was selected, they were generally asked to identify a healthy stool donor. Most patients would identify their spouse or other close family member as a potential donor, or a friend if there was no suitable family member. More recently, use of standard 'anonymous' pre-screened healthy volunteer donors who are willing to provide stool for multiple recipients has become another option with cost savings. In a recent systematic review, no differences in clinical outcomes were found based on whether the recipient received FMT from an anonymous or patient-selected donor.[8] Creation of banked frozen stool, from 'anonymous' healthy donors, that can be reconstituted on demand has also been shown to be an equivalent alternative to using a fresh sample.[9] This has greatly enhanced efficiency of completing FMT.

Regardless of the relationship, screening for potential blood or stool borne pathogens should always be performed. Donors must undergo a fairly comprehensive series of screening tests of blood and stool in order to ensure they are not infected with potentially transmissible infectious agents (**Table 2**). Some centers will also screen stool for viruses such as Norovirus and Rotavirus. Routine screening for multiply resistant pathogens such as Methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecium* (VRE), extended spectrum beta-lactamase producing (ESBL) or multiply resistant Gram-negative colonization of stool is generally not being done; however, these tests may be important considerations in some centers. Standard 'anonymous' healthy donor screening takes place every 90 days and these donors are contacted prior to the scheduled procedure to ensure there has not been any recent change in their health status.

Before any testing is completed, potential donors are initially screened in order to determine potential risk that the donor may harbor a transmissible blood-borne or enteric pathogen. Donors with a history of high-risk behaviors, incarceration, recent tattooing or body piercing, illicit drug use, or multiple sexual partners are excluded. Persons with recent international travel to areas at high risk for enteric infections or multiply drug resistant bacteria are also excluded as are donors with chronic gastrointestinal illnesses, obesity/metabolic syndrome, malignancy or allergic/autoimmune disorders. Donors should not have received any antibiotics, chemotherapy or been hospitalized within 3 months before donation.

Table 2. Diagnostic Screening of FMT Donors

Serologic Evaluation

- HIV I/II Antibody
- HTLV I/II Antibody
- RPR or Syphilis Enzyme Immunoassay
- Cytomegalovirus
- Epstein-Barr virus
- Hepatitis A IgM
- Hepatitis B Surface Antigen
- Hepatitis B Core Antibody IgG, IgM
- Hepatitis C Antibody

Stool Studies

- Bacterial Culture – Enteric Pathogens
- O & P (ova and parasites)
- Giardia antigen
- Cryptosporidium antigen
- Microsporidia smear
- *C. difficile* toxin by PCR or EIA
- *Helicobacter pylori* stool antigen

Preparation of the donor stool

In conjunction with an infection prevention and control department, guidelines should be developed regarding processing of donor stool and delivering it to the endoscopist performing the procedure. A donor stool sample that is at least 50 g is preferred as recurrence rates up to four times higher have been reported when less than 50 g of stool is used.[10] The sample is mixed with non-bacteriostatic normal saline until it reaches a suitable consistency; depending upon the amount of stool, this usually ranges from 200 to 300 mL of saline. The mixture is then strained and sieved to remove any remaining solid particles and aspirated into syringes that are set aside until needed during the FMT administration procedure.

Donor stool administration

Prepared donor stool can be administered into either the upper or lower GI tract via nasoenteral tube, upper endoscope, colonoscope, sigmoidoscope or retention enema. The preferred approach seems to be instillation of donor stool via colonoscopy because reported CDI cure rates of colonoscopic FMT are better than those obtained from upper gastrointestinal administration.[8] This approach may also be the most cost-effective.[11] A recent randomized clinical trial compared FMT administered via colonoscopy with FMT administered by oral capsules (40 capsules) in 116 patients with recurrent CDI.[12] The proportion without a recurrence of CDI after 12 weeks was similar in both groups (96.2%

in both groups), and a significantly greater proportion receiving capsules rated their experience as "not at all unpleasant" (66% vs. 44%). As of yet, the encapsulated form of FMT is not widely available for clinical use in the U.S.

Outcomes

FMT appears to be the most effective treatment for recurrent CDI. In a systematic review and meta-analysis of 273 CDI patients from 11 studies who were treated with FMT, 90% (245 of 273) of these difficult to treat patients experienced clinical resolution.[8] No serious adverse events due to FMT were reported. A more recent systematic review included 2 randomized controlled trials and 21 case-series involving 516 patients with recurrent CDI. The overall cure rate was 85%, with 90% cure when colonoscopy was used to administer the FMT, 78% cure via enema and 77% via upper GI route.[13] The first randomized controlled trial involved 43 patients with recurrent CDI and compared FMT infused through a nasoduodenal tube with standard vancomycin treatment, with or without a colonoscopy bowel purge.[14] Overall cure rate of FMT was 94% compared with 4 of 13 patients (31%) in the vancomycin-alone group, and 3 of 13 patients (23%) in the group receiving vancomycin plus colonoscopy bowel lavage. In other randomized clinical trials, FMT has also been shown to be superior to oral vancomycin administered in a pulse-taper regimen, and to fidaxomicin in the setting of recurrent CDI.[15,16] In a randomized clinical trial performed at 2 centers in the U.S., 46 patients were treated with either donor stool or autologous stool.[17] The overall cure rate was 91% in those who received healthy donor FMT compared with 63% in those who received their own stool FMT. Additional open label case series have shown safety and benefit of FMT in patients with severe and complicated CDI (including pseudomembranous colitis), in immunocompromised patients (e.g., on immunosuppressant drugs, transplant patients, cancer patients, HIV patients).[18,19] Main factors associated with FMT failure include inpatient FMT, immunosuppression, and number of prior CDI-related hospitalizations.[20] Other lesser factors include presence of pseudomembranes, hypoalbuminemia, leukocytosis and FMT administered distal to the splenic flexure.

Association of dysbiosis with a variety of other gastrointestinal and non-gastrointestinal diseases, together with the success of FMT for CDI, has led to considerable interest among patients and providers about expanded therapeutic use of FMT for a myriad of other disorders.[21] Indeed, ongoing FMT clinical trials are being conducted in inflammatory bowel diseases, irritable bowel syndrome, chronic constipation, intestinal pseudo-obstruction, non-alcoholic fatty liver disease, hepatic encephalopathy, primary sclerosing cholangitis, obesity/metabolic syndrome, drug-resistant microbes, graft versus host disease, and several others. Importantly, at this time, an FDA IND is needed to offer FMT to patients for conditions other than CDI.

Role of microbiota in FMT success

It is hypothesized that replacement of a diverse colonic microbiota that is more typical of a healthy person is key to success of FMT for treating CDI. In the FECAL trial, diversity of bacterial communities after donor feces infusion markedly improved and approximated that of the donor within 2 weeks.[14] Engraftment of donor gut microbiota in the recipient, such that key elements of the recipient's microbial taxonomy resemble the donor's, has also been shown to be durable following FMT.[22] In another report, the post-FMT microbiota was found to be predominantly composed of *Bacteroides* spp. and an uncharacterized butyrate-producing bacterium, providing further support to their importance in maintaining colonic homeostasis.[23] Given considerable variation in microbial strains evident in the recipient following FMT for CDI, it may be that as yet undefined recipient differences, such as niche competition or availability, could impact microbe composition. There are also other products of microbes (e.g., bacteriocins, flagellins) and changes to the metabolome (e.g., butyrate, bile acids, bile acid hydrolases) that may occur following FMT and contribute to its benefits. This concept is supported by findings of a recent report in which stool was filtered to remove all microbes and small particles and then this sterile filtrate was administered to 5 patients with recurrent CDI.[24] All patients were symptom free after a minimum of 6 months, supporting that bacterial components, metabolites or bacteriophages mediate many effects of FMT. Changes in specific bacteria and metabolites that correlate with clinical outcomes have also been demonstrated after FMT in patients with ulcerative colitis.[25] Perhaps findings such as these may be of value in designing microbe-based therapies. There is currently intense investigation into development of a completely synthetic 'stool' that contains key microbial elements and could be used therapeutically.[26]

Safety concerns

FMT is generally considered safe with few serious side effects.[27] While there can be adverse events related to the method used to administer FMT (e.g., colonoscopy, nasoenteral tube), the major safety concern with therapeutic use of donated human feces is potential for transmission of infectious agents (e.g., viruses, bacteria or parasites) contained in donor stool. This risk may be minimized by obtaining feces from donors who have undergone appropriate screening as described. Little is known about long-term impact of FMT on other illnesses and disorders (e.g., obesity, diabetes, inflammatory bowel disease) or on transmission of drug-resistant organisms. Although case reports have suggested an association of FMT with development of a variety of chronic diseases (e.g., obesity, lymphoma, rheumatoid arthritis), at this time, there is no convincing causal evidence of any long-term adverse event caused by FMT. A patient registry under direction of the American Gastroenterological Association is currently enrolling and plans to enroll 4,000 FMT recipients and follow them for 10 years to assess safety and effectiveness of FMT.

Conclusion

With appropriate screening, FMT is a safe and extremely effective treatment of recurrent CDI. Optimal delivery method of FMT needs to be individualized. Its

limitations continue to be lack of standardization in processing and method of administration and uncertain long-term safety. The role of FMT in diseases other than CDI is the source of intense investigation but, at present, FMT can only be performed for CDI unless an IND for the condition under study is obtained. Despite its enormous potential, identification of a microorganism or communities of microorganisms necessary for clinical response remains a challenge to development of more personalized microbiota-based therapeutics. After beneficial strains have been identified, another barrier concerns culturing and storage of these strains under good manufacturing practice, both of which are expensive and time-consuming.

References

1. Rager KD, George LW, House JK, DePeters ES. Evaluation of rumen transfaunation after surgical correction of left sided displacement of the abomasum in cows. J Am Vet Med Assoc 2004; 225: 915-920.
2. Zhang F, Luo W, Shi Y, Fan Z, Ji G. Should we standardize the 1700 year old fecal microbiota transplantation? Am J Gastroenterol 2012; 107:1755- 1756.
3. Eiseman B, Silen W, Bascom GS, Kauvar AJ. Fecal enema as an adjunct in the treatment of pseudomembranous enterocolitis. Surgery 1958;44:854-859.
4. Cohen S, Gerding D, Johnson S, Kelly C, Loo V, McDonald LC, et al. Clinical practice guidelines for Clostridium difficile infection in adults: 2010 update by the Society for Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of America (IDSA). Infect Control Hosp Epidemiol 2010; 31: 431–455.
5. Foxx-Orenstein AE, Chey WD. Manipulation of the gut microbiota as a novel treatment strategy for gastrointestinal disorders. Am J Gastroenterol 2012; 1: 541-546.
6. Bakken J, Borody T, Brandt L, et al. Treating *Clostridium difficile* infection with fecal microbiota transplantation. Clin Gastroenterol Hepatol 2011; 9: 1044–1049.
7. Ananthakrishnan A, Issa M, Binion D. *Clostridium difficile* and inflammatory bowel disease. Gastroenterol Clin North Am. 2009; 38: 711–728.
8. Kassam Z, Lee CH, Yuan Y, Hunt RH. Fecal microbiota transplantation for Clostridium difficile infection: systematic review and meta-analysis. Am J Gastroenterol 2013;108:500-508.
9. Hamilton M, Weingarden A, Sadowsky M, Khoruts A. Standardized frozen preparation for transplantation of fecal microbiota for recurrent *Clostridium difficile* infection. Am J Gastroenterol. 2012; 107: 761–767.
10. Gough E., Shaikh H., Manges A. Systematic review of intestinal microbiota transplantation (fecal bacteriotherapy) for recurrent Clostridium difficile infection. Clin Infect Dis. 2011; 53: 994–1002.
11. Konijeti GG, Sauk J, Shrime MG, Gupta M, Ananthakrishnan AN. Cost-effectiveness of competing strategies for management of recurrent Clostridium difficile infection: a decision analysis. Clin Infect Dis. 2014 Jun;58:1507-1514.
12. Kao D, Roach B, Silva M, et al. Effect of oral capsule- vs colonoscopy-delivered fecal microbiota transplantation on recurrent Clostridium difficile infection. A randomized clinical trial. JAMA 2017;318:1985-1993.
13. Drekonja D, Reich J, Gezahegn S, et al. Fecal Microbiota Transplantation for Clostridium difficile Infection: A Systematic Review. Ann Intern Med 2015;162:630-638.
14. Van Nood E, BVrieze A, Neiuwdorp M, et al. Duodenal infusion of donor feces for recurrent Clostridium difficile. N Engl J Med 2013;368:407-415.
15. Cammarota G, Masucci L, Ianiro G, et al. Randomised clinical trial: faecal microbiota transplantation by colonoscopy vs. vancomycin for the treatment of recurrent Clostridium difficile infection. Aliment Pharmacol Ther 2015;41:835-843.
16. Hvas CL, Jorgensen SMD, Jorgensen SP, et al. Fecal microbiota transplantation is superior to fidaxomicin for treatment of recurrent Clostridium difficile infection. Gastroenterology 2019;156:1324-1332.
17. Kelly CR, Khoruts A, Staley C, et al. Effect of Fecal Microbiota Transplantation on Recurrence in Multiply Recurrent Clostridium difficile Infection: A Randomized Trial. Ann Intern Med 2016;165:609-616.
18. Kelly CR, Ihunnah C, Fischer M, et al. Fecal microbiota transplant for treatment of Clostridium difficile infection in immunocompromised patients. Am J Gastroenterol. 2014;109:1065-1071.
19. Shogbesan O, Poudel DR, Victor S, et al. A Systematic Review of the Efficacy and Safety of Fecal Microbiota Transplant for Clostridium difficile Infection in Immunocompromised Patients. Can J Gastroenterol Hepatol 2018 Sep 2;2018:1394379.
20. Fischer M, Kao D, Mehta SR, et al. Predictors of Early Failure After Fecal Microbiota Transplantation for the Therapy of Clostridium Difficile Infection: A Multicenter Study. Am J Gastroenterol 2016;111:1024-1031.
21. Malikowski T, Khanna S, Pardi DS. Fecal microbiota transplantation for gastrointestinal disorders. Curr Opin Gastroenterol 2017;33:8-13.
22. Li SS, Zhu A, Benes V, et al. Durable coexistence of donor and recipient strains after fecal microbiota transplantation. Science 2016;352:586-589.
23. Kumar R, Yi N, Zhi D, et al. Identification of donor microbe species that colonize and persist long term in the recipient after fecal transplant for recurrent Clostridium difficile. npj Biofilms and Microbiomes 2017;3:12.
24. Ott SJ, Waetzig GH, Rehman A, et al. Efficacy of Sterile Fecal Filtrate Transfer for Treating Patients With Clostridium difficile Infection. Gastroenterology 2017;152:799-811.
25. Paramsothy S, Nielsen S, Kamm MA, et al. Specific bacteria and metabolites associated with response to fecal microbiota transplantation in patients with ulcerative colitis. Gastroenterology 2019;156:1440-1454.
26. Petroff EO, Gloor GB, Vanner SJ et al. Stool substitute transplant therapy for the eradication of Clostridium difficile infections: RePOOPulating the gut. Microbiome 2013;1:3-15.
27. Wang S, Xu M, Wang W, et al. Systematic review: adverse events of fecal microbiota transplantation. PLoS One 2016;11:e0161174.

Gut Dysbiosis in Dogs and Cats

Nick Cave, DACVN, PhD, MVSc, BVSc (NZ)
Associate Professor in Small Animal Medicine and Nutrition
School of Veterinary Science
Massey University, New Zealand

In this lecture we will discuss the general concept of dysbiosis as it is currently understood. We will examine some of the mechanisms by which an unhealthy intestinal microflora can effect the host, and ways that an unhealthy flora can develop, with an emphasis on the diet. However, our understanding of the complexities, and especially the factors specific to dogs and cats is nascent at best, and we will discuss the risks of oversimplification, and the enormity of the task ahead of us before testing for dysbiosis has any diagnostic utility, or we have any ability to intelligently manipulate the flora therapeutically.

Rather than identifying specific pathogens involved in disease, microbiome research is now focused on characterizing how imbalances in the distribution of typically commensal bacteria, which are typically not harmful and may be beneficial, are associated with disease. The intestinal ecosystem is diverse, but is largely composed of 4 phyla:Firmicutes, Bacteroidetes, Proteobacteria, and Fusobacteria, with a small biomass (albeit a huge number) of bacteriophages, along with Archea. Though unquestionably important both to the ecosystem and host, our understanding of the contribution of viruses and yeasts to health and disease in dogs and cats is not even embryonic. As such, our focus remains on the bacteria.

The word dysbiosis may have been coined in the late 19th century to describe any life in difficult of unpleasant conditions. However, the first use of the term to describe the intestinal microflora may have been in 1926 in the North American Veterinarian, where it was used to denote “an abnormal intestinal flora”. That is not to suggest that the concept of a healthy or unhealthy microflora had not been considered prior to that.

A student of Louis Pasteur, Elie Metchnikof is supposed to have been inspired by the supposed (and probably apocryphal) longevity and vitality of Bulgarian peasants, and conjectured that it was the result of regular ingestion of yoghurt and its associated bacteria.[1] He proposed that bacteria within the intestine could produce toxins that resulted in premature aging, and that an ideal microflora could prolong a healthy life. In Metchnikof’s view, the host immune system was engaged in an adversarial struggle with its microbial inhabitants.

The popular concepts of “good” and “bad” bacteria have plaour own understanding and have created a simplistic view of a highly complex world. Recognition of the complexity of the microbiome has not necessarily improved this simplistic view, and in some respects the good/bad bacterial dichotomy has simply been transferred to good/bad microbiome. This is an understandable human trait, whereby we attempt to simplify complex phenomena by categorising them into a small number of functionally useful categories.

MECHANISMS BY WHICH DYSBIOSIS CAN CAUSE DISEASE

Bacteria synthesise vitamins (e.g. folate, biotin, B_{12}, vitamin K), essential amino acids, deconjugate bile acids, and ferment luminal contents to produce several volatile compounds such as hydrogen, methane, ammonia, sulphur dioxide, and short chain fatty acids (SCFAs). The microflora alter intestinal epithelial gene expression having effects on cell maturation, nutrient absorption, cell to cell adhesion and barrier function, angiogenesis, and the release of neuropeptides from intestinal epithelial cells.[2,3]

The effect of microflora on feed efficiency is not consistent between studies, with some showing an increased requirement for energy and protein, and others showing a decreased requirement.[3,4] Most of these studies were conducted prior to the availability of molecular methods for defining the microbiome of an animal, and thus differences are likely to have resulted

from differences in the microbiome of conventional animals. In one of the classical early studies, mice born by caesarian were reared in isolation, but not germ-free.[5] Although their microbiome was not carefully defined, it was established that it differed from conventional mice in that they were free of gram negative lactose fermenters (such as E.coil). The "clean" mice could survive and even grow on corn, which has an inadequate content of tryptophan and lysine for mammals, whereas conventional mice harboring a "normal" flora lost weight and eventually died. Even when the diet contained protein of more adequate quality but in marginal quantity (e.g. 15% casein), growth of the "clean" mice was always much better than that of the conventional "dirty" mice. Thus the effect of the intestinal microflora on nutritional requirements is highly variable, and is dependent on the species present. The consistent improvement in feed efficiency in animals when oral antibiotics are administered is testament to this fact.

Bug on bug

Pathogens can cause disease in as many ways, including toxin production (enterotoxigenic shiga toxin producing E.coli, enteroadhesion and effacement (E.coli), induction of inflammation, and enteroinvasion (Salmonella spp.). A healthy intestinal microflora forms a physical and functional barrier against potential pathogens through several different mechanisms.

The direct modes of action resulting in the elimination of pathogens include inhibition of pathogen replication by producing antimicrobial substances like bacteriocins, competition for limiting resources in the host, antitoxin effect, inhibition of virulence, antiadhesive and antiinvasive effects, and competitive exclusion by competition for binding sites or stimulation of epithelial barrier function.

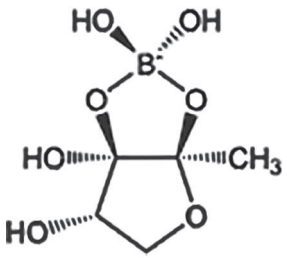
Commensals can prevent pathogen colonisation or overgrowth (colonisation resistance). This can be simply through competition for resources. Fermentation of undigested polysaccharides produces volatile fatty acids such as acetate and lactate, which reduce the luminal pH and inhibit growth of some species. Certain strains of Lactobacillus and bifidobacterium compete with other bacteria for binding sites to epithelial cells, and may even displace attached pathogens.[6] Lactate production by Lactobacilli inhibits the growth of E.coli, but the presence of the whole bacterium appears to directly inhibit E.coli adhesion to the intestinal epithelium.[7]

Recently it has been shown that the production of acetate by specific strains of Bifidobacteria, can protect mice against the lethal oral dosing of enterohaemorrhagic E.coli (EHEC).[8] The luminal production of acetate prevented the absorption of the Shiga toxin, responsible for many cases of mild and fatal diarrhoea in people worldwide. In addition, the specific strains prevent enterocyte apoptosis, inhibited E.coli translocation, and completely protected against death. This finding was highly significant because the authors demonstrated that Bifidobacterium longum 157F produced sufficient lactate to be protective, but the strain JCM 1222 did not, and did not prevent diarrhoea or death. This finding emphasises not simply the mechanism of protection, but the specificity of strain required for the effect.

It has long been observed that certain combinations of enteric flora can prevent colonisation by Salmonella spp in chickens.[9] Deliberate establishment of a specific microflora soon after hatching is effective, especially when combined with vaccination, in reducing carriage of Salmonella spp in production poultry flocks. Certain bacteria can produce antimicrobial peptides, most widely known as bacteriocins. These molecules have a range of effects from cell wall damage to interference with DNA synthesis in certain bacteria. While these peptides are produced by gram +ve and –ve bacteria, generally speaking, bacteriocins are most active against gram +ve bacteria. However, one strain of E.coli (E.coli Nissile) produces a small peptide (microcin) that limits the growth of enteroinvasive E.coli and Salmonella spp to severely limit colonisation during experimental inoculation.[10]

Beneficial commensals restrict access of pathogens to the intestinal mucosa through specific competitive mechanisms, including so-called "quorum sensing" (QS), colonization resistance, direct bactericidal activity, interaction with the host immune system, and counteracting the enteropathogen survival strategies. Through cooperation, functions that would be expensive or futile alone can become efficient and beneficial to all involved, including the production of virulence factors, different metabolites, and biofilm products. The evolutionarily conserved nature of many of the molecules and receptors allows interspecies and inter-genus communication.

Quorum sensing is the way that bacteria sense the presence of others in their vicinity, and regulate gene expression in response to fluctuations in numbers and species present. QS is possible through the production of conserved signalling molecules, their receptors, and appropriate downstream gene regulation.[11] One of the principle, or at least most studied signalling molecules is Autoinducer-2 (AI-2).[12] AI-2 is one of at least three signalling molecules formed from SAMe, where for AI-2 it is metabolised into a furan ring that, in the presence of boron, forms a furanosyl borate diester:



Several different bacterial species have different AI-2 receptors. Antibiotic-induced dysbiosis in mice promotes overgrowth of Firmicutes in excess of Bacteroidetes, and the effect is dependant upon signalling via AI-2.[13] Conversely, oral treatment of

mice with streptomycin massively reduced Firmicutes and allowed Bacteroidetes to proliferate, resulting in a reduced overall diversity. To demonstrate the importance of AI-2, a strain of Escherichia coli that overproduced AI-2 was administered, and it resulted in restoration of the Firmicutes population. Thus, a normal microflora depends on cooperation that is coordinated through specific signalling, and dysbiosis can be characterised by a defect of that signalling. The therapeutic potential of manipulating these quorum-sensing signals has yet to be explored, as has the effect of diet.

MOTILITY

The microflora can affect intestinal motility through the production of fermentative metabolites, through stimulating the production of neuroendocrine mediators by enterochromaffin cells, or by inducing mucosal epithelial or immune cells to release cytokines that alter motility. The enteric nervous system is not fully developed at birth, but continues to develop in parallel with initial colonisation of the gut. Almost nothing is known about the development in dogs or cats, and inference has to be made from human and rodent studies.

In particular, serotonin production by EC cells is influenced by the microflora. Serotonin (5-HT) binds to local 5-HT_3 receptors on the vagal sensory efferents, which then carry signals to the brain. In addition, 5-HT can stimulate the release of acetylcholine, leading to smooth muscle contraction. Oral antibiotics can induce dysbiosis by reducing the diversity and number of bacteria present, and almost inevitably shift the population away from normal. In association, colonic transit times increase significantly with a decrease in spontaneous phasic contractions.[14] The expression of key synthetic enzymes in the EC cells meant a significantly reduced production of serotonin.

Whilst some types of microflora can reduce serotonin production, certain types of dysbiosis may increase the expression of the serotonin transporter (SERT), which is allows non-5-HT3 expressing cells to uptake extracellular serotonin, thus limiting its effects. In human colonic flora, a decrease in Lactobacillus and an increase in Bacteroides (amongst other changes), leads to increased SERT expression, and reduced serotonin signalling and constipation.[15] Thus, altered colonic motility can be a direct consequence of a dysbiotic microflora, via reduced serotonin induced contraction.

In addition to the effects on motility in adulthood, a healthy microflora also affects the development of the enteric nervous system. A disturbed microflora can alter neurodevelopment leading to dysmotility later in life. When mice are administered broad spectrum oral antibiotics early in life, slowed gastric emptying, slowed small intestinal transit time, and reduced colonic propulsion.[16] When their myenteric plexuses were examined microscopically, there were changes in the neuronal morphology, and cholinergic responses were blunted, but substance-P mediated motility (via NK-1 receptors) was increased. Although this experimental model is quite removed from most normal or clinical scenarios, it demonstrates that intestinal motility may be altered throughout life from a dysbiotic microflora, especially if the dysbiosis is sustained.

METABOLISM

The interaction between the host and microbiome is even more complex than simply affecting requirements. Mice fed a high fat diet ad lib, become obese, and develop insulin resistance. In addition, the microflora changes in composition and metabolic activity. Lean germ-free mice that are inoculated with a normal gut microflora harvested from the distal intestine of obese mice results in a greater increase in total body fat than colonization with a "lean gut microflora" leads to weight gain, obesity, and insulin resistance, suggesting that the gut microflora may affect energy absorption.[17] Several mechanisms for this effect of the microflora have been proposed, including increased intestinal fat absorption, increased hepatic VLDL production, reduced lipoprotein lipase expression, increased energy from SCFAs, and altered neuropeptide production. Whatever metabolic changes ensue, it is important to recognise that the control of food intake is altered with different microflora, an effect most notable on high fat diets. When lean mice are switched from a low to a high fat diet, there is a marked change in the microbiome, and it is likely that the high fat diet, rather than obesity, is responsible for changes in the microflora.[18]

When humans ingest a high fat meal (a cup of tea and 3 slices of toast spread with a total of 50 g butter), there is a significant rise in intestinal absorption of lipopolysaccharide (LPS), and is responsible for a transient "mild systemic inflammatory response".[19] In addition, changes in the microflora on high fat diets are associated with altered mucosal immunity that increases mucosal permeability. Bifidobacteria have been reported to reduce intestinal LPS absorption, and improve mucosal barrier function.[20] The addition to a high fat diet of a highly fermentable fibre (oligofructose), resulted in restoration of Bifidobacteria numbers and a decrease in the systemic absorption of LPS.[21] The resulting decrease in systemic inflammatory response, no matter how mild, improves glucose tolerance and insulin sensitivity in those mice. Thus it has been proposed that changes in gut microbiota and integrity of the epithelium may not only be important in inducing these changes but may be the initial events that lead to dysregulation of food intake and body weight in response to high fat, high energy diets.[22]

INTESTINAL INEGRITY AND ARCHITECTURE

Bacteria affect intestinal barrier function and can contribute to limiting translocation of pathogens and the absorption of intact antigens. Enteroinvasive E.coli (EIEC) cause disease, in part, by disrupting epithelial cell cytoskeleton and tight gap-junctional proteins. In mice, the presence of certain strains of Lactobacillus prevents the disruptive effects of EIEC, maintains intestinal integrity, and inhibits pathogen invasion.[23] Other strains of Lactobacilli increase intestinal mucus

production and inhibit bacterial adhesion to epithelial cells.[6] Gastroenteritis of almost any cause is associated with the increased production of several inflammatory cytokines that increase intestinal permeability. The major cytokines that have been shown to increase enterocyte permeability are IFN-γ and TNF-α.[24,25] Exposure to IFN-γ significantly increases both the Cl- secretion and permeability to macromolecules which has been largely attributed to an alteration in paracellular function (i.e. changes in tight junctions) rather than a transcellular one. TNF-α increases permeability by inducing apoptosis which results in much larger leaks than occurs during physiological apoptosis of villus tip cells. However, certain strains of Lactobacillus and soluble factors produced by it, can prevent enterocyte apoptosis by inhibiting intracellular signalling cascades, and stimulating anti-apoptotic factors.[26]

Microflora and immunity

Immediately upon colonisation of the GI tract, there is interaction between the microflora and the host's immune system. This interaction occurs through the production of immunogenic molecules, the production of metabolites that affect cellular responses, direct interaction with intestinal epithelial cells, and direct interaction with leucocytes in the lamina propria, mesenteric lymph nodes, and more distal lymphoid sites.

Bacteria actively stimulate immune responses, principally through engagement with engagement of evolutionarily conserved microbial molecular patterns with specific receptors. The best characterised receptors are the Toll-like receptors (TLRs), of which to date 10 variants have been identified.[27] The membrane-associated TLR-4, which along with other proteins (e.g. CD14) represents the lipopolysaccharide receptor. The TLR-2 homodimer binds to lipoteichoic acid and peptidoglycans derived from Gram-positive bacteria.[28] TLR-9 binds to conserved motifs on bacterial DNA.[27]

Antibiotic treatment of mice increases their susceptibility to infection with the intracellular parasite *Encephalitozoon cuniculi*, but oral treatment with DNA derived from gut flora induces protective immune responses through stimulation of TLR-9.[29] Activation of TLR-4 increases the production of bacteriocidal lectins by enterocytes and provides protection against colonisation by vancomycin-resistant Enterococcus.

Antigen presentation by professional antigen presenting cells, and by intestinal epithelial cells is dependent upon the function of proteosomes, which are the intracellular machinery for proleolytic degradation of endogenous and exogenously derived cytosolic proteins. Foreign antigens require cleavage in a proteosome before peptides can be presented on MHC molecules. A common pathway for the induction of a proinflammatory response by leucocytes is the activation of an intracellular signalling molecule NF-κB. When NF-κB is activated, an inhibitory protein (IκB) is cleaved from it in a proteosome, allowing translocation to the nucleus and induction of the transcription of genes such as IL-1, IL-6, TNF-α, IL-8, and IL-12. Inhibition of proteosome function has shown promise as a therapy in several immune-mediated diseases including rheumatoid arthritis, asthma, and IBD.[30] Some bacteria are capable of inhibiting proteosome function and hence reduce inflammatory cytokine production and reduce antigen presentation in the intestine.[31] This effect appears to be mediated by activation of TLR-9, and thus this particular effect does not require live bacteria, but can be effected by isolated DNA fragments.

MEASURES OF DYSBIOSIS

Diversity

It has been suggested that higher microbial diversity correlates with greater stability and resilience to challenges such as dietary changes or oral antibiotics. Unfortunately, it has not yet been established either what measure of diversity is the best, nor how low on that scale is low enough to cause disease, nor how that might be affected by the specific sequencing technique used to measure it. Overall diversity is significantly influenced by the luminal substrate available to support a complex ecosystem. Feeding dogs a raw meat based diet without any fermentable carbohydrate significantly reduced the faecal diversity, however the dogs remained apparently healthy and did not develop diarrhoea.[32] In dogs with acute diarrhoea, there are commonly significant reductions in the faecal diversity, although in cross-sectional studies it is unknown if that precedes the diarrhoea and actually has a causal connection, or if it is simply the effect of the disturbance and disease.[33] In dogs with IBD, bacterial diversity tends to be lower, although the numbers studied to date are too few to reach firm conclusions.[34] Measures of diversity also tend to be lower in human patients with obesity and inflammatory bowel diseases, although similar to dogs and cats, it remains unclear whether such simplistic measures will be diagnostically useful in the future.

Dysbiosis indices

As with the simplistic score of diversity, the complexities of a microbial ecosystem is such that reducing disturbances to broad categorisations may be misleading. A healthy faecal microflora in dogs and cats is generally dominated by genera of the phyla Firmicutes and Bacteroidetes, with varying contributions from Proteobacteria and Fusobacteria.[35-39] There is significant variability among individuals, and in humans at least, intraindividual and temporal differences tend to be less dramatic than inter-individual differences. However, what could be described as the 'core microbiome', is relatively conserved among healthy individuals of both species. It is also worth noting that at present, our diagnostic approach and current understanding is based upon faecal bacterial populations. However, the populations of bacterial along the GI tract vary significantly, and disturbances in the upper GI flora may not be easily detected in faeces. Thus, although studies of the enteric microfloral are overwhelmingly derived from faecal populations, and though even future diagnostic evaluations will be based on faecal analysis, we should understand the limitations that comes with that focus.

In dogs with acute diarrhoea, increases in faecal *Clostridium* spp (mostly C.perfringens) has been observed, along with decreases in *Bacteroidetes, Faecalibacterium*, and an unclassified genus within *Ruminococcaceae*.[33] Interestingly, a very similar pattern was seen in healthy dogs fed a diversity-depleting raw food diet, which suggests that it might be possible to create a dysbiotic microbiome feeding substrate poor diets, without causing signs of gastrointestinal disease.[32] Although generalisations about chronic enteropathies should be made with caution, given the heterogenous nature of the diseases, both cats and dogs with IBD have been found to have decreased proportions of the genera Faecalibacterium and Bacteroidetes, and increased Enterobacteriaceae.[34,40]

The complexity of evaluating an ecosystem and the obvious limitations of our mental capacities, forces us to look for patterns and to be reductive in our approach, and the concept of classifying what, in human medicine have been referred to as *assemblages*, or '*enterotypes*' is appealing. However, while this is an attractive heuristic to understand the diversity of the microbiome, there is actually much greater plasticity, even within the same individual, and such a reductive approach may not be helpful.

Nonetheless, classifying enterotypes or developing dysbiosis indices is intuitive and may yet have utility. Categorising faecal bacteriome data according to the relative abundance of the major bacterial genera can be done to produce a single value, the "dysbiosis index".[41] This index may be capable of distinguishing between healthy animals and those with dysbiosis associated with a chronic enteropathy. However, in one study of 12 dogs with IBD, the dysbiosis index did not significantly change after 3 weeks of therapy, despite significant clinical improvement.[42] It is not known if that short term study refutes the possibility of that index as a diagnostic test to follow therapy, or if it will only indicate longer term shifts in the population. Alternatively, it may be that even with apparently successful management of IBD, the underlying dysbiosis is difficult to change profoundly. If so, it would be consistent with histological studies of IBD that show surprisingly little microscopic improvement, despite the abatement of clinical signs. Indeed, it may indicate that true resolution of the underlying disease predisposition from an abnormal microflora, may require more profound interventions such as faecal transplantion, before truly long term resolution can be achieved. Clearly, we have much more to learn.

References

1. Mackowiak PA. Recycling metchnikoff: probiotics, the intestinal microbiome and the quest for long life. Front Public Health 2013;1:52-52.

2. Scarpellini E, Campanale M, Leone D, et al. Gut microbiota and obesity. Intern Emerg Med 5 Suppl 1:S53-56.

3. Wostmann BS. The germfree animal in nutritional studies. Annu Rev Nutr 1981;1:257-279.

4. Furuse M, Yokota H. Protein and energy utilization in germ-free and conventional chicks given diets containing different levels of dietary protein. Br J Nutr 1984;51:255-264.

5. Dubos RJ, Schaedler RW. The effect of the intestinal flora on the growth rate of mice, and on their susceptibility to experimental infections. J Exp Med 1960;111:407-417.

6. Wallace TC, Guarner F, Madsen K, et al. Human gut microbiota and its relationship to health and disease. Nutr Rev 69:392-403.

7. Abedi D, Feizizadeh S, Akbari V, et al. In vitro anti-bacterial and anti-adherence effects of Lactobacillus delbrueckii subsp bulgaricus on Escherichia coli. Research in pharmaceutical sciences 2013;8:260-268.

8. Fukuda S, Toh H, Hase K, et al. Bifidobacteria can protect from enteropathogenic infection through production of acetate. Nature 469:543-547.

9. Methner U, Barrow PA, Martin G, et al. Comparative study of the protective effect against Salmonella colonisation in newly hatched SPF chickens using live, attenuated Salmonella vaccine strains, wild-type Salmonella strains or a competitive exclusion product. Int J Food Microbiol 1997;35:223-230.

10. Sassone-Corsi M, Nuccio SP, Liu H, et al. Microcins mediate competition among Enterobacteriaceae in the inflamed gut. Nature 2016;540:280-+.

11. Mukherjee S, Bassler BL. Bacterial quorum sensing in complex and dynamically changing environments. Nature Reviews Microbiology 2019:1.

12. Pereira CS, Thompson JA, Xavier KB. AI-2-mediated signalling in bacteria. FEMS Microbiol Rev 2013;37:156-181.

13. Thompson JA, Oliveira RA, Djukovic A, et al. Manipulation of the quorum sensing signal AI-2 affects the antibiotic-treated gut microbiota. Cell reports 2015;10:1861-1871.

14. Ge X, Ding C, Zhao W, et al. Antibiotics-induced depletion of mice microbiota induces changes in host serotonin biosynthesis and intestinal motility. Journal of translational medicine 2017;15:13.

15. Cao H, Liu X, An Y, et al. Dysbiosis contributes to chronic constipation development via regulation of serotonin transporter in the intestine. Sci Rep 2017;7:10322.

16. Caputi V, Marsilio I, Filpa V, et al. Antibiotic-induced dysbiosis of the microbiota impairs gut neuromuscular function in juvenile mice. Br J Pharmacol 2017;174:3623-3639.

17. Tilg H, Moschen AR, Kaser A. Obesity and the microbiota. Gastroenterology 2009;136:1476-1483.

18. Hildebrandt MA, Hoffmann C, Sherrill-Mix SA, et al. High-fat diet determines the composition of the murine gut microbiome independently of obesity. Gastroenterology 2009;137:1716-1724 e1711-1712.

19. Erridge C, Attina T, Spickett CM, et al. A high-fat meal induces low-grade endotoxemia: evidence of a novel mechanism of postprandial inflammation. Am J Clin Nutr 2007;86:1286-1292.

20. Conterno L, Fava F, Viola R, et al. Obesity and the gut microbiota: does up-regulating colonic fermentation protect against obesity and metabolic disease? Genes & Nutrition 6:241-260.

21. Cani PD, Neyrinck AM, Fava F, et al. Selective increases of bifidobacteria in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia. Diabetologia 2007;50:2374-2383.

22. de Lartigue G, de La Serre CB, Raybould HE. Vagal afferent neurons in high fat diet-induced obesity; intestinal microflora, gut inflammation and cholecystokinin. Physiol Behav 105:100-105.

23. Resta-Lenert S, Barrett KE. Live probiotics protect intestinal epithelial cells from the effects of infection with enteroinvasive Escherichia coli (EIEC). Gut 2003;52:988-997.

24. Gitter AH, Bendfeldt K, Schulzke JD, et al. Leaks in the epithelial barrier caused by spontaneous and TNF-alpha-induced single-cell apoptosis. The Federation of American Societies for Experimental Biology Journal 2000;14:1749-1753.

25. Adams RB, Planchon SM, Roche JK. IFN-gamma modulation of epithelial barrier function. Time course, reversibility, and site of cytokine binding. J Immunol 1993;150:2356-2363.

26. Yan F, Polk DB. Probiotic bacterium prevents cytokine-induced apoptosis in intestinal epithelial cells. J Biol Chem 2002;277:50959-50965.

27. Akira S. Mammalian Toll-like receptors. Curr Opin Immunol 2003;15:5-11.

28. Heine H, Lien E. Toll-like receptors and their function in innate and adaptive immunity. Int Arch Allergy Immunol 2003;130:180-192.

29. Jarchum I, Pamer EG. Regulation of innate and adaptive immunity by the commensal microbiota. Curr Opin Immunol 23:353-360.

30. Elliott PJ, Zollner TM, Boehncke WH. Proteasome inhibition: a new anti-inflammatory strategy. J Mol Med (Berl) 2003;81:235-245.

31. Jijon H, Backer J, Diaz H, et al. DNA from probiotic bacteria modulates murine and human epithelial and immune function. Gastroenterology 2004;126:1358-1373.

32. Cave NJ, Young W, Thomas DG, et al. Raw red meat diets decrease faecal microbial diversity in the dog. In: Waltham International Nutritional Sciences Symposium. Chicago: 2016.

33. Guard BC, Barr JW, Reddivari L, et al. Characterization of Microbial Dysbiosis and Metabolomic Changes in Dogs with Acute Diarrhea. PLoS ONE 2015;10:e0127259.

34. Suchodolski JS, Markel ME, Garcia-Mazcorro JF, et al. The Fecal Microbiome in Dogs with Acute Diarrhea and Idiopathic Inflammatory Bowel Disease. PLoS ONE 2012;7:e51907.

35. Middelbos IS, Boler BMV, Qu A, et al. Phylogenetic Characterization of Fecal Microbial Communities of Dogs Fed Diets with or without Supplemental Dietary Fiber Using 454 Pyrosequencing. Plos One 2010;5.

36. Panasevich MR, Kerr KR, Dilger RN, et al. Modulation of the faecal microbiome of healthy adult dogs by inclusion of potato fibre in the diet. Br J Nutr 2015;113:125-133.

37. Sandri M, Dal Monego S, Conte G, et al. Raw meat based diet influences faecal microbiome and end products of fermentation in healthy dogs. BMC Vet Res 2017;13:65.

38. Adler CJ, Malik R, Browne GV, et al. Diet may influence the oral microbiome composition in cats. Microbiome 2016;4.

39. Young W, Moon CD, Thomas DG, et al. Pre- and post-weaning diet alters the faecal metagenome in the cat with differences in vitamin and carbohydrate metabolism gene abundances. Sci Rep 2016;6:34668.

40. Suchodolski JS, Foster ML, Sohail MU, et al. The Fecal Microbiome in Cats with Diarrhea. Plos One 2015;10.

41. Suchodolski JS. Diagnosis and interpretation of intestinal dysbiosis in dogs and cats. Vet J 2016;215:30-37.

42. Minamoto Y, Otoni CC, Steelman SM, et al. Alteration of the fecal microbiota and serum metabolite profiles in dogs with idiopathic inflammatory bowel disease. Gut Microbes 2015;6:33-47

Prebiotic and Probiotic Therapies for Gut Dysbiosis in Humans

Kelly A. Tappenden, Ph.D., R.D., FASPEN
Professor and Head
University Distinguished Teacher-Scholar
University of Illinois at Chicago
Editor-in-Chief, *Journal of Parenteral and Enteral Nutrition*
Chicago, Illinois, USA

Introduction

The central physiological role of the intestine is being recognized. Beyond digestive and absorptive functions, the intestine holds the largest immune system and coordinates functions throughout the rest of the body. The intestine is constantly challenged by an enormous number of food-borne antigens and microbes. This is particularly fascinating when one considers that the intestinal epithelium manages the intimate contact it has with these pathogens and the resident commensal microbiota. Though reviewed in detail by other presenters in this symposium, the intestinal microbiota is a *dynamic* mixture of essential microbes that should be viewed as a living, changing ecosystem that differs along the length of the gut, within any one segment from the lumen versus the mucosa, and from person to person.

Evidence confirming the physiological benefits of the commensal microbiota is well-established. Key contributions from a robust, healthy microbiota include:

1. Production of essential mucosal nutrients, such as short-chain fatty acids;
2. Control of intestinal structure and function;
3. Prevent overgrowth of pathogenic organisms;
4. Maturation of intestinal immune system;
5. Powerful anti-inflammatory activity;
6. Development of the gut-brain axis.

When the intestinal microbiota is poorly established or compromised by factors such as environment, diet and drugs, the suboptimal or **dysbiotic** community is associated with infectious, metabolic and inflammatory disorders. Table 1 outlines a variety of diagnoses associated with dysbiosis in children and adults.

Table 1. Dysbiosis associated with human disease

Children	Adults
Celiac Disease	Acute diarrhea
Inflammatory Bowel Disease	Inflammatory bowel disease
Irritable Bowel Syndrome	Functional bowel disorders
Necrotizing enterocolitis	Liver disease
Immune impairment	Energy regulation
Obesity	Gastrointestinal malignancy
Cystic fibrosis	*Clostridium difficile* disease

The majority of studies show a simple associated between dysbiosis and human disease; however, evidence is emerging in conditions such as childhood allergies wherein the dysbiotic phenotype precedes manifestation of the disease by several years. Such information may provide predictive markers for individuals at high-risk for certain diagnoses; however, this knowledge may also provide the opportunity for corrective intervention.

Strategies for optimizing the intestinal microbiota in humans

Given the benefits of a healthy and robust commensal microbiota, it is not surprising that efforts to optimize this microbiome date back >2000 years ago when the Nomads consumed soured milks. The concept of **probiotics** – the consumption of live microorganisms in adequate amounts to confer a beneficial health effect on the host – has received much attention in clinical and lay arenas. However, lesser well known is the concept of prebiotics that can be consumed as part of a regular healthy diet. A **prebiotic** is defined as a selectively fermented ingredient that allows specific changes, both in the composition and/or activity in the microbiota that confers benefits upon host well-being and health. Evidence indicates that consumption of prebiotics and/

or probiotics can be a therapeutic strategy for correcting dysbiosis and supporting a microbiota associated with health in humans from infancy through old age.

Probiotics

Oral probiotics are living microorganisms that upon ingestion in specific numbers, exert health benefits beyond those of inherent basic nutrition. Characteristics of probiotics include that they are nonpathogenic, resistant to technological processing, storage and delivery, resistant to gastric acidity and lysis by bile, viable in the gastrointestinal environment, may adhere to the epithelium and produces antimicrobial substances. Strong evidence exists for the use of specific probiotics in the conditions outlined on Table 2.

Table 2. Strong Evidence Supporting Probiotic Use

Clinical Condition	Organism
Diarrhea	
Infectious adult – treatment	*Saccharomyces boulardii*, LGG
Infectious child - treatment	LGG, *Lactobacillus reuteri*
Prevention of antibiotic-associated diarrhea	*S. boulardii,* LGG, *L. casei, Bulgaricus, S. thermophilus*
Inflammatory bowel disease	
Pouchitis - Preventing and maintaining remission	VSL#3
Ulcerative colitis – remission maintenance	*E. coli* Nissle, VSL#3
Immune response	LGG, *L. acidophilus, L. plantarum, B. lactis, L. johnsonii,* VSL#3
Clinical Condition	Organism
Atopic eczema associated with cow's milk allergy – treatment and prevention	LGG, *B. lactis*
Hepatic encephalopathy	VSL#3

An important concept when making recommendations for probiotic use is to be specific about the genus, species and strain wherein published evidence of benefit exists. Health benefits of probiotics are specific to strain and may not occur with another strain even of the same species.

Prebiotics

Prebiotics can be found in plants consumed as part of our diet or isolated for their functional value and consumed alone or supplemented in products. Many fermentable dietary fibers are prebiotics; however, the two words are not synonymous. Not all dietary fibers are prebiotics and not all prebiotics are dietary fibers. Just as probiotic recommendations must be specific, the physiological attributes of prebiotics vary and should be selected just as strategically. Though far less studied, many of the clinical conditions the benefit from a specific probiotic have also shown to response to a carefully chosen and administered prebiotic.

Fructans are a type of prebiotic that are incorporated into many consumer and medical products. They are derived from plants such as chicory, artichoke and bananas. True to the 'form equals function' concept, fructans can be found in differing degrees of polymerization (ie. chain length) which impacts the rate of fermentation within the gut and the associated clinical utility (Table 3).

Table 3. Different types of fructans and associated benefit

Fructan	Abbreviation	Degree of polymerization	Fermentation rate	Fermentation location	Potential application
Short chain fructo-oligosaccharide	scFOS	2-5 units	Rapid	Distal ileum Proximal colon	Support commensal microbiota Short bowel syndrome Intestinal pathogens targeting distal ileum (e.g. Salmonella) Combined with other, more resistant substrates to provide fermentation along length of distal gut
Oligofructose	Oligofructose	≤10 units	Moderate	Proximal-mid colon	Support commensal microbiota Antibiotic associated/pathogenic diarrhea Mucosal immunity Intestinal health
Inulin	Inulin	>10 units	Slow	Entire length of distal gut	Support commensal microbiota Antibiotic associated/pathogenic diarrhea Mucosal immunity Intestinal health Combined with rapidly fermented substrates to extend location of fermentation

The colonization process occurs very early in life and is influenced by factors such as genetics, mode of birth (cesarean versus vaginal delivery), environmental exposure and diet (human milk versus formula). Breast-fed infants establish a stable microbiota that is rich with bifidobacteria; whereas, formula-fed infants host a more complex, shifting microbiota that is dominated by Enterobacteria and gram-negative organisms.

Oligosaccharides are one of the main components of human milk averaging >12g/L. The composition of human milk oligosaccharides is very complex with >100 structures identified. These naturally occurring prebiotics in human milk have led scientists to hypothesize that the addition of prebiotics to infant formula may result in modifications to the intestinal microbiota that infers advantages, such as resistance to intestinal pathogens and diarrheal disease.

Consumption of prebiotic containing infant formulas enhances microbiota, immunity and susceptibility to infections and allergies. Supplementation of a term infant's formula with 4-8 g/L galacto- and fructooligosaccharides has a dose-dependent stimulating effect on the growth of commensal bifidobacteria and lactobacilli in the intestine and result in softer, more breast-fed like stool. Importantly, infants consuming prebiotic containing infant formula have higher fecal concentration of secretory immunoglobulin A, indicative of enhanced mucosal immunity. However, the most convincing evidence supporting the benefits of prebiotic consumption in formula-fed infants relates to the clinical susceptibility of these children to infections and allergies.

Arslanoglu and colleagues (2007) completed a prospective, randomized, double-blind, placebo-controlled trial, wherein healthy term infants were fed either prebiotic-supplemented or placebo-supplemented infant formula during the first 6 months of life. Infants in the prebiotic group had fewer episodes of all types of infections combined. They also tended to have fewer upper respiratory tract infection episodes and fewer infections requiring antibiotic treatment. Similarly, the cumulative incidence of recurring infections was significantly lower in the prebiotic group. The cumulative incidence of any recurring infection and recurring respiratory infections was 3.9 and 2.9% in the prebiotic group and 13.5 and 9.6% in the placebo group, respectively.

Remarkably, these researchers demonstrated that protective effects of prebiotics lasted beyond the intervention during the first 6 months of life (Arslanoglu et al., 2008). Blind follow-up occurred in 88% of the participants from the initial study until 2 years of life. The protection against infections was maintained even after prebiotic-formula consumption ceased, as the previous prebiotic infants had fewer episodes of physician-diagnosed overall and upper respiratory tract infections, fever episodes, and fewer antibiotic prescriptions. During this follow-up period, previous prebiotic infants had significantly lower incidence of allergic manifestations. Cumulative incidences for allergic dermatitis, recurrent wheezing, and allergic urticaria were higher in the placebo group, (27.9, 20.6, and 10.3%, respectively) than in the intervention group (13.6, 7.6, and 1.5%). The lasting clinical benefit of prebiotic consumption during the early months of life appears to be very important for protecting against both allergic manifestations and infections. The observed dual protection lasting beyond the intervention period suggests that an immune modulating effect through establishment of a healthy commensal microbiota during the important colonization period. The beneficial effect of prebiotic consumption is not limited to the early years of life. Other examples of clinical conditions that benefit from prebiotic consumption in humans are found in Table 4.

Table 4. Clinical conditions that benefit from prebiotic use

Clinical condition	Prebiotic
Dysbiosis	Inulin, pectin, short-chain fructooligosaccharides, long-chain galactooligosaccharies, plant based diet
Metabolic syndrome	Fructooligosaccharides
Travellers diarrhea	Galactooligosaccharides
Pouchitis	Inulin
Coronary heart disease	High mixed fiber diet
Stroke	High mixed fiber diet
Diabetes	High mixed fiber diet
Obesity	High mixed fiber diet

Conclusion

Both the intestine and its commensal microbiota serve a critical role in health and disease. Efforts to support this important ecosystem by the consumption of prebiotics and probiotics are beneficial for humans across the lifespan. Clinicians should consider, and feed, this complex ecosystem in humans consuming diets wherein fermented foods and fibers are limited.

References

1. Arslanoglu S, Moro GE, Boehm G. Early supplementation of prebiotic oligosaccharides protects formula-fed infants against infections during the first 6 months of life. J Nutr. 2007;137:2420-2424.
2. Arslanoglu S, Moro GE, Schmitt J, et al. Early dietary intervention with a mixture of prebiotic oligosaccharides reduces the incidence of allergic manifestations and infections during the first two years of life. J Nutr. 2008;138:1091-1095.
3. Bartholome AL, Albin DM, Baker DH, et al. Supplementation of total parenteral nutrition with butyrate acutely increases structural aspects of intestinal adaptation after an 80% jejunoileal resection in neonatal piglets. JPEN 2004;28:210-222; discussion 222-223.
4. Bauer E, Williams BA, Smidt H, et al. Influence of the gastrointestinal microbiota on development of the immune system in young animals. Curr Issues Intest Microbiol 2006;7:35-51.

5. Boehm G, Stahl B. Oligosaccharides. In: Mattila-Sandholm T (ed): Functional Dairy products. Woodhead Publ Cambridge, 2003;pp203-243.
6. Druart C, Alligier M, Salazar N, et al. Modulation of the gut microbiota by nutrients with prebiotic and probiotic properties. Adv Nutr. 2014 Sep 15;5(5):624S-633S.
7. Falk PG, Hooper LV, Midtvedt T, Gordon JI. Creating and maintaining the gastrointestinal ecosystem: what we know and need to know from gnotobiology. Microbiol Mol Biol Rev 1998;62:1157-1170.
8. Gibson GR, Probert HM, Loo JV, et al. Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. Nutr Res Rev 2004;17:259-275.
9. Gibson GR, Hutkins R, Sanders ME, et al., Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nat Rev Gastroenterol. Hepatol, 2017:14:291-502.
10. Harmsen HJM, Wildeboer-Veloo ACM, Raangs GC, et al. Analysis of intestinal flora development in breast-fed formula-fed infants by using molecular identification and detection methods. J Pediatr Gastroenterol Nutr 2000;30:62-67.
11. Hill C, Guarner F, Reid G, et al. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol. 2014 Aug;11(8):506-14.
12. Husebye E, Hellstrom PM, Midtvedt T. Intestinal microflora stimulates myoelectric activity of rat small intestine by promoting cyclic initiation and aboral propagation of migrating myoelectric complex. Dig Dis Sci 1994;39:946-956.
13. Macpherson AJ, Harris NL. Interactions between commensal intestinal bacteria and the immune system. Nat Rev Immunol 2004;4:478-485.
14. Moro G, Minoli I, Mosca M, et al. Dosage-related bifidogenic effects of galacto- and fructooligosaccharides in formula-fed term infants. J Pediatr Gastroenterol Nutr. 2002;34:291-295.
15. Peña JA, Rogers AB, Ge Z, et al. Probiotic Lactobacillus spp. diminish Helicobacter hepaticus-induced inflammatory bowel disease in interleukin-10-deficient mice. Infect Immun. 2005;73:912-920.
16. Roberfroid MB. Prebiotics: the concept revisited. J Nutr 2007;137:830S-837S.
17. Savage DC. Gastrointestinal microflora in mammalian nutrition. Annu Rev Nutr 1986;6:155-178.
18. Scholtens PA, Alliet P, Raes M, et al. Fecal secretory immunoglobulin A is increased in healthy infants who receive a formula with short-chain galacto-oligosaccharides and long-chain fructo-oligosaccharides. J Nutr. 2008;138:1141-1147.
19. Sood A, Midha V, Makharia GK, et al. The probiotic preparation VSL#3 induced remission in patients with mild-to-moderately active ulcerative colitis. Clin Gastroenterol Hepatol 2009;7:1202-1209.
20. Strandberg K, Sedvall G, Midtvedt T, Gustafsson B. Effect of some biologically active amines on the cecum wall of germfree rats. Proc Soc Exp Biol Med 1966;121:699-702.
21. Szajewska H, Ruszczynski M, Radzikowski A. Probiotics in the prevention of antibiotic-associated diarrhea in children: a meta-analysis of randomized controlled trials. J Pediatr 2006;149:367-372.
22. Tappenden KA, Deutsch AS. The physiological relevance of the intestinal microbiota – contributions to human health. J Am Coll Nutr 2007;26(6):679S-683S.
23. Woloszynek S, Pastor S, Mell JC, et al. Engineering Human Microbiota: Influencing Cellular and Community Dynamics for Therapeutic Applications. Int Rev Cell Mol Biol. 2016;324:67-124.

Therapeutic Manipulation of the Gut Microbiome in Veterinary Patients

Stanley L. Marks, BVSc, PhD, DACVIM (Internal Medicine, Oncology), DACVN
University of California, Davis, School of Veterinary Medicine
Davis, CA, USA

The mammalian intestinal tract contains a complex, dynamic, and diverse population of non-pathogenic bacteria. Researchers have estimated that the average human contains 100 trillion microbes in the gut, which is 10 times more than the cells of the human body.[1] The intestinal microbiota influences the health of the host by providing nutritional substrates, modulating the immune system, and providing a support in the defence against intestinal pathogens.[2] The term *microbiome* refers to the total number of microorganisms and their genetic material and is contrasted from the term *microbiota*, which is the microbial population present in different ecosystems in the body.

There has been a plethora of research focusing on the mechanisms by which pathogenic bacteria influence intestinal function and induce disease; however, recent attention has focused on the indigenous non-pathogenic microbiota and the ways in which it may benefit the host. Initial colonization of the sterile newborn intestine occurs with maternal vaginal and fecal bacterial flora. The first colonizers have a high reduction potential and include species such as enterobacter, streptococcus, and staphylococcus. These bacteria metabolize oxygen, favouring the growth of anaerobic bacteria, including lactobacilli and bifidobacteria. Colonization with these bacteria is significantly delayed in caesarean deliveries,[3] leading to delayed activation of the efferent limb of the mucosal immune response.[4] Additional beneficial effects of developing a normal bacterial flora is seen in germ free mice that have small intestines that weigh less than their healthy counterparts. This effect occurs partly due to underdevelopment of lymphoid constituents, with a lack of plasma cells in the lamina propria and Peyer's patches, and subsequent reduction in IgA production. Exposure to bacteria results in a reversal of this phenomenon within 28 days of exposure.[5]

The intestinal microbiota has been associated with Crohn's disease and ulcerative colitis, as well as irritable bowel syndrome in humans.[6-8] In addition, the intestinal microbiota has also been implicated in the pathogenesis of various canine GI disorders, either associated with the presence of specific enteropathogens such as Salmonella, *Clostridium perfringens*, and viruses in acute episodes of diarrhea or a non-specific dysbiosis precipitating inflammatory bowel disease.[9,10] Molecular –phylogenetic studies have revealed a bacterial and/or fungal dysbiosis in the duodenum of dogs with IBD. A decrease in the proportion of Clostridiales and an increase in Proteobacteria is most commonly observed.[10] Only a few studies have described the fecal microbiota of dogs with acute and chronic GI disorders. Dogs with acute diarrhea, particularly those with acute hemorrhagic diarrhea (AHD) have the most profound alterations in their microbiome characterized by decreases in Blautia, Ruminococcaceae including *Faecalibacterium* and *Turicibacter* spp, and significant increases in genus Sutterella and *Clostridium perfringens* compared to healthy dogs. Dogs with clinically active IBD had decreased *Faecalibacterium* spp. and Fusobacteria that increased during resolution of the IBD.[10] The bacterial species that are commonly decreased during diarrhea are thought to be important short-chain fatty acid producers and could promote intestinal health. A deeper understanding of the gut microbiome will provide reference values for healthy populations and assist in diagnosing and treating diseased animals. In addition, manipulation of the intestinal microbiome via dietary intervention, administration of probiotics, prebiotics, or synbiotics, and fecal transplantation is currently being performed to maintain gut health in people and companion animals.

Manipulation of canine GI microbiota to improve health via dietary intervention did not begin until the early 1990's. Dietary fiber, prebiotics and probiotics have been the major nutritional strategies studied to modulate the canine and feline GI microbiota. Unfortunately, most of the research studies published to date have evaluated the effects of dietary manipulation of the GI microbiome in clinically healthy dogs and cats, and many of these studies have used traditional plating techniques or qPCR to quantify a limited number of bacteria (e.g., *Lactobacillus, Bifidobacteria, C. perfringens,* and *E. coli*) to assess efficacy.

PROBIOTICS

Probiotics refer to live microorganisms which when administered in adequate amounts confer a health benefit on the host. The term probiotic was derived from the Greek, meaning "for life." The Food and Agricultural Organization of the United States (FAO) and the World Health Organization (WHO) have stated that there is adequate scientific evidence to indicate that there is potential for probiotic foods to provide health benefits and that specific strains are safe for human use.[11] There has been a literal explosion of interest and research on the subject in recent years. Despite this activity, much still remains to be done to determine the specific indications and applications of probiotics in dogs and cats. There has been tremendous interest among veterinary pet food companies and manufacturers of animal health and wellness products to market probiotic formulations that are safe, pure, stable, and confer a beneficial effect in dogs and cats. These products are generally preferred to the multitude of over-the-counter probiotics marketed for veterinary use, given the concerns pertaining to quality control of the over-the-counter products.[12] A number of criteria are essential for efficacy and safety of probiotics. These include resistance to gastric acid and bile, ability to colonize the gastrointestinal tract, efficacy against pathogenic microorganisms, and modulation of the immune system.[13] Several potential mechanisms have been proposed for how probiotics reduce the severity or duration of diarrhea: competition with pathogenic bacteria or viruses for nutrients, competition for receptor sites, modification of the metabolic activity of the intestinal microflora, and the direct antagonism through the action of antimicrobial metabolites.[14,15]

Evidence for the Benefits of Probiotics in People

There is currently level 1 evidence (i.e., data from either high-quality, randomized controlled trials with statistically significant results and few design limitations or from systematic reviews of trials) for effectiveness of probiotics in treating lactose intolerance/maldigestion, treating acute infectious or nosocomial diarrhea in children, preventing or treating antibiotic-associated diarrhea, preventing and maintaining remission of pouchitis in adults, and maintaining remission of ulcerative colitis in adults.[16] In addition, there is level 2 evidence (evidence obtained from randomized trials that have limitations in methodology or results that have wide confidence intervals) for using probiotics to treat traveler's diarrhea, prevent sepsis secondary to severe acute pancreatitis, and prevent infections in postoperative patients.[16] Unfortunately, a similar level of evidence critically evaluating the benefits of specific probiotic strains in dogs and cats is currently lacking in the veterinary profession.

Evidence for the Benefits of Probiotics in Dogs

To date, only a relatively small number of studies have been published evaluating the effects of probiotics in dogs, and many of these have focused on the intestinal microbiota in apparently healthy dogs. Probiotic strains of human or canine origin (*Lactobacilli, Bifidobacter,* and *Enterococcus*) were used in healthy adult dogs or dogs with food-responsive diarrhea to assess their effects on intestinal microbial populations, their ability to reduce specific pathogens in feces, and effectiveness as immunomodulators.[17-22] In many of these studies, probiotics added to the food in healthy dogs had an equivocal effect on fecal microflora and pathogens.[19,22] However, it is important to note that most of these studies were not randomized, controlled trials, and the strains of probiotic varied from study to study, making interpretation of findings more challenging. In addition, many studies focused on fecal isolation and quantitative cultures of putative pathogenic bacteria such as *C. perfringens*, rather than on the evaluation of more meaningful end points such as phylogenetic characterization of the microbiota, mucosal immunopathology, and alterations in intestinal integrity. Only two studies addressing the role of probiotics in management of dietary sensitivity and food-responsive diarrhea have been published to date, with overall positive results.[17,18] Only one of those studies was a randomized, placebo-controlled clinical trial,[17] and the results of that study, although clinically positive (all of the dogs in the study improved when they were placed on the elimination diet) showed no specific changes in the inflammatory cytokine patterns or a specific benefit of the probiotic. The immunomodulatory effects of *Enterococcus faecium* SF68 have been studied in dogs, and the probiotic was associated with increased fecal IgA concentrations and increased vaccine-specific circulating IgG and IgA concentrations.[23] Although increased immune globulins may suggest enhanced immune response, the clinical relevance of this finding is not known.

Additional studies are warranted in dogs to further assess the immunomodulatory effects of probiotics and to evaluate their safety. The latter issue is particularly important given the recent finding of increased intestinal adhesion of *Campylobacter jejuni* in an *in vitro* model of canine intestinal mucus following incubation with *Enterococcus faecium*.[24] It should be noted that this *E. faecium* strain is different from the *E. faecium* SF68 strain available commercially; moreover, to date there has been no clinical or anecdotal evidence of *Campylobacter*-associated diarrhea in dogs associated with *E. faecium* administration. Short-term treatment (6 weeks) with *E. faecium* SF68 to 20 dogs with chronic naturally acquired subclinical giardiasis failed to affect giardial cyst shedding or fecal giardial antigen and did not alter innate or adaptive immune responses at multiple time points.[25] These results are in contrast to those shown following the oral feeding of *E. faecium* strain SF68 to mice experimentally infected with *Giardia intestinalis* trophozoites.[26] Oral feeding of *E. faecium* strain SF68 starting 7 days before inoculation with *Giardia* trophozoites significantly increased the production of specific anti-*Giardia* intestinal IgA and blood IgG. This humoral response was mirrored at the cellular level by an increased percentage of CD4+ T-cells in the Peyer's patches and in the spleens of SF68-fed mice. The improvement of specific immune responses in probiotic-fed mice was associated with a diminution in the number of active trophozoites in the small intestine as well as decreased shedding of *fecal Giardia* antigens (GSA65 protein). The latter findings underscore the importance of carefully evaluating the animal model, the timing of probiotic administration (prior to infection or following infection), and the specific end-points assessed.

Evidence for the Benefits of Probiotics in Cats

Unfortunately, there is little published information pertaining to probiotic use in cats, and only one clinical

study has reported a beneficial effect of probiotic therapy for any feline disease to date. In that study, administration of *Enterococcus faecium* SF68 to 217 cats housed in an animal shelter was associated with a significantly lower percentage of cats with diarrhea ≥ 2days (7.4%) compared with a placebo group (20.7%).[27] One study evaluating the effect of dietary supplementation with the probiotic strain of *Lactobacillus acidophilus* DSM 13241 (2×10^8 CFU/d for 4.5 weeks) administered to 15 healthy adult cats demonstrated that recovery of the probiotic from the feces of the cats was associated with a significant reduction in *Clostridium* spp. and *Enterococcus faecalis*.[28] However, the immunomodulatory effects were reported based on decreased lymphocyte and increased eosinophil populations and increased activities of peripheral blood phagocytes. The relevance of these findings is unclear, because this study was not a randomized trial and the changes reported in the populations of peripheral blood cells cannot be extrapolated into evidence of systemic health benefits. Evaluation of the effect of supplementation with *Enterococcus faecium* strain SF68 on immune function responses following administration of a multivalent vaccine was evaluated in specific pathogen-free kittens.[29] This prospective, randomized, placebo-controlled study resulted in the recovery of *E. faecium* SF68 from the feces of seven of nine cats treated with the probiotic, and a nonsignificant increase in feline herpesvirus 1–specific serum IgG levels. Concentrations of total IgG and IgA in serum were similar in the probiotic and placebo groups, and the percentage of CD4+ lymphocytes was increased significantly only in kittens at 27 weeks and not at any other time points. Probiotics also have been evaluated in juvenile captive cheetahs, a population with a relatively high incidence of bacteria-associated enteritis. Administration of a species-specific probiotic containing *Lactobacillus* Group 2 and *Enterococcus faecium* to 27 juvenile cheetahs was associated with a significantly increased body weight in the treatment group, with no increase in the control group.30 In addition, administration of the probiotic was associated with improved fecal quality in the probiotic group.

PREBIOTICS

A prebiotic is defined as a "nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth of and/or activates the metabolism of one or a limited number of health promoting bacteria in the intestinal tract."[31] The most common prebiotics studied are fructans, although other prebiotics such as mannans, lactosucrose, and lactulose are also being evaluated.

Evidence for the Benefits of Prebiotics in Dogs and Cats:

Unfortunately, there is a paucity of information evaluating the clinical benefits of prebiotics in dogs and cats. Most of the outcomes published in the literature are limited to nutrient digestibility, microbial concentrations in feces, and fecal protein catabolites that may not necessarily denote a health benefit to the patient. The effects of short chain fructooligosaccharides (scFOS) were evaluated in a group of German Shepherd dogs suspected to have IgA deficiency.[32] Although the scFOS supplemented dogs had decreased aerobic and anaerobic bacteria in intestinal biopsies, the findings of the study were clouded in light of the fact that anaerobic bacterial counts did not decrease in the intestinal fluid samples of the dogs supplemented with scFOS. The effects of various oligosaccharides have been tested in adult ileal cannulated dogs to evaluate the effects on ilial and total tract nutrient digestibilities, microbial populations, ileal pH, ammonia, blood glucose, fecal consistency, and SCFA concentrations. Oligosaccharides (oligofructose, mannanoligossacharides, and xylooligosaccharides) were each given at 0.5% of the diet in a Latin square design. The only significant finding was a decrease in *fecal Clostridium perfringens* populations in dogs fed MOS.[33] The effects of 3% inulin supplementation of elimination and hydrolyzed protein diets to healthy dogs was associated with a slight increase in fecal moisture content (not clinically relevant), decreased apparent nutrient digestibility coefficients of crude protein in dogs on the elimination diet, and no effect on fecal IgA concentrations.[34] Adult beagles fed diets containing cellulose or beet pulp plus oligofructose for 6 weeks were found to have similar fecal concentrations of total anaerobes and aerobes; however, the dogs fed oligofructose had fewer Enerobacteriaceae and clostridia and greater numbers of lactobacilli. In addition, dogs fed oligofructose had longer and heavier small intestines (35% heavier), and 37% more mucosal mass with consequent greater absorptive surface area.[35] Administration of 1% scFOS or 1% inulin to weanling puppies (12-weeks-old) during a pathogen (*Salmonella* Typhimurium) challenge was associated with a lower severity of enterocyte sloughing in puppies consuming the fructans versus the control diet. In addition, puppies fed inulin also had higher fecal acetate, total SCFA concentrations and lactobacilli, indicating that prebiotics appear to attenuate some of the adverse effects of Salmonella challenge, and may provide protection against infection in weanling puppies. Cats fed diets containing 0 or 0.75% oligofructose had significantly increased fecal concentrations of lactobacilli and decreased concentrations of *C. perfringens* and *E. coli* compared with controls.[36] A study evaluating the effects of short-chain fructooligosaccharides (0.5%) and galactooligosaccharides (0.5%) in healthy cats showed no effect on fecal protein catabolites, including ammonia, 4-methylphenol, indole, and biogenic amines, underscoring the fact that concentrations of oligosaccharides > 0.5% should be used to elicit a positive response.[37]

The first nutritional intervention study in dogs that used pyrosequencing to evaluate the effects of beet pulp fiber on fecal microbial composition was performed in 2010.[38] Dog fed a control diet were compared to dogs fed a diet containing 7.5% beet pulp in a crossover design with 14 day periods. *Eubacterium balii* and *Faecalibacterium prausnitzii*, both of which are butyrate producers, were overrepresented in the dogs fed the beet-pulp containing diet, suggesting a possible anti-inflammatory effect of the beet-pulp. In contrast, Fusobacteria was under-represented in dogs fed he beet-pulp-containing diet. The effects of a synbiotic formulation to privately owned dogs for 21 days was evaluated via fecal 454 pyrosequencing.[39] The most abundant phylum in feces of all dogs was Firmicutes, followed by Actinobacteria and Bacteroidetes, regardless of synbiotic treatment. Synbiotic administration was associated with increased abundance of family Eubacteriaceae and phylum Fusobacteria. Recent studies have attempted to characterize the fecal microbiota of diarrheic dogs, as

well as dogs with IBD. Reduced bacterial diversity as well as significantly higher proportion of Enterobacteriaceae were observed in duodenal brush borders from dogs with IBD compared to healthy controls.[9] Suchodolski et al. confirmed a bacterial dysbiosis in fecal samples of dogs with chronic diarrhea (IBD) and acute hemorrhagic diarrhea, and observed changes in the microbiome between acute and chronic disease states. The bacterial groups that were commonly decreased are important producers of short-chain-fatty acids and may play an important role in caninc intestinal health.[10]

Fecal Microbiotia Transplantation (FMT):

Fecal microbiotal transplantation or infusion of a fecal suspension from a healthy individual into the gastrointestinal tract of another person to cure a specific disease, is best known as a treatment for recurrent *Clostridium difficile* infection (RCDI) in people,[40] and experience with FMT for ulcerative colitis and Crohn's disease is somewhat limited. Re-establishment of the wide diversity of intestinal microbiotia via infusion of donor feces into the colon is the proposed mechanism in patients with RCDI and IBD. FMT has been performed in dogs with a variety of chronic enteropathies (Scott Weese, personal communication), and the author (SLM) is currently completing a clinical trial evaluating the efficacy of FMT in Macaques with chronic colitis. There are a variety of application methods to inoculate the donor feces into the patient, and most studies have relied upon colonoscopy or retention enemas over nasogatric tubes or lyophilized fecal capsules to administer donor feces.

Donor stool is most often used within 8 hours of passage; however, frozen stool samples from standardized donors have been thawed and colonoscopically administered 1-8 weeks after passage for treatment of RCDI with similar success rates to fresh stool.[41] Donor fecal samples must be carefully screened for bacterial, viral, and parasitic enteropathogens, and human donors are excluded if they have taken antibiotics within the preceding 3 months or are on immunosuppressive or chemotherapeutic agents. In addition, patients with IBD, atopy, GI malignancy, and chronic diarrhea are excluded from being donors. The amount of donor stool used has varied; however, in a recent review, relapse was four-fold greater when < 50 g of stool was used in people with RCDI.[42] Stool is most commonly suspended in nonbacteriostatic saline; however, other diluents (e.g., yoghurt and milk) have been successfully used. The donor stool is mixed with diluent to a consistency that can be injected via the biopsy channel of a colonoscope. The suspension should be filtered through gauze pads or strainer to remove large particulate matter before aspiration into the syringe. The volume of stool suspension that is deposited in the colon varies tremendously, although volumes of 300-500 mL are commonly used. A larger volume allows the clinician to deposit aliquots of 90-100 mL into multiple locations within the intestinal tract, including the jejunum, ileum, ascending colon, transverse colon, and upper descending colon.

Conclusions and Future Directions:

The potential benefits and specific indications for the administration of pro- and prebiotics to dogs and cats have yet to be fully defined, although our knowledge and understanding of the nature and diversity of the feline and canine intestinal microbiome during health and disease has expanded rapidly following the advent of high-throughput DNA-sequencing platforms. Defining a role for pro- and prebiotics as well as FMT in dogs and cats will require completion of prospective, randomized, placebo-controlled studies that rely on clinically relevant end points related to a particular physiological or pathological condition. Further studies are warranted to determine the need for probiotics to be live microorganisms following the provocative studies of Rachmilewitz et al., who documented that the beneficial effects of probiotics are mediated by their DNA, circumventing the need for live, viable bacteria.[43] Pro- and prebiotics do appear to have a potential role in the prevention and treatment of various gastrointestinal illnesses, but it is likely that benefits achieved are specific to the bacterial species used and to the underlying disease context.

References

1. Savage DC. **Annu Rev Microbiol** 1977;31:107-33
2. Hooper LV, et al. **Science** 2001;291:881-884
3. Gronlund MM, et al. **J Pediatr Gastroenterol Nutr** 1999;28:19-25
4. Insoft RM, et al. **Pediatr Clin N Am** 1996;43:551-71
5. McCraken VJ, et al. **Cell Microbiol** 2001;3:1-11
6. Seksik P, et al. **Gut** 2003;52:237-242
7. Sokol H, et al. **Inflamm bowel dis** 2006;12:106-111
8. Nobaek S, et al. **Am J Gastroenterol** 2000;95:1231-1238
9. Xenoulis PG, et al. **FEMS Microbiol Ecol** 2008;66:579-589
10. Suchodolski JS, et al. **Plos ONE** 2012;7:e51907
11. Food and Agricultural Organization of the United States and World Health Organization (Online), 2001
12. Weese JS. **Can Vet J,** 2003;44:982-3
13. Tuomola E, et al. **Am J Clin Nutr** 2001;73:393S-398S
14. Coconnier MH, et al. **FEMS Microbiol Lett** 1993;110:299-305
15. Rolfe RD, et al. **J Infect Dis** 1981;143:470-475
16. Guarner F. In Versalovic J, Wilson M, editors: **Therapeutic Microbiology: Probiotics and Related Strategies.** ASM Press, Washington DC 2008; p.255
17. Sauter SN, et al. **Anim Nutr** 2006;90:269
18. Pascher M, et al. **Arch Anim Nutr** 2008;62:107
19. Vahjen W, et al. **Arch Anim Nutr** 2003;57:229
20. Swanson KS, et al. **J Nutr** 2002;132:3721
21. Biagi G, et al. **Vet Microbiol** 124:160, 2007
22. Baillon MLA, et al. **Am J Vet Res** 65:338, 2004
23. Benyacoub J, et al. **J Nutr** 133:1158-1162, 2003
24. Rinkinen M, et al: **Vet Microbiol** 2003;92:111
25. Simpson KW, et al. **J Vet Int Med** 2009;23:476-481
26. Benyacoub J, et al. **J Nutr** 2005;135:1171-6
27. Bybee SN, et al. **J Vet Int Med** 2011;25:856-860
28. Marshall-Jones ZV, et al. **Am J Vet Res** 2006;67:1005
29. Veir JK, et al: **Vet Ther** 2007;8:229
30. Koeppel KN, et al: **J So Afr Vet Assoc** 2006;77:127
31. Gibson GR, et al. **J Nutr** 1995;125:1401-12
32. Willard MD, et al. **Am J Vet Res** 1994;55:654-659
33. Strickling JA, et al. **Anim Feed Sci Technol** 2000;86:205-219
34. Verlinden A, et al. **Br J Nutr** 2006;96:936-944
35. Buddington RK, et al. **Am J Vet Res** 1999;60:354-358
36. Sparkes AH, et al. **Am J Vet Res** 1998;59:436-440
37. Kanakupt A, et al. **J Anim Sci** 2011;89:1376-1384
38. Middlebos IS, et al. **PloS ONE** 2010;5:e9768
39. Garcia-Mazcorro JF, et al. **FEMS Microbiol Ecol** 2011;78:542-554
40. Song Y, et al. **Plos ONE** 2013;8:e81330
41. Hamilton MJ, et al. **Am J Gastroenterol** 2012;107:761-767
42. Gough E, et al. **Clin Infect Dis** 2011;53:994-1002
43. Rachmilewitz D, et al. **Gastroenterol** 2004;126:520-528.

Harnessing the Power of Nutrition to Improve Gut Health in Adult Cats and Dogs

Susan Wernimont, PhD, MS, RDN
Associate Director, Clinical Nutrition, Claims and Clinical Studies
Hill's Pet Nutrition, Inc.
Topeka, KS, USA

Fibers have a long history of use in pet foods, particularly beet pulp and cellulose[1] Other pet food fiber sources in common use include corn, fruit, rice bran, and whole grains such as barley and oats[1]. Fibers are classically defined based on characteristics such as solubility (the ability to disperse in water) and fermentability (the capability of the fiber to be fermented by bacteria to produce metabolites such as short chain fatty acids)[2]. Soluble fibers include fructo-oligosaccharides (FOS), gums, and pectin, while insoluble fibers include cellulose and lignin; other fiber sources such as beet pulp and psyllium are considered mixed (i.e., contain both soluble and insoluble fibers)[3,4]. Fermentable fibers include pectins, gums, inulin and oligofructose, while non-fermentable fibers include cellulose, lignin, and cereal fibers rich in cellulose such as wheat bran **(Table 1)**.[3] However, not all fibers grouped within these classification systems confer similar health effects; the physiochemical characteristics of the fiber including particle size, bulk volume, surface area characteristics and hydration properties underpin the mechanism of action and have been suggested to be better predictors of the health effects associated with consumption[2,5]. For example, large/coarse insoluble fibers (e.g., wheat bran) stimulate water and mucus secretion in the large bowel by mechanically irritating the gut mucosa while gel-forming soluble fibers (e.g., psyllium) hold water and resist dehydration of the intestinal contents **(Figure 1)**.[5] To confer these effects, fibers must resist fermentation in the small bowel and reach the large bowel intact[5].

Domestic cats, *Felis catus*, are true carnivores with short gastrointestinal (GI) tracts, rapid GI transit, minimal production of enzymes for carbohydrate digestion and vestigial ceca. These characteristics suggest cats have limited ability to effectively metabolize fiber; however, the microbiota of cats[6] and dogs[7,8] have been shown to be capable of fermenting a range of plant fibers. The companion animal microbiota can be defined as the collection of microbes that live inside and on cats and dogs[7,9]. The intestinal microbiota can be defined as the dynamic collection of microorganisms within the GI tract and the system of interactions these organisms have with each other and with the host cells[10]. The microbiota includes bacteria, archaea, fungi, protozoa, and viruses and together forms a community or ecosystem much like a city neighborhood. The microbiome is involved in a range of processes important to the health of the host including energy homeostasis, metabolism, gut epithelial health, immunologic activity, and neurobehavioral development[7]. Bacteria in the canine and feline microbiota are capable of fermenting fibers and certain types of fiber have been long-recognized to function as prebiotics[11] nourishing the gut microbiome and undergoing fermentation to produce postbiotic metabolites such as short chain fatty acids[12].

Recently, other properties of fibers have been recognized including the presence of various bioactive compounds. Fruits and vegetables in particular serve as rich sources of both plant fibers and polyphenols[13]. Polyphenols comprise a class of compounds including flavonoids, tannins and phenolic acids and their derivatives[14]. While the bioavailability of polyphenols was previously not well understood[13], it is now known that the gut microbiota plays an important role in metabolizing naturally occurring polyphenol conjugates and oligomers to more bioavailable forms; in turn, dietary polyphenols also help shape the composition and function of gut microbiota populations[15]. Catabolism of polyphenols by the gut microbiota may occur by one of three mechanisms: hydrolysis, cleavage, and reduction[15]. The net result of these catabolic processes is microbe-derived metabolites with bioavailability that may be even greater than the parent compound. Rich sources of plant-derived polyphenols and fermentable carbohydrates may provide benefits canine and feline GI health.

Analysis of fecal postbiotics (metabolites produced by gut bacteria) is a state-of-the-art tool that provides

a window to understand intestinal microbial function. Production of postbiotics can be influenced by dietary fiber type. For example, prebiotic fibers can be metabolized to yield short chain fatty acids (SCFA) such as butyrate and propionate which are energy sources for colonocytes and the liver[16,17]. Fibers in flaxseed can be metabolized to yield postbiotics such as enterodiol with antioxidant activity[15], while plant polyphenols can be metabolized to yield postbiotics such as hesperetin and 4-hydroxycinnamate which have anti-inflammatory activity[15,18,19].

Given the importance of the gut microbiome to canine and feline digestive health, studies on the gut microbiome are a natural extension of Hill's research to provide optimal nutrition for companion animals. In fact, Hill's has been conducting microbiome research for over 20 years, starting before the term "microbiome" was coined[20]. Recently, Hill's has invested in state-of-the-art tools and expertise and developed the ActivBiome+™ technology, a proprietary blend of fibers shown to nourish and activate gut microbiome to promote digestive health and well-being. The ActivBiome+™ technology is comprised of both soluble and insoluble fibers specifically chosen for their unique properties, including prebiotic activity, water holding, stool bulking and antioxidant characteristics **(Figure 2)**. Hill's has compiled a substantial body of evidence supporting the benefits of the ActivBiome+™ technology in both cats and dogs[8,21-26].

In one study[21,23], 39 adult dogs were fed a dry control food for 4 weeks, and then fed a dry test food for 4 weeks. (control food: 3,411 kcals/kg, 2.2 g total dietary fiber; 0.5 g soluble fiber; 1.7 g insoluble fiber per 100 kcal; test food: 3,273 kcal/kg, 5.0 g total dietary fiber, 0.8 g soluble fiber, 4.2 g insoluble fiber per 100 kcal). Foods were complete and balanced and met 2017 AAFCO nutritional guidelines. Feces were collected at the end of each feeding period, scored on a 5-point scale (1=liquid stool to 5=firm stool), homogenized and frozen at -80C. Untargeted metabolomics analysis was performed by a commercial laboratory. Fecal microbiome 16s rRNA sequencing was performed (Illumina MiSeq, processed through Mothur). Predicted microbial functions were determined (PICRUSt) and analyzed statistically (PERMANOVA). Fecal short chain fatty acids (SCFA) were analyzed using liquid-liquid extraction and gas chromatography with flame ionization detection. Linear mixed models were used to analyze fecal pH, ammonium, and metabolites. The test food significantly decreased total putrefactive fecal branched SCFAs (isobutyric, 2-methylbutyric, and isovaleric acids), increased fecal acetic acid, and decreased fecal ammonium compared with control food. The test food improved stool scores compared with control food. Compared with control food, the test food increased fecal saccharolytic products ribulose/xylulose and arabinose derived from fiber. Furthermore, the test food increased fecal antioxidant and anti-inflammatory plant compounds such as hesperidin, poncirin, limonin, sinensetin, naringenin, diosmetin, eriodictyol, and narirutin compared with control food **(Figure 3).** The acetate- and lactate-producing genera *Bacteroides* and *Faecalibacterium* were significantly increased while *Streptococus* and *Enterococcus* were significantly decreased compared with control food. Predicted microbial functions representing butyrate, phenylalanine and tyrosine metabolic pathways were significantly different from the control food. In this study, the test food shifted the GI microbiome composition and metabolism of dogs toward saccharolytic fermentation and decreased putrefactive metabolites, characteristics which may provide benefits for GI health.

The test food was subsequently evaluated in a prospective clinical study involving 31 adult dogs with predominantly large bowel diarrhea (21. 8 ± 15. 3 kg, age: 5. 4 ± 3. 3 years), recruited from private veterinary practices across the United States[22]. Dogs were required to be currently experiencing an episode of diarrhea at the time of enrollment. Physical examinations, clinical evaluations and fecal collections were performed on days 1, 2, 3, 14, 28, and 56. Untargeted metabolomics analysis was performed by a commercial laboratory and analyzed using repeated measures ANOVA. Results significant at $p<0.05$ are reported. The test food significantly decreased fecal putrefactive metabolites isobutyric, 2-methylbutyric, and isovaleric acids, and decreased fecal ammonium compared with baseline. In addition, the test food increased fecal ribulose/xylulose and arabinose (saccharolytic products derived from fiber) compared with baseline. Furthermore, the test food significantly increased fecal antioxidant and anti-inflammatory plant

Table 1. Classification of fibers based on solubility and fermentability

	Fermentable	**Non-Fermentable**
Soluble	Some resistant starches (e.g. wheat dextrin) Some pectins β-Glucans Guar gum Partially hydrolyzed guar gum Inulin Fructo-oligosaccharides (FOS) Beet pulp	Methylcellulose Psyllium seed husk
Insoluble	Some pectins Some resistant starches Beet pulp	Cellulose Lignin Wheat bran Psyllium seed husk

Adapted from [1,3,27,28]

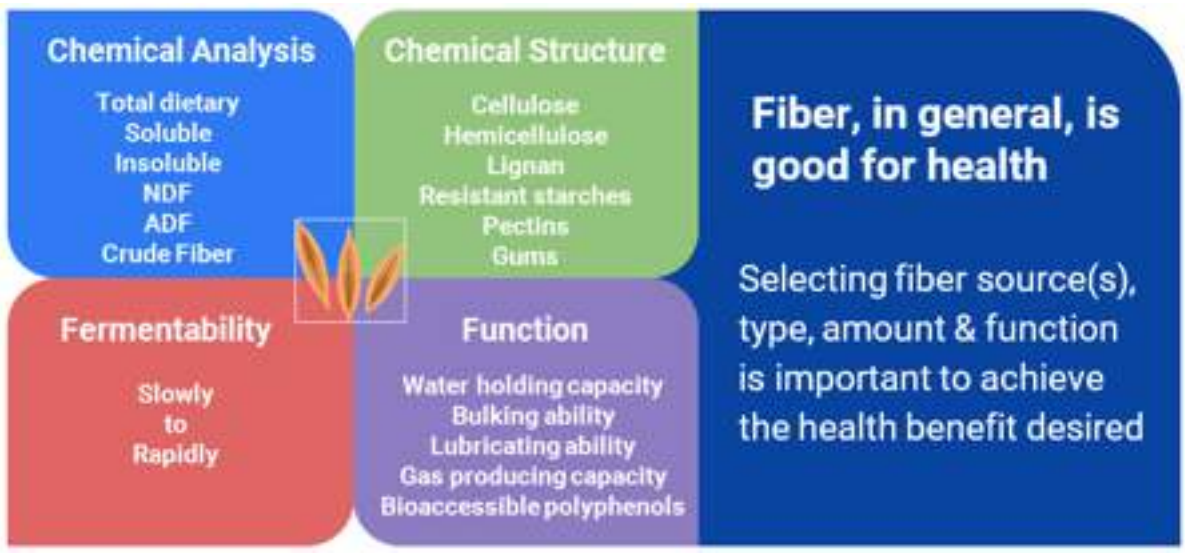
compounds such as limonin, nomilin, diosmetin, tangeritin, sinensetin, eriodictyol, secoisolariciresinol diglucoside, vanillate, hesperidin, neoponcirin, and narirutin, as well as postbiotics produced by microbial metabolism such as secoisolariciresinol, hesperetin, ponciretin, naringenin, and 4-hydroxycinnamate as compared with baseline. In this study, the test food increased metabolites associated with saccharolytic fermentation, decreased putrefactive metabolites, and increased antioxidant and anti-inflammatory plant compounds and postbiotics in dogs with chronic enterocolitis, suggesting that fiber sources rich in antioxidant and anti-inflammatory compounds may contribute to long term GI health.

An additional study in dogs evaluated the fiber technology in different food backgrounds, including hydrolyzed meat and grain rich foods[8]. In both cases, the fiber inclusion provided several GI benefits, including improved stool quality, lowered stool pH, increased beneficial gut microbes, and changed microbial metabolites to indicate improved colonic health. However, addition of the fiber to the grain-rich food increased SCFA (acetate, propionate, and butyrate) levels while addition of the fiber to hydrolyzed meat decreased branched SCFAs (bSCFA; 2-methyl propionate, 2-methyl butyrate, and 3-methyl butyrate). These results indicate that the ActivBiome+™ technology can beneficially impact canine health by modulating microbiome metabolites.

In a feline study[24,25,] 46 healthy adult cats were fed control food for 4 weeks and test food for 8 weeks (control food: 4129 kcal/kg, 1.6 g total dietary fiber; 0.7 g soluble fiber; and 0.9 g insoluble fiber per 100 kcal; test food: 4010 kcal/kg, 3.2 g total dietary fiber, 0.3 g soluble fiber, 2.9 g insoluble fiber per 100 kcal). Untargeted metabolomics analysis was performed by a commercial laboratory. Feces were collected after 4 weeks of control food and after 4 and 8 weeks of test food, scored on a 6 point scale (1=watery to 6=very hard), cleaned of litter, homogenized, and frozen at -70C. Fecal microbiome 16s rRNA sequencing was performed (Illumina MiSeq, processed through Mothur). Predicted microbial functions were determined (PICRUSt) and analyzed statistically (PERMANOVA). Fecal short chain fatty acids (SCFA) were analyzed using liquid-liquid extraction and gas chromatography with flame ionization detection. Statistical analysis was performed using mixed models. At 4 and 8 weeks, the test food significantly increased fecal acetic and propionic acids, decreased isobutyric, 2-methylbutyric, and isovaleric acids, increased moisture and decreased pH compared with control food while maintaining acceptable stool scores. The test food significantly increased fecal saccharolytic products ribulose/xylulose, maltose and arabinose as well as fecal anti-inflammatory and antioxidant plant compounds naringenin, eriodictyol, hesperidin, hesperetin, limonin, ponciretin, secoisolariciresinol and secoisolariciresinol diglucoside compared with control food at weeks 4 and 8 **(Figure 4)**. The genera *Peptococcus, Succinivibrio* and *Enterococcus* were significantly decreased compared with control food at 4 and 8 weeks while *Blautia, Bacteroides,* and *Turicibacter* were significantly increased compared with control food at 4 and 8 weeks. Predicted microbial functions representing arginine, benzoate, butyrate, phenylalanine, propionate, tryptophan and tyrosine metabolic pathways were significantly different from the control food at 4 weeks. Here too, the test food shifted the GI microbiome composition and metabolism of cats toward saccharolytic fermentation and decreased putrefactive metabolites, characteristics which may provide benefits for GI health.

In another study using the same test and control foods, 30 adult cats (healthy or with mild GI distress) consumed the control food during a 3 week prefeed. Cats were randomized to either control food or test food for a 4 week treatment period then crossed to the opposite food for 4 weeks. Feces were collected on prefeed day 18 and on treatment days 24 and 52 and scored on a 5 point scale (a score of 1 was given to a sample that was greater than 75% liquid while a score of 5 was given to a sample that was greater than 90% firm). Feces were cleaned, homogenized, and frozen at -70C. Fecal SCFA were analyzed using liquid-liquid extraction and gas chromatography with flame ionization detection. Results were compared at 4 weeks using a paired t-test approach with a significance threshold of $p<0.05$. The test food significantly increased fecal propionic acid and decreased pH compared with the control food while maintaining acceptable stool scores; acetate was

Figure 1. Fibers vary in their physical and chemical properties as well as in their health effects



also nominally higher in the test food group although this difference did not reach statistical significance. The test food increased fecal saccharolytic products including erythrose, fucose, maltose, ribulose/xylulose, glucose, arabinose, glucuronate, fructose, xylose, and mannose. Fecal anti-inflammatory and antioxidant plant compounds including eriodictyol, hesperidin, naringenin, limonin, and ponciretin from citrus, secoisolariciresinol diglucoside from flax, and ferulate, quinate, sinapate, vanillate and gentisate organic acids were increased in test food-fed cats. The test food also increased fecal hesperetin, secoisolariciresinol and dihydroferulic acid. In this study, the test food with fibers rich in polyphenols and fermentable carbohydrates increased fecal saccharolytic and fermentative metabolites, lowered fecal pH and increased fermentative bacterial taxa, indicating increased fiber metabolism. The test food increased anti-inflammatory and antioxidant plant polyphenols in the lower GI tract of cats.

In conclusion, while many pet foods contain fiber, not all fibers are the same. Hill's ActivBiome+™ technology builds on an extensive history of microbiome research and contains a specialized blend of fibers chosen for their unique properties including prebiotic, water holding, stool bulking and antioxidant characteristics. In multiple studies involving multiple background foods tested in healthy dogs and cats, the ActivBiome+™ technology impacts the intestinal microbiome in ways that benefit GI health. These studies have consistently demonstrated GI benefits that include lowered fecal pH, increased anti-inflammatory and antioxidant plant polyphenols and microbe-derived anti-inflammatory postbiotics indicating that Hill's ActivBiome+™ nourishes and activates the gut microbiomes of dogs and cats to promote digestive health and well-being. Similar results were seen in dogs with GI disease, suggesting that the specialized polyphenol-rich fiber sources found in the test food may be beneficial in optimizing health of dogs with clinical signs of chronic enterocolitis. In the future, this technology may also have positive health effects when used for chronic GI conditions commonly observed in cats such as diarrhea and constipation.

Figure 2. The ActivBiome+™ technology includes soluble and insoluble fibers specifically chosen for their unique properties

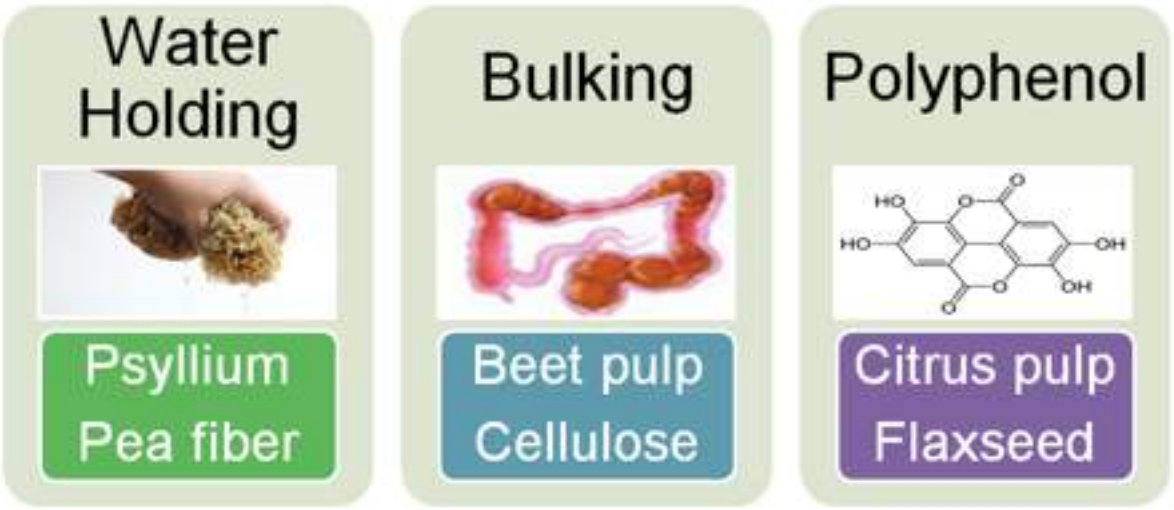
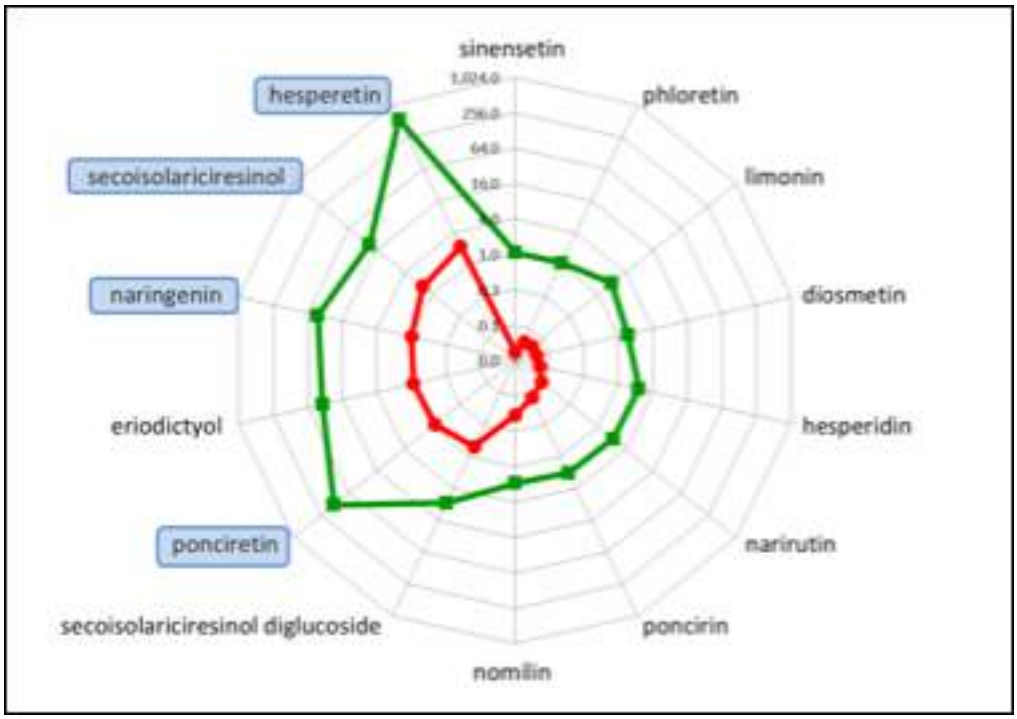
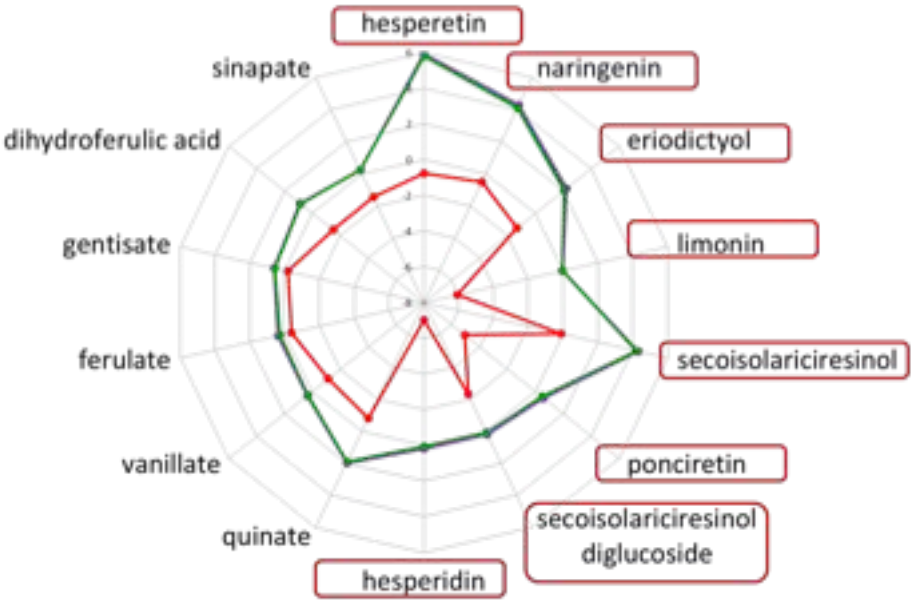


Figure 3. Antioxidant and anti-inflammatory plant compounds and beneficial microbe-derived postbiotics were significantly higher in feces from dogs fed the test food for 4 weeks compared with the control food



Plot shows log-transformed data. Red: Control food; Green: Test food

Figure 4. Antioxidant and anti-inflammatory plant compounds and beneficial microbe-derived postbiotics were significantly higher in feces from cats fed the test food for 4 and 8 weeks compared with the control food



Plot shows log-transformed data. Red: Control food; Purple: Test food 4 weeks; Green: Test food 8 weeks.

References

1. de Godoy MR, Kerr KR, Fahey GC, Jr. Alternative dietary fiber sources in companion animal nutrition. *Nutrients* 2013;5:3099-3117.
2. Dhingra D, Michael M, Rajput H, et al. Dietary fibre in foods: a review. *J Food Sci Technol* 2012;49:255-266.
3. Slavin JL, Savarino V, Paredes-Diaz A, et al. A review of the role of soluble fiber in health with specific reference to wheat dextrin. *J Int Med Res* 2009;37:1-17.
4. Linder D. Featuring fiber: understanding types of fiber & clinical uses. *TVPJournalcom* 2017:69-74.
5. McRorie JW, Jr., McKeown NM. Understanding the physics of functional fibers in the gastrointestinal tract: an evidence-based approach to resolving enduring misconceptions about insoluble and soluble fiber. *J Acad Nutr Diet* 2017;117:251-264.
6. Rochus K, Janssens GP, Hesta M. Dietary fibre and the importance of the gut microbiota in feline nutrition: a review. *Nutr Res Rev* 2014;27:295-307.
7. Barko PC, McMichael MA, Swanson KS, et al. The gastrointestinal microbiome: a review. *J Vet Intern Med* 2018;32:9-25.
8. Jackson MI, Jewell DE. Balance of saccharolysis and proteolysis underpins improvements in stool quality induced by adding a fiber bundle containing bound polyphenols to either hydrolyzed meat or grain-rich foods. *Gut Microbes* 2018:1-23.
9. Turnbaugh PJ, Ley RE, Hamady M, et al. The human microbiome project. *Nature* 2007;449:804-810.
10. Blake A, Suchodolski J. Importance of gut microbiota for the health and disease of dogs and cats. *Animal Frontiers* 2016;6:37-42.
11. Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J Nutr* 1995;125:1401-1412.
12. Verbeke KA, Boobis AR, Chiodini A, et al. Towards microbial fermentation metabolites as markers for health benefits of prebiotics. *Nutr Res Rev* 2015;28:42-66.
13. Palafox-Carlos H, Ayala-Zavala JF, Gonzalez-Aguilar GA. The role of dietary fiber in the bioaccessibility and bioavailability of fruit and vegetable antioxidants. *J Food Sci* 2011;76:R6-R15.
14. Williamson G, Clifford MN. Role of the small intestine, colon and microbiota in determining the metabolic fate of polyphenols. *Biochem Pharmacol* 2017;139:24-39.
15. Espin JC, Gonzalez-Sarrias A, Tomas-Barberan FA. The gut microbiota: A key factor in the therapeutic effects of (poly)phenols. *Biochem Pharmacol* 2017;139:82-93.
16. Carlson JL, Erickson JM, Lloyd BB, et al. Health Effects and Sources of Prebiotic Dietary Fiber. *Curr Dev Nutr* 2018;2:nzy005.
17. Rios-Covian D, Ruas-Madiedo P, Margolles A, et al. Intestinal Short Chain Fatty Acids and their Link with Diet and Human Health. *Front Microbiol* 2016;7:185.
18. Masek A, Chrzescijanska E, Latos M. Determination of antioxidant activity of caffeic acid and p-coumaric acid by using electrochemical and spectrophotometric assays. *Int J Electrochem Sci* 2016;11:10644-10658.
19. Galato D, Ckless K, Susin MF, et al. Antioxidant capacity of phenolic and related compounds: correlation among electrochemical, visible spectroscopy methods and structure–antioxidant activity. *Redox Report* 2001;6:243-250.
20. Prescott SL. History of medicine: origin of the term microbiome and why it matters. *Human Microbiome Journal* 2017;4:24-25.
21. Fritsch DA, Wernimont SM, Jackson MI, et al. Select dietary fibers alter GI microbiome composition & promote fermentative metabolism in the lower gastrointestinal tract of healthy adult dogs. *Nutrition 2019 conference.* Baltimore, MD, 2019.
22. Fritsch DA, Wernimont SM, Jackson MI, et al. Food with novel fiber blend improves clinical outcomes and changes gastrointestinal microbiome metabolism in dogs. *ACVIM Forum, 2019. Phoenix, AZ,* 2019.
23. Fritsch DA, Wernimont SM, Jackson MI, et al. Select dietary fiber sources improve stool parameters, decrease fecal putrefactive metabolites, and deliver antioxidant and anti-inflammatory plant polyphenols to the lower gastrointestinal tract of adult dogs. *The FASEB Journal* 2019;33:587.581-587.581.
24. Wernimont SM, Fritsch DA, Jackson MI, et al. Specialized dietary fibers alter microbiome composition & promote fermentative metabolism in the lower gastrointestinal tract of healthy adult cats. *Nutrition 2019 conference.* Baltimore, MD, 2019.
25. Wernimont SM, Fritsch DA, Jackson MI, et al. Specialized dietary fiber sources improved stool parameters, increased fecal saccharolytic and fermentative metabolites, & delivered antioxidant & antiinflammatory polyphenols to the lower gastrointestinal tract of healthy adult cats. *The FASEB Journal* 2019;33:587.582-587.582.
26. Wernimont SM, Paetau-Robinson I, Jackson MI, et al. Bacterial metabolism of polyphenol-rich fibers in a true carnivore, Felis catus. *The FASEB Journal* 2019;33:723.723-723.723.
27. McRorie JW, Jr. Evidence-Based Approach to Fiber Supplements and Clinically Meaningful Health Benefits, Part 1: What to Look for and How to Recommend an Effective Fiber Therapy. *Nutr Today* 2015;50:82-89.
28. McRorie JW, Jr. Evidence-Based Approach to Fiber Supplements and Clinically Meaningful Health Benefits, Part 2: What to Look for and How to Recommend an Effective Fiber Therapy. *Nutr Today* 2015;50:90-97.

A Food with a Unique Prebiotic Technology Benefits Dogs with Chronic Large Bowel Diarrhea

Dana Hutchinson, DVM, DACVN
Senior Manager of Scientific Communications,
Hill's Pet Nutrition, USA
Clinical Nutritionist,
Angell Animal Medical Center, MA, USA

In a short amount of time the scientific community has gone from simply recognizing that the gastrointestinal (GI) microbiome exists, to understanding that it plays an essential role in both health and disease of mammals. While much research investigating the role of the microbiome in health and disease has been conducted in humans, the veterinary community has begun to invest significant resources in studies aimed at using modulation of the microbiome as part of multimodal therapy for conditions such as stress-related diarrhea, constipation, and chronic enteropathies.[1-3] Hill's Pet Nutrition is one of the key contributors in this area and has conducted studies investigating benefits of a unique prebiotic technology (ActivBiome+) in both healthy dogs and cats, and those with GI disease.[4-6] ActivBiome+ technology is also a key attribute of new Hill's Prescription Diet Gastrointestinal Biome, shown to resolve diarrhea in dogs in as little as 24 hours.[7]

Using Nutrition to Modulate the Microbiome

Like humans, pets with GI disease are frequently in a state of dysbiosis (a change in an individual's microbiota in composition or function that negatively impacts the host).[8] Dysbiosis is important to host health because it contributes to altered intestinal barrier function, damage to the intestinal brush border and enterocytes, and increased competition for nutrients.[9] In the majority of GI diseases it is still unknown whether dysbiosis is the cause or result of disease, never-the-less, modulation of the microbiome through nutrition has the potential to shift the function of a pet's microbiota and GI tract towards a healthier state. This has been the driving force behind the development of Hill's® Prescription Diet® Gastrointestinal Biome with ActivBiome+™.

Key benefits of ActivBiome+™ prebiotic technology include a unique blend of prebiotics rich in fermentable fibers and fiber-bound polyphenols. Prebiotic fibers work to nourish beneficial bacteria present in the GI microbiome, enhance their metabolic function, and increase production of beneficial postbiotics (metabolic end products of bacterial metabolism). Published evidence supports that many polyphenols are fiber-bound and are released and activated to more bioactive forms by bacterial enzymes in the colon.[10] Through this process the fiber-bound polyphenols are made available to the pet and may have numerous potential health benefits both locally, in the colon, and systemically once absorbed.[10] In humans, certain polyphenols have anti-inflammatory, antioxidant, and even anti-carcinogenic properties among other benefits.[11,12] Hill's® Prescription Diet® Gastrointestinal Biome with ActivBiome+™includes an optimal balance of soluble and insoluble fiber including ActivBiome+ technology, which utilizes fiber-bound polyphenols, making it the ideal nutrition for pets with GI disease and suspected dysbiosis. These features are believed to be responsible for positive results seen in studies at Hill's Pet Nutrition Center and external studies in veterinary practices when Hill's® Prescription Diet® Gastrointestinal Biome with ActivBiome+™was fed to dogs.

Results of a Clinical Study in which Prescription Diet Gastrointestinal Biome was Fed to Dogs with Chronic Large Bowel Diarrhea

In 2018, a prospective, multicenter clinical trial was conducted in the United States in adult, client-owned dogs with clinical signs consistent with chronic large bowel diarrhea. The objective of the study was to determine if a fiber-supplemented nutritional intervention (Hill's® Prescription Diet® Gastrointestinal Biome with ActivBiome+™) could improve clinical signs of GI health.

Table 1: Enrollment Criteria

Inclusion Criteria	Exclusion Criteria
Experiencing unresolved diarrhea lasting at least 2 weeks and likely to become chronic based on veterinary assessment, and be currently experiencing diarrhea and have a history of persistent GI signs including episodes of diarrhea or loose stools for a minimum of 2 weeks	Known foreign body, intestinal parasites, musculoskeletal issues, or any concurrent systemic disease
1-10 years of age	Receiving oral antibiotics within the past 4 weeks
Body condition score 2-4/5	Fed a therapeutic diet within the past 3 months
History of successfully consuming dry food	Planned surgery, dogs that were pregnant or likely to become pregnant during the trial period
Diarrhea that includes one or more of the following concurrent signs: frequent emission of feces, liquid or loose stool consistency, straining while defecating (dyschezia), frequent attempts to evacuate bowels (tenesmus), displays of abdominal discomfort, blood in stool (hematochezia), mucus in stool, vomiting, loss of appetite.	Multi-dog households, unless pet owner could assure independent feeding of each dog throughout the study, and prevent access to other foods
Live predominantly or exclusively indoors	History of chronic use of drugs and medications that significantly influence colonic motility
	Participating in another clinical trial within the previous 6 months
	Fractious dogs
	Dogs who were unwilling or unable to consume the study food

Table 2: Timeline of Diagnostic Evaluation and Specimens Collected

	Baseline	Dogs stayed in-clinic			Outpatient		
Study Day	**0**	**1**	**2**	**3**	**14**	**28**	**56**
Medical History	√						
Physical Exam	√	√	√	√		√	√
Veterinary Clinical Evaluation	√	√	√	√		√	√
Medication use	√	√	√	√		√	√
Stooling Behavior Questionnaire	√				√	√	√
Stool Frequency and Quality (daily)	√	√	√	√	√	√	√
Collect Feces	√	√	√	√	√	√	√
Collect Blood	√	√	√	√		√	√
Collect Urine	√						

Study Design

The eligibility of each dog was assessed by medical, drug, and nutritional histories, physical examination, and laboratory analysis of blood and urine. Strict inclusion and exclusion criteria were followed (**Table 1**). Upon enrollment, all dogs were assigned to the test food, Hill's® Prescription Diet® Gastrointestinal Biome with ActivBiome+™Canine dry formula. Dogs were housed at the veterinary clinic for the first 3 days of the study while veterinarians monitored clinical signs, performed stool scoring, and collected blood and fecal specimens (**Table 2**). Stool quality was graded by the veterinary staff during the first 72 hours of the study, and by pet owners on a daily basis for the remainder of the 8-week study. Stool quality was graded on a 5-point scale (1=liquid stools, 5=firm, formed stools) using the Hill's Pet Nutrition Fecal Scoring Chart (**Figure 1**). Veterinarians evaluated changes in overall clinical signs, stool consistency, stool characteristics (presence of blood and mucus), and stool frequency compared with baseline at days 2, 3, 4, 28, and 56. These changes were classified into one of four categories: negative response, non-response, positive response, and complete resolution. In addition, veterinarians also evaluated recurrence of overall clinical signs. Pet owners evaluated nausea/vomiting, and stooling behaviors (straining, unproductive attempts, defecation accidents) on a 0 (never) – 100 (always) scale at days 1, 14, 28, and 56. Quality of life was evaluated on a 0 (very poor) – 10 (excellent) scale during the same time period.

Results

Thirty one dogs were enrolled and 22 had complete medical records and were included in the analysis. Dogs representing several breeds completed the 8-week study according to protocol. The mean age of dogs enrolled in the study was 5.4 years, and comprised 15 females (14 spayed, 1 intact) and 16 males (14 neutered, 2 intact). Veterinarians observed rapid improvements in stool scores and clinical signs during the first few days of the study. Mean stool quality increased significantly (P<0.0001) from 2.6 to 3.8 within the first 24 hours of consuming the test food. Mean stool quality continued to improve on day 2 (4.3), day 3 (4.5), and day 4 (4.7), and was ≥ 4.5 for the remainder of the study (**Figure 2**). Veterinarians reported that all dogs had either a positive (32%) or complete response (68%) by study Day 56 compared with baseline, and no dogs experienced recurrence during the study period (**Figure 3**). Veterinarians also reported a positive response or complete resolution in stool consistency (32% and 68%) and stool characteristics (41% and 59%) on day 56 versus baseline, respectively. As early as Day 14, pet owners reported a significant reduction in frequency of clinical signs (P<0.05) including nausea/vomiting, straining, unproductive attempts to defecate, and defecation accidents. Pet owners also reported a significant improvement in quality of life in dogs at Day 28 (P=0.003) and Day 56 (P=0.02) compared with Day 1.

This study demonstrated that when dogs with signs of chronic large bowel diarrhea were fed Hill's® Prescription Diet® Gastrointestinal Biome with ActivBiome+™, stool quality improved significantly (P<0.0001) within 24 hours and all dogs had improved stool consistency and characteristics during the study. Additionally, owners reported both improvements in clinical signs and quality of life during the study period. These findings suggest that Hill's® Prescription Diet® Gastrointestinal Biome with ActivBiome+™may have important clinical implications for veterinarians and owners managing dogs with GI disease as well as for dogs suffering from these conditions. A similar study is currently underway in cats with chronic diarrhea or recurrent constipation and may further broaden our knowledge of the benefits of this innovative nutritional solution.

Figure 1: Hill's Pet Nutrition Canine Fecal Scoring Chart for evaluating stool quality on a 5-point scale (1=liquid stools, 5=firm, formed stools).

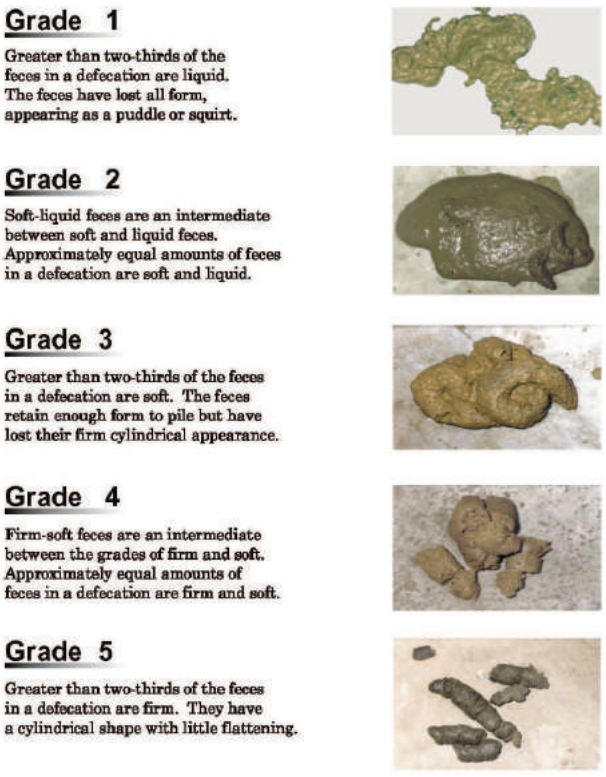


Figure 2: Mean stool quality of dogs with chronic large bowel diarrhea improved significantly within 24 hours of starting test food

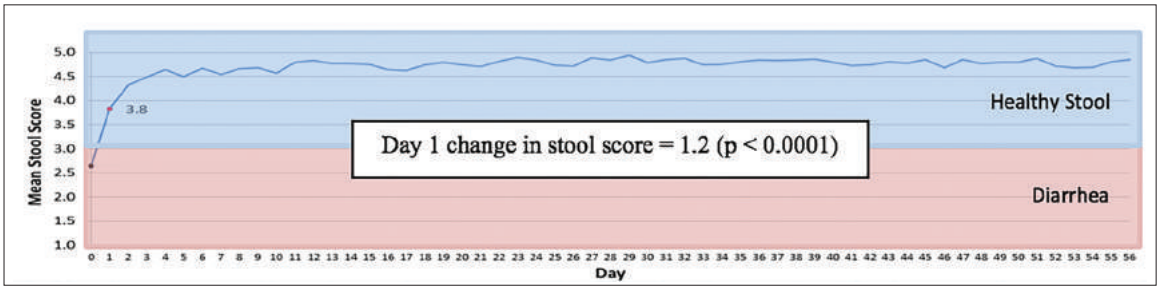
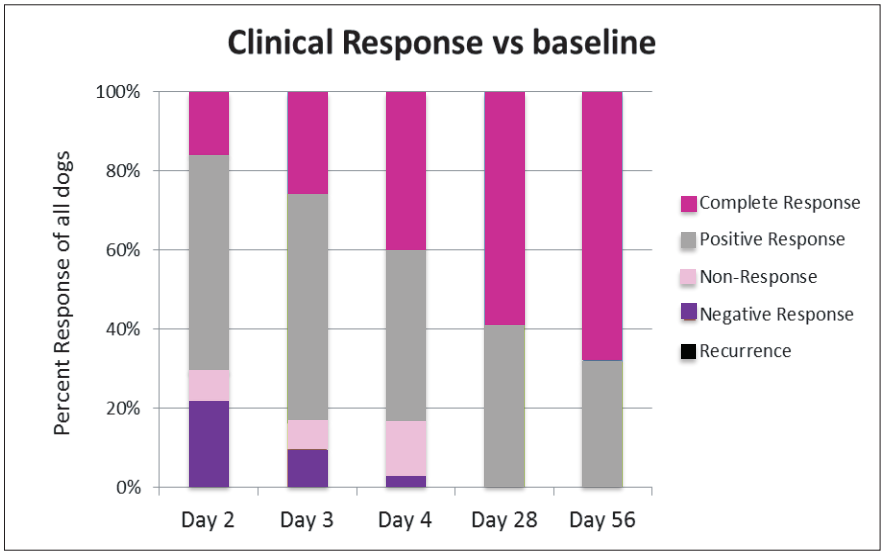


Figure 3: Compared with baseline, on Day 56 of the study veterinarians reported that all dogs had either a positive or complete response. No dogs were reported to have had recurrence of large bowel diarrhea during the 8-week study.



References:

1. Nixon SL, Rose L, Muller AT. Efficacy of an orally administered anti-diarrheal probiotic paste (Pro-Kolin Advanced) in dogs with acute diarrhea: A randomized, placebo-controlled, double-blinded clinical study. *J Vet Intern Med* 2019; Mar 18. doi: 10.1111/jvim.15481.
2. Rossi G, Jergens A, Cerquetella M, et al. Effects of a probiotic (SLAB51™) on clinical and histologic variables and microbiota of cats with chronic constipation/megacolon: a pilot study. *Beneficial Microbes* 2018; 9(1):101-110. doi: 10.3920/BM2017.0023.
3. D'Angelo S, Fracassi F, Bresciani F, et al. Effect of *Saccharomyces boulardii* in dogs with chronic enteropathies: double-blinded, placebo controlled study. *Vet Record* 2018; 182 (9): 258. DOI: 10.1136/vr.104241.
4. Fritsch DA, Wernimont SM, Jackson MI, et al. Select dietary fiber sources improve stool parameters, decrease fecal putrefactive metabolites, and deliver antioxidant and anti-inflammatory plant polyphenols to lower the gastrointestinal tract of adult dogs. *FASEB* (abstract) 2019; 33(1).
5. Wernimont SM, Fritsch DA, Jackson MI, et al. Specialized Dietary Fiber Sources Improved Stool Parameters, Increased Fecal Saccharolytic and Fermentative Metabolites, & Delivered Antioxidant & Anti-inflammatory Polyphenols to the Lower Gastrointestinal Tract of Healthy Adult Cats. *FASEB* (abstract) 2019; 33(1).
6. Jackson MI, Jewell DE. Balance of saccharolysis and proteolysis underpins improvements in stool quality induced by adding a fiber bundle containing bound polyphenols to either hydrolyzed meat or grain-rich foods. *Gut Microbes* 2018; Oct 30:1-23. doi: 10.1080/19490976.2018.1526580.
7. MacLeay MM, Fritsch DA, Wernimont SM, et al. Food with Novel Fiber Blend Improves Clinical Outcomes and Changes Gastrointestinal Microbiome Metabolism in Dogs. Proceedings.*ACVIM* Forum 2019.
8. DeGruttola AK, Low D, Mizoguchi A, et al. Current understanding of dysbiosis in disease in human and animal models. *Inflamm Bowel Dis* 2016; 22(5): 1137-1150. doi: 10.1097/MIB.0000000000000750.
9. Honneffer JB, Minamoto Y, Suchodolski JS. Microbiota alterations in acute and chronic gastrointestinal inflammation of cats and dogs. *World J Gastroenterol* 2014; 20(44): 16489-16497. doi: 10.3748/wjg.v20.i44.16489.
10. Kawabata K, Yoshioka Y, Terao J. Role of Intestinal Microbiota in the Bioavailability and Physiological Functions of Dietary Polyphenols. *Molecules* 2019; 24: 370. doi: 10.3390/molecules24020370.
11. Sahu, BD. Hesperidin attenuates cisplatin-induced acute renal injury by decreasing oxidative stress, inflammation and DNA damage. *Phytomedicine* 2013; 20(5):453-60. doi: 10.1016/j.phymed.2012.12.001.
12. Lall RK, Syed DN, Adhami VM, et al. Dietary Polyphenols in Prevention and Treatment of Prostate Cancer. *Int J Mol Sci* 2015; 16: 3350-3376.

The Gut-Kidney Axis

Jan Suchodolski, MedVet, DrVetMed, PhD, AGAF
Diplomate ACVM (Immunology)
Associate Professor, Small Animal Medicine
Associate Director for Research, Head of Microbiome Sciences
Gastrointestinal Laboratory
Texas A&M University, College of Veterinary Medicine, USA

INTRODUCTION

The intestinal microbiota is defined as all live microorganisms that live within the gastrointestinal (GI) tract. It has been estimated that a highly complex microbial load of 100 trillion cells live within the intestine. This ecosystem contains approximately 10 times more cells than the host body, and 100 times more genes than the number of host genes. The intestinal microbiome has a crucial impact on host health. The commensal bacteria provide nutritional support (e.g., vitamins, short chain fatty acids), regulate intestinal permeability, and affect local and systemic immunity. Microbiota-derived metabolites (postbiotics) are important factors affecting host health inside and outside the intestine. For example, dietary carbohydrates are fermented by bacteria, resulting in the production of short chain fatty acids (SCFA). These SCFA are utilized by the host as energy sources, they modulate intestinal motility, and are important growth factors for epithelial cells.

External factors such as antibiotic usage and major dietary changes affect the intestinal microbiome. Many ingested nutrients, as well as drugs and other xenobiotics, are metabolized by intestinal microbes and then absorbed by the host. Therefore, it is obvious that a balanced composition of the intestinal microbiota will play an important part in intestinal homeostasis. Any changes in the microbiota may directly or indirectly influence metabolic host pathways, and these can affect also organ systems other than the GI tract itself. Especially convincing evidence has been gathered associating alterations in the composition of the intestinal microbiota with acute and chronic inflammation in dogs and cats.[1,2] While the focus of the last few years has been on describing phylogenetic changes within the microbiome in various diseases, the field is now moving more into assessing functional changes due to intestinal microbial dysbiosis. Novel metabolomics approaches using multiple mass spectrometry platforms allow us to assess changes in metabolite profiles,[3] whether produced by the host or by the microbiota; taken together this information yields a better understanding of the pathophysiology of intestinal dysbiosis and associations with extra-intestinal diseases. Various bacterially derived metabolites such as indole, a byproduct of tryptophan degradation, or secondary bile acids, have been found to be immuno-modulatory, thereby maintaining immune homeostasis and strengthening intestinal barrier function. These beneficial effects of the gut microbiota reach beyond the GI tract, and can also affect other organ systems such as the brain, pancreas, liver, and kidney.

MICROBIOTA AND CHRONIC KIDNEY DISEASE

Initial studies in humans and animal models with chronic kidney disease (CKD) have reported an altered intestinal microbiota. Due to the importance of normal intestinal microbiota for maintaining immune and metabolic homeostasis, alterations in the intestinal microbiota are thought to add to intestinal as well as systemic inflammation, and abnormal clearance of uremic toxins derived from the intestine. Therefore, microbiota dysbiosis may be a contributing factor to the various systemic complications of CKD.[4]

The gut-kidney axis, a term which refers to the metabolic interactions between gut microbiota and the kidney, is thought of as a bidirectional communication between both organ systems. The metabolic changes caused by CKD (e.g., uremia) may affect the GI tract through mechanisms such as intestinal hypoperfusion, changes in luminal pH, and changes in intestinal motility. These changes may in turn cause intestinal dysbiosis, which all affect the intestinal barrier system, leading to increased intestinal permeability and potential translocation of bacterial endotoxin. This mechanism is thought to contribute to systemic low grade inflammation. In mouse models it has been shown that

dietary and therapeutic changes as commonly used in humans or animals during treatment periods of CKD, such as low fiber intake, antibiotics, and phosphate binders, can cause changes in the intestinal microbiota (i.e., dysbiosis).[4] The increased systemic levels of uremic toxins in CKD may also contribute to intestinal dysbiosis. The dysbiosis together with increased intestinal permeability potentiates endotoxemia and low-grade systemic inflammation, which in turn may affect the progression of CKD.[4]

Most data derived so far underlining these concepts have been derived in rodent models of CKD. For example, rats with experimentally induced CKD showed marked azotemia, systemic oxidative stress, and depletion of the key protein constituents of the epithelial tight junctions (claudin-1, occludin, and ZO1) in the stomach and small intestine.[5] Similar results have also been observed in cultured human enterocytes.6 Alterations in microbial populations were identified in fecal samples of 24 patients with end-stage renal disease when compared to 12 healthy persons.[7] There were differences in the abundance of 190 bacterial operational taxonomic units (OTUs) between the groups.[7] An example of a microbiota-derived metabolic change in humans is trimethylamine-N-oxide (TMAO), a gut microbial-dependent metabolite of dietary choline. TMAO is increased in CKD and is associated with a poorer prognosis. In animal models, an increase in dietary choline led to increased TMAO, which in turn led to progressive renal tubulointerstitial fibrosis.[8] A recent study described changes in the gut microbiota of cats with CKD that showed increased serum concentrations of indoxyl sulfate and p-cresol sulfate.[9]

These cats with CKD also had decreased diversity and species richness of the fecal microbiota compared to healthy cats.

These important changes in the gut-kidney axis are raising the therapeutic interest in modulating the intestinal microbiota using either dietary fibers (e.g., prebiotics) or probiotics. Initial data in humans and animal models have suggested that specific dietary fibers (such as arabic gum, oligofructose, and pectins) may reduce some of the uremic toxins (i.e., serum BUN, creatinine, p-cresyl sulphate), but more randomized clinical trials are needed to confirm such findings.[4] Less data are available on the use of probiotics to either alter intestinal microbiota and their metabolites, or to enhance intestinal barrier function. A recent pilot study using a high-dose probiotic in dogs with CKD suggested that the probiotic was useful to reduce the deterioration of glomerular filtration rate over time when compared to the placebo group.[10] Clearly, further research is warranted to study the kidney—gut axis and how novel therapeutic approaches aimed at microbiota can potentially improve patient outcomes in CKD.

References

1. Honneffer J, Guard B, Steiner JM, et al. Mo1805 Untargeted Metabolomics Reveals Disruption Within Bile Acid, Cholesterol, and Tryptophan Metabolic Pathways in Dogs With Idiopathic Inflammatory Bowel Disease. *Gastroenterology* 2015;148:S-715.
2. Guard BC, Barr JW, Reddivari L, et al. Characterization of Microbial Dysbiosis and Metabolomic Changes in Dogs with Acute Diarrhea. *PLoS ONE* 2015;10:e0127259.
3. Pavlidis P, Powell N, Vincent RP, et al. Systematic review: bile acids and intestinal inflammation-luminal aggressors or regulators of mucosal defence? *Aliment Pharmacol Ther* 2015;42:802-817.
4. Sabatino A, Regolisti G, Brusasco I, et al. Alterations of intestinal barrier and microbiota in chronic kidney disease. *Nephrol Dial Transplant* 2015;30:924-933.
5. Vaziri ND, Yuan J, Nazertehrani S, et al. Chronic kidney disease causes disruption of gastric and small intestinal epithelial tight junction. *Am J Nephrol* 2013;38:99-103.
6. Vaziri ND, Yuan J, Norris K. Role of urea in intestinal barrier dysfunction and disruption of epithelial tight junction in chronic kidney disease. *Am J Nephrol* 2013;37:1-6.
7. Vaziri ND, Wong J, Pahl M, et al. Chronic kidney disease alters intestinal microbial flora. *Kidney Int* 2013;83:308-315.
8. Tang WH, Wang Z, Kennedy DJ, et al. Gut microbiota-dependent trimethylamine N-oxide (TMAO) pathway contributes to both development of renal insufficiency and mortality risk in chronic kidney disease. *Circ Res* 2015;116:448-455.
9. Summers SC, Quimby JM, Isaiah A, Suchodolski JS, Lunghofer PJ, Gustafson DL. The fecal microbiome and serum concentrations of indoxyl sulfate and p-cresol sulfate in cats with chronic kidney disease. *J Vet Intern Med* 2019; 33:662-669.
10. Lippi I, Perondi F, Ceccherini G, et al. Effects of probiotic VSL#3 on glomerular filtration rate in dogs affected by chronic kidney disease: A pilot study. *Can Vet J* 2017;58:1301-1305.

The Gut-Brain Axis

Prof. Caroline Mansfield BSc BVMS MANZCVSc PhD DECVIM-CA
Director of U-Vet Animal Hospital
Victoria, Australia

The role of the gut microbiome has gained increasing attention over the past decade. Initially, interest was placed in changes in the microbiome that occurred during intestinal disease and inflammation (Crohn's disease, ulcerative colitis. etc), but later expanded to changes evident in metabolic diseases such as obesity and diabetes mellitus.[1] This interest aligned with the findings that gastrointestinal (GI) microbes have metabolic as well as local functions and impacts on the host.

Along with the premise that potentially every disease begins in the gut, a lot of attention has been placed on the gut-brain-GI microbiome axis in recent years, with emphasis in the human field in the areas of autism, Parkinson's disease and stress/anxiety.[3] There is increased understanding of interactions, particularly in pre-clinical models, but no clear recommendations or treatment options have resulted from research to date.

There is bi-directional interaction between the gut and the brain. The brain modulates the autonomic nervous system (ANS) and hypothalamus-pituitary-adrenal (HPA) axis in response to stress, and these are then inputted into the gut via target cells in the gut wall (enteric neurons). Thus, the brain can affect the GI microbiome neurologically and hormonally by changing intestinal motility, permeability, pH, and mucus secretion over the length of the intestine, or regionally.[2] The central nervous system (CNS) can also modulate immune response to microbes by enhancing production of antimicrobial peptides by Paneth cells. Although most ANS function is delivered via the vagus nerve, there does appear to be vagus-independent mechanisms by which the ANS-enteric nervous system (ENS) interaction occurs.[5]

The ENS is in constant communication with the GI microbiome, mediated by a variety of signals including short-chain fatty acids, bile acids and neuroactive metabolites. There is also a mounting interest in a direct microbial signalling system, or quorum sensing.[5] The quorum sensing allows bacteria to regulate their gene expression in response to signals from other bacteria, but also in response to the host. Bacteria may impact the gut-brain axis by altering the intestinal barrier, modulating sensory nerves, up- or down-regulating production of neurotransmitters and regulating the mucosal immune system (which will have an impact on the brain via circulating cytokines).

Pre-clinical evidence, mainly in rodent experimental models, clearly shows that bacteria and bacterial metabolites also are involved in modulating behaviour, social interactions and learning.[4] Of interest in many of these experiments is demonstration of increased HPA axis responsiveness and reduced expression of brain-derived neurotropic factor (BDNF) in germ-free mice.[5] There have also been studies that demonstrate changes in other neurotransmitter receptors in the brain (NDMA receptors, GABA receptors) and tryptophan (or tryptophan metabolites like tryptamine) expression in germ-free mice, which correlated with emotional behaviour.[2, 4, 5] Similarly, ketogenic diets, used to treat epilepsy, also induce changes in the microbiota that may contribute to anti-seizure activity.

Acute post-natal stress (such as maternal separation, antibiotic administration) may have an impact on the gut microbiome, which in turn may impact the developing brain.[2] In fact, the gut microbiome is essential to development of the central nervous system. Additionally, research has been looking at the concept of chronic stress and resultant brain plasticity that results from this. In mice with chronic stress (resulting from physical restraint), the relative abundance of *Bacteroides* spp decreased, whilst *Clostridium* spp. increased. Additionally, in people with chronic stress or depressive disease, increased intestinal permeability has been identified along with increased circulating concentrations of bacterial lipopolysaccharide (LPS).

It is apparent from interest in the medical field and pre-clinical experimental models that there is great potential for further investigation of manipulation of the gut-brain axis in veterinary medicine. Areas of interest include stress-associated diarrhea, cognitive and behavioural disorders, hepatic encephalopathy, and more idealistically, manipulation of the microbiome during the early development stage (i.e. post-weaning to 1 year) to ensure a stable and functional GI microbiome.

References

1. Bailey TM, Dowd SE, Galley JD et al. Exposure to a social stressor alters the structure of the intestinal microbiota: implications for stressor-induced immunomodulation. *Brain Behav Immun* 2011; 25: 397-407

2. Cryan JF & Dinan TG. Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nature Reviews Neuroscience* 2012; 13: 701- 712

3. Leprun PMB & Clarke G. The gut microbiome and pharmacology: a prescription for therapeutic targeting of the gut-brain axis. *Current Opinion in Pharmacology* 2019; 49: 17-23

4. Mayer EA, Tillisch K & Gupta A. Gut/brain axis and the microbiota. *The Journal of Clinical Investigation* 2015; 125:926-938

5. Rhee SH, Pothoulakis C & Mayer EA. Principles and clinical implications of the brain-gut-enteric microbiota axis. *Nat Rev Gastroenterol Hepatol* 2009; 6: 306-314

Gut Microbiome and Obesity

Joe Bartges, DVM, PhD, DACVIM (SAIM), DACVN
Professor of Medicine and Nutrition
The University of Georgia
Athens, GA, USA

OBESITY

What if there was a disease that affected over 1/3 of all human beings and over 1/2 of all dogs and cats in the United States, was easy to diagnose, had reasonable treatment and preventative strategies, and if controlled would result in less associated comorbidities and longer life span? Obesity is that epidemic. Obesity affects one-third of humans and is associated with over 3 million deaths and loss of quality of life and life expectancy. Approximately 60% of dogs and cats are overweight or obese with a 2:1 ratio of dogs being considered overweight versus obese while a 1:1 ratio of cats being considered overweight versus obese.[1-3] Associated diseases of obesity in dogs and cats include orthopedic, dermatologic, intestinal, hepatic, urogenital, cardiopulmonary, endocrine, and neoplastic, and shortened life span.[4-12] Obesity is a disease and meets the American Medical Association definition of a disease: (1) an impairment of normal functioning of some aspect of the body, (2) demonstration of characteristic signs or symptoms, and (3) causes harm or morbidity. Further, this implies that being overweight is a pre-disease (pre-obese) state and that intervention should have benefit at this stage.

Obesity is often defined as weighing approximately 25 to 30% or more over ideal while being overweight is defined as weighing up to 25% over ideal due to an increase in body adiposity. Body condition scoring provides a valid and accurate estimate of body fat content,[13-15] and a score of 8 to 9 out of 9 equates to 25% or more over ideal body fat content while a score of 6 to 7 equates to less than 25% over ideal body fat content. There is a continuum between being overweight and obese.

Although an oversimplification of the etiopathogenesis, obesity results from an imbalance between energy intake and energy expenditure where intakes exceeds expenditure. Development of overweight and obese conditions involves a complex interaction between genetic and environmental factors. Identified risk factors for obesity in dogs and cats include animal factors (breed, gender, neuter status, growth rate, and age) and owner factors (diet choice, feeding method, exercise and living environment, age and body composition of owners, income, and underestimation of a pet's body condition score).[4,16]

Management of obese dogs and cats involves decreasing caloric consumption so that energy expenditure exceeds intake. The goal is to induce fat mobilization resulting in weight loss and (hopefully) preferential loss of adipose tissue with minimal loss of lean body mass. This may be accomplished by increasing energy expenditure with exercise, such as walking, playing games, etc. or by modifying the diet or both.[8,17,18] Dietary strategies for inducing weight loss in dogs and cats include diet nutrient modification (either high fiber/low fat or high protein/low carbohydrate), altering food texture, altering feeding patterns, or decreasing amount of food consumed.[17,19-26] While these approaches sound like success is all but guaranteed, experience has shown that success is less than ideal and veterinary weight loss diets may have differing levels of acceptance among pets.[27] Additionally, weight rebound after weight loss is achieved is a real concern.[23,24,28-31] One of the reasons for this rebound is that focusing on energy intake only deals with half of the problem (energy intake) while ignoring the other half (energy expenditure) and does not consider factors that mediate energy homeostasis.

GUT MICROBIOME

In recent years, attention has turned to gut microbiome and its role in obesity. Gut microbiome represents an interface with the environment and influences the body's metabolic and immune functions. Gut microbiome is defined as the collection of all living microbes (bacteria, fungi, protozoa, and viruses) residing in the gastrointestinal tract.[32] In humans, an estimated 100 trillion bacterial cells populate the gastrointestinal tract and the total sum of bacteria is approximately 10 times more than the number of host cells. The genome of these microbes exists in close relationship with the host and, through its immunologic and metabolic functions, this microbial-host ecosystem impacts host

health. Resident bacteria are beneficial and function to suppress enteropathogens, aid in nutrition and digestion, affect dietary energy availability, provide metabolites for enterocyte function, and stimulate the immune system.[32,33]

Bacterial phyla that comprise gut microbiome are relatively conserved across mammals including dogs and cats but vary throughout the length of the gastrointestinal tract.[34,35] Gut microbiome presents a low diversity at the phylum level with only one archaeal phylum (prokaryotic), Euryarchaeota, and six primary bacterial phyla (eukaryotic) represented by *Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, and Fusobacteria,* (Table 1).[36-41] Healthy adult gut microbiome are dominated by three bacterial phyla, *Firmicutes, Bacteroidetes,* and *Actinobacteria,* and one major methanogenic archaeon, *Methanobrevibacter.*[36,37,42] The composition of gut microbiome also depends on its functional role in digestion.

Table 1: The 6 major phyla of the human gut microbiome and their predominant species

Phyla	Genera
Firmicutes	*Ruminococcus* *Clostridium* *Lactobacillus* *Eubacterium* *Faecalibacterium* *Roseburia* *Erysipelotrichaceae*
Bacteroidetes	*Bacteroides* *Prevotella* *Xylanibacter*
Actinobacteria	*Collinsella* *Bifodobacterium*
Proteobacteria	*Escherichia* *Desulfovibrio*
Fusobacteria	*Fusobacterium prausnitzii*
Euryarchaeota	*Methanobrevibacter*

Gut microbiome establishes within hours to days of birth in humans, dogs, cats, and rodents.[43-47] After weaning with a transition in diet, gut microbiome undergoes change in terms of species and numbers of bacteria with variability between animals.[48] Dietary changes throughout life account for up to 57% of gut microbiome changes, whereas host genes account for no more than 12% in humans.[49]

Gut microbiome intervenes mainly in the colon where no digestive enzymes are secreted to metabolize macronutrients not digested in the ileum.[36,37] Each type of macronutrient influences gut microbiome with changes occurring more at a gene expression (metabolic) level than at a taxonomic level.[50-52] Nevertheless, transient changes are observed in the diversity of gut microbiome associated with each macronutrient. These changes affect only specific species whose metabolic activity are affected by the investigated macronutrient (Table 2).[53] Oligo- and polysaccharides fermentation by commensal bacteria of the colon results in synthesis of short chain fatty acids, and phenolic compounds that are metabolized to bioactive compounds.[37] Absorbed short chain fatty acids serve as an energy source for the animal (e.g. propionate and acetate), regulate intestinal motility, are enterocyte growth factors, and help to maintain intestinal barrier.[54,55] It has been estimated that microbial short chain fatty acid production provides 2 to 7% of adult dog maintenance energy requirements.[56,57] Saccharolytic species include species belonging to the *Bacteroides, Bifidobacterium, Clostridium, Eubacterium,*

Table 2: Dietary influences on composition of human gut microbiome.[62]

Macronutrient	Increased species	Decreased species
High-carbohydrate diet	*High fermenting power* *Firmicutes* *Clostridium cluster XVIII* *Lachnospiraceae (Clostridium clostridioforme)* *Ruminococcaceae (Faealiacterium prausnitzii)* *Bacteroidetes* *Prevotella*	*Bacteroidetes* *Bacteroides* *Actinobacteria* *Bifodobacterium* *Proteobacteria* *Enterobacteriaceae*
High-fat diet	*Bile tolerant* *Bacteroidetes* *Alisitpes* *Bacteroides* *Proteobacteria* *Bilophila*	
High-protein diet	*Butyrate producing species* *Firmicutes* *Clostridium cluster XIV* *Roseburia* *Eubacterium rectale* *Faecalibacterium prausnitzii* *Lactobacilli* *Proteolytic species* *Bacteroidetes* *Bacteroides*	

Lactobacillus and Ruminococcus genera.[36] Dietary fiber consumption leads to an increase in butyrate-producing species that ferment these fibers (*Roseburia, Blautia, Eubacterium rectale, Faecalibacterium prausnitzii*), in the Actinobacteria phylum (*Bifidobacteria, Lactobacilli*) and variations in *Bacteroidetes* proportion depending on the type of dietary fiber.[36,50,58-60] Fermentation of proteins by gut microbiome takes place via bacterial proteinase and peptidase due to species such as *Clostridia, Propionibacterium spp., Prevotella spp., Bifidobacterium* spp. and *Bacteroides* spp.[36,37]A high protein diet, which is usually a low carbohydrate diet stimulates a decrease in butyrate producing species and an increase of proteolytic species such as *Bacteroides* spp.[36,50] Dietary fat has an indirect impact on gut microbiome diversity: a high fat diet stimulates production of bile acids that in turn select species with ability to metabolize bile acids and/or induce loss of some species due to antimicrobial activity of bile acids.[36,50] Composition of diet may result in dysbiosis, which is an imbalance or alteration of gut microbiome associated with various diseases including obesity.[61]

Data evaluated and extrapolated from studies of humans and rodents may not be applicable to dogs and cats. Dogs, as omnivorous carnivores, and cats, as carnivores, have no nutritional requirement for carbohydrates and can thrive on a diet high in protein and fat and low in carbohydrate and fiber.[63,64] Commercial heat processed diets tend to be carbohydrate-based. Comparisons of canine and feline gut microbiome composition and influence with diet to data from humans and rodents may not be wholly applicable. When dogs are fed a high protein, meat-based diet, fecal weight and short chain fatty acid production were lower and digestibility of protein and energy were higher when compared with dogs consuming a lower fat, dry dog food.[65] Dogs fed an extruded dry kibble (EXT), high-moisture grain-free roasted refrigerated (HMGFRR), raw (RAW), or high-moisture roasted refrigerated (HMRR) diet in a Latin Square design showed greatest abundance of Firmicutes and lowest abundance of Bacteroidetes when dogs ate the extruded diet while the opposite occurred when dogs ate the raw diet (Figure 1). Lightly cooked and raw diets tested were highly palatable, highly digestible, reduced blood triglycerides, maintained fecal quality and serum chemistry, and modified the fecal microbial community of healthy adult dogs.[66]

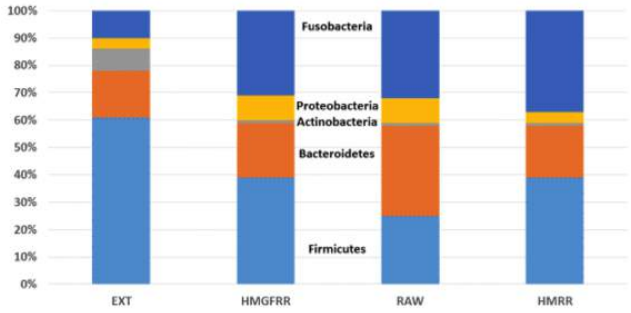


Figure 1: Relative abundances of 5 microbial phyla in feces of dogs fed an extruded dry kibble (EXT), high-moisture grain-free roasted refrigerated (HMGFRR), raw (RAW), or high-moisture roasted refrigerated (HMRR) diet (*n* = 8/treatment).[66]

GUT MICROBIOME AND OBESITY

In recent years, the role of the gut microbiome in obesity has received considerable attention and the association is multifactorial (Figure 2).

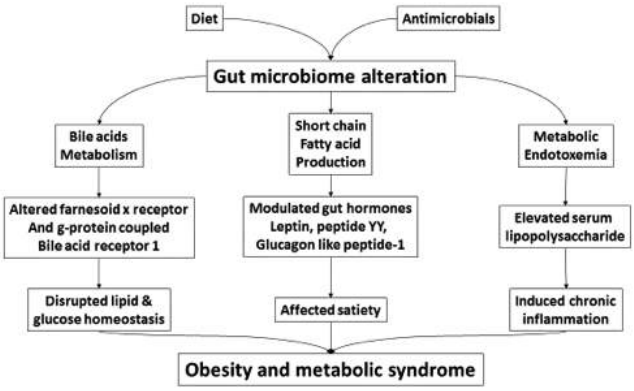


Figure 2: Links between obesity and gut microbiome[67]

Gut microbiome is modified in obesity and related-comorbidities such as type 2 diabetes mellitus, metabolic syndrome, and cardiovascular disease in humans.[68,69] Hypothesized or proven links between gut microbiome and obesity include energy extraction capacity from food, alteration of gut barrier integrity, modulation of chronic inflammation and immune system, and production of specific metabolites that affect the gut-associated immune system and intestinal barrier as well as other organs including the brain, liver, and adipose tissue.

Genetic and environmental factors have significant impacts on structure and composition of gut microbiome with diet being one of the greatest influences that can alter it.[70-72] In humans and mice, changes in predominant gut microbiome phyla are linked to obesity with more Firmicutes and fewer Bacteroidetes in obese individuals than in lean ones.[73,74] This is reversible as Bacteroidetes to Firmicutes ratios (B/F ratios) in obese mice became similar to those in lean mice after diet-induced weight loss.[73,75] Increased Firmicutes may increase efficiency in energy extraction from the diet resulting in higher levels of short-chain fatty acids that may alter metabolism of obese individuals.[74,76,77]

Rodent models have demonstrated a causal relationship between dysbiosis and control of body weight. Transplanting gut microbiome from obese mice to lean mice increased adiposity of recipients and recipient germ free mice that received gut microbiome from obese versus lean donors developed obesity or remained lean depending on body condition of the donor.[77] In humans, transplantation of gut microbiome from lean donors to patients with metabolic syndrome resulted in improved insulin sensitivity.[78,79] Mechanisms involved are not defined but may include, in part, immune system modulation. Gut microbiome gene profiling is better at distinguishing type 2 diabetic humans from control subjects than use of body mass index.[80,81]

Overweight and obese cats had a significantly different gut microbiome compared with lean cats ($p < 0.05$),

but this finding could not be linked to differences in specific bacterial groups.[82] In a small study of obese and lean dogs, there were differences in gut microbiome; Firmicutes, *Fusobacteria* and *Actinobacteria* were the predominant bacterial phyla. Actinobacteria and the genus *Roseburia* were significantly more abundant in obese pet dogs.[83] Gut microbiome are less diverse in obese dogs when compared with lean dogs, and Firmicutes predominated in lean dogs while Proteobacteria predominated in obese dogs.[84] Obese dogs also had higher circulating leptin concentrations and lower cerebrospinal fluid concentrations of adiponectin and 5-hydroxytryptamine than lean dogs, which may increase risk of obesity due to increased appetite.[84] In a study comparing normal weight, overweight, and obese dogs, there were differences in microbiome and metabolome profiles between groups especially plasma phospholipid profiles demonstrating alteration in metabolic status with obesity.[85] Obese and lean Labrador retrievers and Beagles when fed a low protein, high carbohydrate diet (26% protein, 39% carbohydrate, dry matter basis) favored growth of *Bacteroides* and *Clostridium* while a high protein, low carbohydrate diet (50% protein, 11% carbohydrate, dry matter basis) favored *Clostridium* and *Ruminococcus* with a decrease in *Bacteroidetes* to *Firmicutes* ratio and an increase in *Bacteroidetes* to *Prevotella* ratio.[86] The effect was more evident in obese dogs than in lean dogs and was not dependent on breed. These data suggest differences in lean, overweight, and obese dogs and cats similar to observations in humans and rodents.

Management of obesity in dogs and cats involves decreasing energy intake, increasing energy expenditure, or a combination of the two. Dietary fiber is known as microbiota-accessible carbohydrates that are composed of monosaccharides connected through glycosidic linkages that may be modified by chemical substituents such as acetyl and sulfate groups. Variation in their chemical composition, solubility, and size differentiates these carbohydrates into a vast array of ecological niches.[87] Gut microbiome depend on dietary fiber to thrive and provide energy. Diet, especially consumption of dietary fiber, appears to be a critical determinant for gut bacterial ecology, diversity, and function in humans and rodents.[88] Increased dietary fiber is often used in weight reduction diets in dogs and cats with lower fat (and thus energy) intake. Initial benefits were thought to be due to providing a sensation of satiety and decreased energy intake; however, an additional benefit is altering gut microbiome. Weight reduction diets are usually lower in fat than maintenance diets; this also changes the gut microbiome. Obesity is an inflammatory condition and inclusion of omega-3 fatty acids can reduce body fat and decrease inflammation through modulation of gut microbiome.[89]

In a small study inducing weight loss in obese Beagles, feeding an energy-restricted, high-fiber, low-fat diet resulted in a decrease in *Firmicutes* and an increase in *Bacteroidetes* with an increase in *Bacteroidetes* to *Firmicutes* ratio and biodiversity of gut microbiome.[90] Restricted feeding of a low-fat, high-fiber dry diet modifies gut microbiome in obese dogs, increasing biodiversity with a different representation of microbial genus and metabolic pathways. Feeding a high-protein diet, higher in total dietary fiber and insoluble fiber but lower in soluble fiber, to lean and obese Beagles was associated with increased abundance and activity of butyrate-producing bacteria, Clostdrial clusters IV and XIVa independent of body condition; however, gut microbiome was more diverse in the obese Beagles.[91] When 15 obese dogs were fed a high-protein, high-fiber diet compared with 25 obese dogs fed a high-protein, medium-fiber diet with equivalent caloric density, percentage of weight loss was greater, mean rate of weight loss was faster, and percentage of body fat mass decrease was greater.[26] In a study evaluating weight-loss and exercise, 18 obese pet dogs were recruited for a 12-week weight-loss intervention using a commercial high-protein/high-fiber dry diet, and eight of these dogs were enrolled in an exercise program in addition to the diet intervention. Total weight loss, food allowance and gut microbiome were not changed by exercise. Acetic and propionic acid concentrations decreased in dogs with a faster weight loss rate; thus, having a gut microbiome that favors short-chain fatty acid production may negatively affect weight loss rate in dogs.[92]

In a study comparing 8 lean cats with 8 obese cats undergoing weight reduction, gut microbiome of lean cats was similar to that found in obese rodents and humans with a greater abundance of *Firmicutes* and lower abundance of *Bacteroidetes*. Weight loss in the obese cats was associated with a reduction in *Firmicutes* as seen with weight reduction in dogs, rodents, and humans.[93] However, in another study of obese cats, feeding a moderate protein, high fiber (36% crude protein and 17% total dietary fiber dry matter), weight loss was associated with a greater proportion of *Actinobacteria* and lower proportion of *Bacteroide*tes, with *Firmicutes* being unchanged.[94] Reduction in *Bacteroidetes* with weight loss was primarily attributable to a reduction in *Prevotella* spp. Increase in *Actinobacteria* with weight loss was primarily attributable to an increase in *Bifidobacterium* spp and *Collinsella* spp.[94]

We are only beginning to understand the role of gut microbiome in health and disease. Obesity is the most common nutritional disease of dogs and cats and is associated with many related disorders. Diet plays a major role in its development, treatment, and prevention and is more than just modification of energy intake. Macronutrient influence gut microbiome, which in turn alters digestion, energy metabolism, whole body metabolism, and immune response, all being important with obesity. Data from studies of humans and rodents, both omnivores, may not be completely applicable to dogs, a carnivorous omnivore, and cats, an obligate carnivore. Therefore, species-specific studies are required to advance knowledge of the role of gut microbiome in canine and feline obesity and to improve health, well-being, and longevity of our four-legged family members.

REFERENCES

1. Ward E. U.S. Pet Obesity Steadily Increases, Owners and Veterinarians Share Views on Pet Food. http://www.PetObesityPrevention.org: Association for Pet Obesity Prevention, 2017.
2. Lund EM, Armstrong PJ, Kirk CA, et al. Prevalence and risk factors for obesity in adult cats from private veterinary practices. *Int J Appl Res Vet Med* 2005;3:88-96.
3. Lund EM, Armstrong PJ, Kirk CA, et al. Prevalence and risk factors for obesity in adult dogs from private veterinary practices. *Int J Appl Res Vet Med* 2006;4:177-186.
4. Larsen JA, Villaverde C. Scope of the Problem and Perception by Owners and Veterinarians. *Vet Clin North Am Small Anim Pract* 2016;46:761-772.
5. Clark M, Hoenig M. Metabolic Effects of Obesity and Its Interaction with Endocrine Diseases. *Vet Clin North Am Small Anim Pract* 2016;46:797-815.
6. Weeth LP. Other Risks/Possible Benefits of Obesity. *Vet Clin North Am Small Anim Pract* 2016;46:843-853.
7. Chandler ML. Impact of Obesity on Cardiopulmonary Disease. *Vet Clin North Am Small Anim Pract* 2016;46:817-830.
8. Frye CW, Shmalberg JW, Wakshlag JJ. Obesity, Exercise and Orthopedic Disease. *Vet Clin North Am Small Anim Pract 2016*;46:831-841.
9. Saker KE, Selting KA. Cancer In: Hand MS, Thatcher CD, Remillard RL, et al., eds. *Small Animal Clinical Nutrition.* 5th ed. Topeka: Mark Morris Institute, 2010;588-607.
10. Scarlett JM, Donoghue S. Associations between body condition and disease in cats. *J Am Vet Med Assoc* 1998;212:1725-1731.
11. Kealy RD, Lawler DF, Ballam JM, et al. Evaluation of the effect of limited food consumption on radiographic evidence of osteoarthritis in dogs. *J Am Vet Med Assoc* 2000;217:1678-1680.
12. Kealy RD, Lawler DF, Ballam JM, et al. Effects of diet restriction on life span and age-related changes in dogs. *J Am Vet Med Assoc* 2002;220:1315-1320.
13. Laflamme D. Devlopment and validation of a body condition score system for dogs. *Canine Practice* 1997;22:10-15.
14. Laflamme D. Development and validation of a body condition score system for cats: A clinical tool. *Feline Practice* 1997;25:13-18.
15. Mawby DI, Bartges JW, d'Avignon A, et al. Comparison of various methods for estimating body fat in dogs. *J Am Anim Hosp Assoc* 2004; 40:109-114.
16. Backus R, Wara A. Development of Obesity: Mechanisms and Physiology. *Vet Clin North Am Small Anim Pract* 2016;46:773-784.
17. Chapman M, Woods GRT, Ladha C, et al. An open-label randomised clinical trial to compare the efficacy of dietary caloric restriction and physical activity for weight loss in overweight pet dogs. *Vet J* 2019;243:65-73.
18. Kushner RF, Blatner DJ, Jewell DE, et al. The PPET Study: people and pets exercising together. *Obesity (Silver Spring)* 2006;14:1762-1770.
19. Sagols E, Hours MA, Daniel I, et al. Comparison of the effects of different kibble shape on voluntary food intake and palatability of weight loss diets in pet dogs. *Res Vet Sci* 2019;124:375-382.
20. Murphy M. Obesity Treatment: Environment and Behavior Modification. *Vet Clin North Am Small Anim Pract* 2016;46:883-898.
21. Linder DE, Parker VJ. Dietary Aspects of Weight Management in Cats and Dogs. *Vet Clin North Am Small Anim Pract* 2016;46:869-882.
22. German AJ. Outcomes of weight management in obese pet dogs: what can we do better? *Proc Nutr Soc* 2016;75:398-404.
23. German AJ. Obesity Prevention and Weight Maintenance After Loss. *Vet Clin North Am Small Anim Pract* 2016;46:913-929.
24. German AJ. Weight management in obese pets: the tailoring concept and how it can improve results. *Acta Vet Scand* 2016;58:57.
25. Pena C, Suarez L, Bautista-Castano I, et al. Effects of low-fat high-fibre diet and mitratapide on body weight reduction, blood pressure and metabolic parameters in obese dogs. *J Vet Med Sci* 2014;76:1305-1308.
26. German AJ, Holden SL, Bissot T, et al. A high protein high fibre diet improves weight loss in obese dogs. *Vet J* 2010;183:294-297.
27. Hours MA, Sagols E, Junien-Castagna A, et al. Comparison of voluntary food intake and palatability of commercial weight loss diets in healthy dogs and cats. *BMC Vet Res* 2016;12:274.
28. German AJ, Holden SL, Morris PJ, et al. Long-term follow-up after weight management in obese dogs: the role of diet in preventing regain. *Vet J* 2012;192:65-70.
29. Laflamme DP, Kuhlman G. The effect of weight loss regimen on subsequent weight maintenance in dogs. *Nutr Res* 1995;15:1019-1028.
30. Deagle G, Holden SL, Biourge V, et al. Long-term follow-up after weight management in obese cats. *J Nutr Sci* 2014;3:e25.
31. German AJ, Holden SL, Mather NJ, et al. Low-maintenance energy requirements of obese dogs after weight loss. *Br J Nutr* 2011;106 Suppl 1:S93-96.
32. Suchodolski JS. Gut Brain Axis and Its Microbiota Regulation in Mammals and Birds. *Vet Clin North Am Exot Anim Pract* 2018;21:159-167.
33. Kil DY, Swanson KS. Companion animals symposium: role of microbes in canine and feline health. *J Anim Sci* 2011;89:1498-1505.
34. Honneffer JB, Minamoto Y, Suchodolski JS. Microbiota alterations in acute and chronic gastrointestinal inflammation of cats and dogs. *World J Gastroenterol* 2014;20:16489-16497.
35. Tropini C, Earle KA, Huang KC, et al. The Gut Microbiome: Connecting Spatial Organization to Function. *Cell Host Microbe* 2017;21:433-442.
36. Maukonen J, Saarela M. Human gut microbiota: does diet matter? *Proc Nutr Soc* 2015;74:23-36.
37. Jandhyala SM, Talukdar R, Subramanyam C, et al. Role of the normal gut microbiota. *World J Gastroenterol* 2015;21:8787-8803.
38. Honneffer JB. Variation of the microbiota and metabolome along the canine gastrointestinal tract. *Metabolomics : Official journal of the Metabolomic Society* 2017;13:1-20.

39. Swanson KS, Dowd SE, Suchodolski JS, et al. Phylogenetic and gene-centric metagenomics of the canine intestinal microbiome reveals similarities with humans and mice. *The ISME journal* 2011;5:639-649.
40. Suchodolski JS, Foster ML, Sohail MU, et al. The fecal microbiome in cats with diarrhea. *PLoS One* 2015;10:e0127378.
41. Coelho LP, Kultima JR, Costea PI, et al. Similarity of the dog and human gut microbiomes in gene content and response to diet. *Microbiome* 2018;6:72.
42. Gill SR, Pop M, Deboy RT, et al. Metagenomic analysis of the human distal gut microbiome. *Science* 2006;312:1355-1359.
43. Garcia-Mantrana I, Collado MC. Obesity and overweight: Impact on maternal and milk microbiome and their role for infant health and nutrition. *Mol Nutr Food Res* 2016;60:1865-1875.
44. Garcia-Mantrana I, Bertua B, Mrtinez-Costa C, et al. Perinatal nutrition: How to take care of the gut microbiota. *Clin Nutr Exp* 2016;6:3-16.
45. Buddington RK. Postnatal changes in bacterial populations in the gastrointestinal tract of dogs. *Am J Vet Res* 2003;64:646-651.
46. Jia J, Frantz N, Khoo C, et al. Investigation of the faecal microbiota of kittens: monitoring bacterial succession and effect of diet. FEMS *Microbiol Ecol* 2011;78:395-404.
47. Guard BC, Mila H, Steiner JM, et al. Characterization of the fecal microbiome during neonatal and early pediatric development in puppies. *PLoS One* 2017;12:e0175718.
48. Schaible UE, Kaufmann SH. A nutritive view on the host-pathogen interplay. *Trends Microbiol* 2005;13:373-380.
49. Clark A, Mach N. Exercise-induced stress behavior, gut-microbiota-brain axis and diet: a systematic review for athletes. *J Int Soc Sports Nutr* 2016;13:43.
50. Graf D, Di Cagno R, Fak F, et al. Contribution of diet to the composition of the human gut microbiota. *Microbial ecology in health and disease* 2015;26:26164.
51. Flint HJ, Duncan SH, Scott KP, et al. Links between diet, gut microbiota composition and gut metabolism. *Proc Nutr Soc* 2015;74:13-22.
52. Aguirre M, Eck A, Koenen ME, et al. Diet drives quick changes in the metabolic activity and composition of human gut microbiota in a validated in vitro gut model. *Res Microbiol* 2016;167:114-125.
53. Costabile A, Deaville ER, Morales AM, et al. Prebiotic Potential of a Maize-Based Soluble Fibre and Impact of Dose on the Human Gut Microbiota. *PLoS One* 2016;11:e0144457.
54. Rondeau MP, Meltzer K, Michel KE, et al. Short chain fatty acids stimulate feline colonic smooth muscle contraction. *J Feline Med Surg* 2003;5:167-173.
55. Scheppach W. Effects of short chain fatty acids on gut morphology and function. *Gut* 1994;35:S35-38.
56. Herschel DA, Argenzio RA, Southworth M, et al. Absorption of volatile fatty acid, Na, and H2O by the colon of the dog. *Am J Vet Res* 1981;42:1118-1124.
57. Stevens CE, Hume ID. Contributions of microbes in vertebrate gastrointestinal tract to production and conservation of nutrients. *Physiol Rev* 1998; 78:393-427.
58. Chung WS, Walker AW, Louis P, et al. Modulation of the human gut microbiota by dietary fibres occurs at the species level. *BMC Biol* 2016;14:3.
59. Chung WSF, Walker AW, Vermeiren J, et al. Impact of carbohydrate substrate complexity on the diversity of the human colonic microbiota. *FEMS Microbiol Ecol* 2019;95.
60. Dominianni C, Sinha R, Goedert JJ, et al. Sex, body mass index, and dietary fiber intake influence the human gut microbiome. *PLoS One* 2015;10:e0124599.
61. Wang B, Yao M, Lv L, et al. The human microbiota in health and disease. *Engineering* 2017;3:71-82.
62. Alou MT, Lagier J-C, Raoult D. Diet influence on the gut microbiota and dysbiosis related to nutritional disorders. *Hum Microbiome* J 2016;1:3-11.
63. Verbrugghe A, Hesta M. Cats and Carbohydrates: The Carnivore Fantasy? *Veterinary sciences* 2017;4.
64. Loftus JP, Yazwinski M, Milizio JG, et al. Energy requirements for racing endurance sled dogs. *J Nutr Sci* 2014;3:e34.
65. Bermingham EN, Maclean P, Thomas DG, et al. Key bacterial families (Clostridiaceae, Erysipelotrichaceae and Bacteroidaceae) are related to the digestion of protein and energy in dogs. *PeerJ* 2017;5:e3019.
66. Algya KM, Cross TL, Leuck KN, et al. Apparent Total Tract Macronutrient Digestibility, Serum Chemistry, Urinalysis, and Fecal Characteristics, Metabolites and Microbiota of Adult Dogs Fed Extruded, Mildly Cooked, and Raw Diets. *J Anim Sci* 2018.
67. Tseng CH, Wu CY. The gut microbiome in obesity. J *Formos Med Assoc* 2019;118 Suppl 1:S3-s9.
68. Dao MC, Clement K. Gut microbiota and obesity: Concepts relevant to clinical care. *Eur J Intern Med* 2018;48:18-24.
69. Tremaroli V, Backhed F. Functional interactions between the gut microbiota and host metabolism. *Nature* 2012;489:242-249.
70. David LA, Maurice CF, Carmody RN, et al. Diet rapidly and reproducibly alters the human gut microbiome. *Nature* 2014;505:559-563.
71. Xu Z, Knight R. Dietary effects on human gut microbiome diversity. *Br J Nutr* 2015;113 Suppl:S1-5.
72. De Filippo C, Cavalieri D, Di Paola M, et al. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural *Africa. Proc Natl Acad Sci U S A* 2010;107:14691-14696.
73. Ley RE. Obesity and the human microbiome. *Curr Opin Gastroenterol* 2010;26:5-11.
74. Ley RE, Backhed F, Turnbaugh P, et al. Obesity alters gut microbial ecology. *Proc Natl Acad Sci U S A* 2005;102:11070-11075.
75. Turnbaugh PJ, Backhed F, Fulton L, et al. Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. Cell *Host Microbe* 2008;3:213-223.
76. Turnbaugh PJ, Hamady M, Yatsunenko T, et al. A core gut microbiome in obese and lean twins. *Nature* 2009;457:480-484.
77. Turnbaugh PJ, Ley RE, Mahowald MA, et al. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 2006;444:1027-1031.

78. Qin J, Li Y, Cai Z, et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature* 2012;490:55-60.
79. Vrieze A, Van Nood E, Holleman F, et al. Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology* 2012;143:913-916.e917.
80. Karlsson FH, Tremaroli V, Nookaew I, et al. Gut metagenome in European women with normal, impaired and diabetic glucose control. *Nature* 2013;498:99-103.
81. Osto M, Lutz TA. Translational value of animal models of obesity-Focus on dogs and cats. *Eur J Pharmacol* 2015;759:240-252.
82. Kieler IN, Molbak L, Hansen LL, et al. Overweight and the feline gut microbiome - a pilot study. *J Anim Physiol Anim Nutr (Berl)* 2016;100:478-484.
83. Handl S, German AJ, Holden SL, et al. Faecal microbiota in lean and obese dogs. *FEMS Microbiol Ecol* 2013;84:332-343.
84. Park HJ, Lee SE, Kim HB, et al. Association of obesity with serum leptin, adiponectin, and serotonin and gut microflora in beagle dogs. *J Vet Intern Med* 2015;29:43-50.
85. Forster GM, Stockman J, Noyes N, et al. A Comparative Study of Serum Biochemistry, Metabolome and Microbiome Parameters of Clinically Healthy, Normal Weight, Overweight, and Obese Companion Dogs. *Top Companion Anim Med* 2018;33:126-135.
86. Li Q, Lauber CL, Czarnecki-Maulden G, et al. Effects of the Dietary Protein and Carbohydrate Ratio on Gut Microbiomes in Dogs of Different Body Conditions. *MBio* 2017;8.
87. Sonnenburg ED, Sonnenburg JL. Starving our microbial self: the deleterious consequences of a diet deficient in microbiota-accessible carbohydrates. C*ell metabolism* 2014;20:779-786.
88. Tan J, McKenzie C, Vuillermin PJ, et al. Dietary Fiber and Bacterial SCFA Enhance Oral Tolerance and Protect against Food Allergy through Diverse Cellular Pathways. *Cell reports* 2016;15:2809-2824.
89. Kaliannan K, Wang B, Li XY, et al. A host-microbiome interaction mediates the opposing effects of omega-6 and omega-3 fatty acids on metabolic endotoxemia. *Scientific reports* 2015;5:11276.
90. Salas-Mani A, Jeusette I, Castillo I, et al. Fecal microbiota composition changes after a BW loss diet in Beagle dogs. *J Anim Sci* 2018;96:3102-3111.
91. Xu J, Verbrugghe A, Lourenco M, et al. The response of canine faecal microbiota to increased dietary protein is influenced by body condition. *BMC Vet* Res 2017;13:374.
92. Kieler IN, Shamzir Kamal S, Vitger AD, et al. Gut microbiota composition may relate to weight loss rate in obese pet dogs. *Veterinary medicine* and *science* 2017;3:252-262.
93. Fischer MM, Kessler AM, Kieffer DA, et al. Effects of obesity, energy restriction and neutering on the faecal microbiota of cats. *Br J Nutr* 2017;118:513-524.
94. Pallotto MR, de Godoy MRC, Holscher HD, et al. Effects of weight loss with a moderate-protein, high-fiber diet on body composition, voluntary physical activity, and fecal microbiota of obese cats. *Am J Vet Res* 2018;79:181-190.

Practical Communication Tips for Partnering with Clients in Framing their Nutrition Truths

Jason B Coe, DVM, PhD
Associate Professor
Ontario Veterinary College, University of Guelph
Ontario, Canada

"The truth is rarely pure and never simple." Oscar Wilde

Introduction

Today, information is more available and accessible than ever before. As a result, clients often attend to a veterinary practice armed with information, both reliable and unreliable, which has a role in their understanding and acceptance of their pet's healthcare. When a client's understanding of their pet's healthcare does not align with a practitioner's, tension can often exist within the veterinarian-client-patient relationship. In this instance, each party is likely to possess knowledge that has informed their understanding of the truth. For clients, their perception of the truth is often the result of investing a considerable amount of time and resources in exploring and uncovering information about their pet's healthcare from various sources. Equally, a practitioner' perception of the truth is the result of invested time and resources ensuring they provide high-quality evidence-based information to clients regarding a pet's healthcare. As a result, both parties are committed to their own understanding and knowledge of the truth. When these truths align, the interaction often proceeds smoothly towards a common plan of action. Yet, when these truths do not align, friction can exist, which often results in the client's truth prevailing and the veterinarian-client relationship being strained.

Research in healthcare suggests when a client enters an appointment holding a pre-established viewpoint not consistent with the practitioner's, the client is likely to reject the practitioner's view in favor of their own unless the practitioner is attentive and responds appropriately to the client's viewpoint.[1] Therefore, seeking to understand a client's truth upfront provides a foundation for identifying common ground. This common ground offers a starting point for veterinary professionals to re-evaluate their own understanding of the truth or, when appropriate, to begin sharing their own understanding and perceptions of the truth in relation to the client's. Through an active process of discussion and discovery the veterinary professional gains an appreciation of the underlying bases for a client's version of the truth; as well, through this process of being heard a client is also likely to become more receptive to hearing another perspective on the truth. This unpacking of a client's truth provides an opportunity to identify common ground from where the development of a collaborative recommendation, in which all parties are invested, becomes possible by reframing the truths held by all parties. Specific to pet nutrition, observational research conducted from the Ontario Veterinary College found that, when study veterinarians attempted to initiate proposals for long-term dietary change, their proposals were often met with client resistance.[2] When examining the basis for clients' resistance, the researchers identified that proposal-relevant information was often shared by clients during their resistance to the veterinarian's proposal, highlighting an opportunity to further explore a client's viewpoint upfront in order to include it in a proposed dietary change.

Given the essential importance of nutrition to life, veterinary guidelines promote that a nutritional assessment and a nutritional recommendation should be a part of every small-companion animal's visit to a veterinarian.[3, 4] In order for these guidelines to be successful, it is important that veterinary personnel approach nutrition conversations in a non-judgmental way, and with a curiosity to explore a client's nutrition truths (i.e., beliefs, goals and expectations). With six in ten pet owners found to access pet health information online before or after a visit to their veterinarian,[5] it should not be unexpected that clients hold their own ideas and understandings about their pet's nutrition. Pet nutrition specifically is an area where veterinary clients are likely to have their own perceptions of the truth, given the plethora of online sites dedicated to informing the public about pet nutrition. In addition, nutrition information is not limited to online sources

and is available to pet owners via an endless number of avenues including breeders, trainers, pet-store employees and the lay media to mention a few. Whether a veterinary professional views a client's information (or the source of the information) as credible does not change the fact that this information is likely to inform a client's perception of the truth about their pet's nutrition. Therefore, it is important when performing a nutritional assessment to not only gather a complete diet history but also to gather information specific to the client's perceptions of the truth (e.g., their beliefs, their goals, their expectations). By investing time to unpack and understand a client's nutrition truths, one provides the groundwork for either reinforcing or reframing a client's nutrition truths.

A relationship-centered approach to nutrition conversations with clients

Relationship-centered veterinary care is based on the recognition that within any interaction there are at least two or more individuals involved and through a process of sharing of information back and forth each participant gains an appreciation of the other's perspective,[6] which can often result in the discovery of common ground. This two-way approach to veterinarian-client interactions recognizes discovery of common ground as being essential to successful delivery of veterinary care. Within veterinary medicine, a relationship-centered approach has been found to have a positive association with veterinarian satisfaction,[7] client satisfaction[8] and client adherence[9].

Specifically, a study examining a collection of 83 video-recorded veterinarian-client-patient interactions that included a dentistry recommendation, a surgery recommendation, or both, by the veterinarian were examined using the Roter Interaction Analysis System to assess the nature of both verbal and nonverbal communication used by the veterinarian and client.[9] For each video-recorded interaction a relationship-centered communication score was established based on the nature and distribution of verbal communication between the veterinarian and client. Clients' adherence was assessed 6 months after the video-recorded interaction by reviewing each patient's medical record. The study found that the video-recorded veterinarian-client-patient interactions leading to client adherence scored significantly higher for a relationship-centered approach than interactions where the clients did not adhere.

As a result, investing in the use of relationship-centered communication tools that allow veterinary personnel to explore a client's beliefs and values about their pet's nutrition, to investigate the client's and their pet's living situation and to engage the client in the nutrition decision-making process, allows veterinary personnel to assess a client's starting point, to identify areas of common ground and to co-develop a nutrition recommendation that has value for the client.[10]

Communication tools for partnering with clients in framing their nutrition truths

Open-ended inquiry

Open-ended inquiry is the communication tool at the foundation of exploring a client's nutrition truths. Using open-ended inquiry promotes a collaborative rather than expert-in-charge approach to client interactions and provides an opportunity for a client to share their thoughts rather than respond specifically to direct questions that the veterinarian deems important. Open-ended inquiry is a statement framed in a way that encourages a client to broadly share their perspective rather than a focused question that encourages a one-word response, typically "yes" or "no".[11] Questions leading to one-word answers can be valuable during information gathering; yet are typically best reserved for pursuing finer details and clarifying information following the use of open-ended inquiry.

Specific to a pet's nutrition, observational research conducted in small-animal veterinary practices found veterinarians participating in the study often initiated a nutrition conversation with a client by using a simple what-prefaced question (e.g., "*What kind of food is he/she on?*").[12] The study found clients often treated this question as a closed-ended question, reporting only 1 or 2 food items that their pet was currently eating 89% of the time. Rarely (8% of the time) did the client disclose information about treats or human foods. Upon further examination, the researchers identified both veterinarians and clients oriented to the what-prefaced question as a closed-ended question. In addition, within 75% of the interactions containing a what-prefaced question, the veterinarian did not ask about any additional food items consumed by the pet, suggesting the scope of many veterinarians' nutritional-information gathering is very narrow. Gathering a detailed nutritional history from a client is an important area of exploration for beginning to develop an understanding of a client's nutritional truth. A comprehensive nutritional history is also one of several key components to developing and delivering an acceptable and appropriate nutritional recommendation to a client.

Examples of open-ended inquiry:

> *"Tell me everything he/she eats throughout a day, starting first thing in the morning right through to the end of the day"*[12]

> *"Walk me through the things that are important to you when choosing a new food for Harley?"*

Listening to the client

Listening to the client is the second step in unpacking a client's nutrition truth, that immediately follows open-ended inquiry. Observational research conducted in small-animal veterinary practice found that following veterinarians' solicitation of their client's concerns at the beginning of an appointment, the participating

veterinarians interrupted their clients before they were finished 55 percent of the time.[13] Further examination identified that the study veterinarians allowed the clients on average to speak for 15.3 seconds before interrupting (median, 11 seconds; range, 1 to 139 seconds). Yet potentially more important, once interrupted, clients were not provided the opportunity to return to and complete their response following 72 percent of the interruptions. By interrupting the client and specifically by not allowing them the opportunity to return to and complete their response, the veterinarian has potentially lost valuable information on their client's perspective. Taking the time to listen upfront allows veterinary professionals the opportunity to more fully appreciate their client's perspective in order to use this understanding to partner with the client in planning their pet's healthcare.

Accepting the client's perspective without judgment

As a client's perspective on a topic is being explored, it is important to be aware of one's own reaction to a client's thoughts or ideas and how one verbally and non-verbally manages this reaction. It has been suggested that it is important to have an immediate response of acceptance rather than judgment.[14] By accepting the client's views and beliefs about their pet's nutrition (i.e., their nutrition truth), it does not mean one is agreeing or disagreeing with the client; rather, it is an opportunity to acknowledge the client's nutrition truth and let the client know they have been heard.[11] Reassuring, agreeing or disputing a client's nutrition truth before all of the appropriate and relevant information has been explored is likely to lead to problems. Acceptance allows the veterinarian to remain open, avoid judgment and build trust with the client.

Examples of accepting responses:

> *"I can see you have done a fair amount of research into a new food for Harley and that you have questions about the food he is currently on. [Pause]"*

> *"I appreciate you letting me know your concerns with changing Harley's diet right now. [Pause]"*

Relating explanations to the client's perspective

As indicated above, when clients have a pre-established viewpoint regarding an issue that is not consistent with their veterinarian's, the client is likely to reject the veterinarian's viewpoint in favor of their own.[1] Once a client's perspective on their pet's nutrition has been elicited, the veterinarian becomes better positioned to adjust and explain nutrition-related information in a way that holds relevance to the client.[11] If a client has a different nutrition truth from their veterinarian, it becomes important for the veterinarian to explain information in a manner that acknowledges the client's perspective. This may mean the veterinarian will need to reframe their own perspective (i.e., truth) in order to present information in a way the client will be open to receiving it. In the end, the goal is for the veterinarian and client to respectfully recognize their differences in perspective, to acknowledge these differences, to establish common ground and to co-develop an acceptable and appropriate nutritional plan moving forward.

An example of relating explanations to the client's perspective:

> *"You mentioned earlier you had concerns about changing Harley's diet because of the byproducts in many commercial foods. I can understand your interest in choosing a food that you feel is safe and of a high quality for Harley. There are a number of diets we can consider that are safe and of a high quality. I'm confident with a little more discussion we can find a diet that will work for you and Harley and that will address my concerns as well."*

Summary

Today's clients often enter a veterinary practice armed with information, both reliable and unreliable, which has a role in their understanding and acceptance of their pet's healthcare. Taking the time to listen upfront allows veterinary professionals the opportunity to more fully appreciate their client's perspective in order to use this understanding to partner with the client in planning their pet's healthcare. Using a relationship-centered approach, including open-ended inquiry and active listening, allows the veterinary healthcare team to explore a client's beliefs and values about their pet's nutrition and has a positive impact on veterinarian satisfaction, client satisfaction and client adherence.

References

1. Tuckett D, Boulton M, Olson C, Williams A. *Meetings between experts: an approach to sharing ideas in medical consultations.* Tavistock Publications, London, 1985.

2. MacMartin C, Wheat HC, Coe JB. Veterinarian-initiated long-term dietary recommendations: Practitioners' management of clients' responses (poster presentation). *Waltham International Nutr Sci Symp*, Portland, OR, 2013.

3. Baldwin K, Bartges J, Buffington T, et al. AAHA nutritional assessment guidelines for dogs and cats. *J Am Anim Hosp Assoc* 2010;46:285–296.

4. WSAVA Nutritional Assessment Guidelines Task Force. WSAVA nutritional guidelines. *J Small Anim Pract* 2011;52:385–396.

5. American Animal Hospital Association. 2015 AAHA State of the Industry Fact Sheet. Available at: aaha.org/graphics/original/professional/resources/library/aaha_state_of_the_industry_2015_fact_sheet.pdf. Accessed May 15, 2019.

6. Shaw JR, Bonnett BN, Adams CL, et al. Veterinarian-client-patient communication patterns used during clinical appointments in companion animal practice. *J Am Vet Med Assoc* 2006;228:714–721.

7. Shaw JR, Adams CL, Bonnett BN, et al. Veterinarian satisfaction with companion animal visits. *J Am Vet Med Assoc* 2012;240:832–841

8. Coe JB. Communication during veterinarian-client-patient interactions in companion animal practice. PhD thesis, University of Guelph, Canada. 2008.

9. Kanji N, Coe JB, Adams CL, et al. Effect of veterinarian-client-patient interactions on client adherence to dentistry and surgery recommendations in companion-animal practice. *J Am Vet Med Assoc* 2012;240:427-436.

10. Coe JB. It's not what you know – it's what your clients know. Available at: http://veterinarymedicine.dvm360.com/vetmed/article/articleDetail.jsp?id=768618. Accessed May 15, 2019.

11. Coe JB. Taking down the wall: Overcoming communication barriers in the exam room, in *Proceedings*. American Animal Hospital Association Yearly Conference 2013.

12. MacMartin C, Wheat HC, Coe JB, et al. Effect of question design on dietary information solicited during veterinarian-client interactions in companion animal practice in Ontario, Canada. *J Am Vet Med Assoc* 2015;246:1203-1214.

13. Dysart LMA, Coe JB, Adams CL. Analysis of solicitation of client concerns in companion animal practice. *J Am Vet Med Assoc* 2011;238:1609–1615.

14. Adams CL, Kurtz SA. *Skills for communicating in veterinary medicine.* Parsippany, USA: Dewpoint Publishing, 2017.