



Emerging Research: Microbiome & Protein Needs

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The symbiotic relationship that exists between a host species and the microbiota that inhabits the gastrointestinal tract has been well known and appreciated for several decades. In all host species, the commensal microbiota are important in developing and maintaining the gastrointestinal immune system, resisting pathogenic colonization, and fermenting fibrous materials into short-chain fatty acids (SCFAs) that provide energy to colonocytes, reduce pH to limit pathogen growth, and act as secondary messengers that stimulate gut peptide synthesis and initiate other signal transduction pathways.¹⁻³ Dogs and cats evolved as members of Carnivora and have traditionally relied on high-protein, high-fat diets containing relatively low dietary fiber concentrations. Despite having a relatively simple gastrointestinal tract designed to digest this type of diet, dogs and cats harbor a rich microbial community, especially in the large intestine, capable of fermenting a variety of substrates.

Although the indigenous microbiota play many important roles in the gastrointestinal tract, they may also be a burden on the body. Greater mucus production, epithelial cell turnover, and production of immune cells are needed to protect the body from potential pathogens like *Salmonella*, *Campylobacter*, *Listeria*, and some *Clostridium* and *E. coli* strains that commonly contribute to pet illness and/or commercial pet food recalls. Because most of today's pet dogs and cats live inside the home, they serve as a potential vector for pathogen exposure to humans. Recent studies have shown that co-inhabiting humans and pets share microbiota,⁴ that pets may be the source of owner illness,^{5,6} and that antibiotic resistance in pets may affect human therapeutic options.⁷ Given their proximity to humans, similar disease incidence and etiology, and unique metabolism, dogs provide a microbiome research model and such research may not only lead to improved pet nutrition and veterinary care but may also increase our understanding of host-

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microbe interactions, with relevance to human metabolism and diseases/public health at large.

The gastrointestinal microbiota populations are known to be affected by many factors, including diet, life stage, environment, disease status, and medications. Because a variety of gastrointestinal diseases are common in both humans and companion animals, this has been a rich area for research in the recent past. Clinical studies have been conducted to identify pathogens, understand their metabolism and physiology, and develop methods for disease prevention or treatment. The pet food industry has also been actively involved in this area of research. In addition to studying disease, they have developed and tested a variety of dietary strategies to promote gastrointestinal health, including dietary fibers, prebiotics, and probiotics.

A probiotic is a live microbial food supplement that beneficially affects the host by improving its intestinal microbial balance.⁸ In contrast, a prebiotic is a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, thus improving host health.⁹ These definitions and the criteria by which they are evaluated continue to evolve, but the general concepts remain the same.

In addition to measuring the microbes themselves, there is value in measuring the metabolites they produce because they provide useful information about the gastrointestinal environment and microbial metabolism. Fecal, urinary, and blood samples may be useful in

this regard, but most of the current data have been derived from feces. Microbial fermentation of dietary fibers and prebiotics leads primarily to the production of SCFAs (acetate, propionate, butyrate), which possess many beneficial properties. While most gastrointestinal microbes prefer to metabolize carbohydrate-based substrates, others are able to ferment proteins and become more active when it is more abundant in the colon.¹⁰ The amount and type of protein reaching the colon is not only dictated by the dietary protein concentration, but also the ingredient composition, nutrient profile, the amount and type of processing, and digestibility of the diet. The fermentation of protein produces some SCFA, but also a lot of putrefactive compounds including phenols, indoles, ammonia, and biogenic amines that contribute to fecal odor and may be detrimental to colon health.¹¹ Although much of the research in this area has focused on prebiotics and probiotics, the entire ingredient and nutrient composition of the diet, processing method, and nutrient digestibility should be considered.

Since the human microbiome project (HMP) was launched in 2007, the microbiome field has made great research strides. While microbial populations relevant to human health were the main priority, most of the tools, concepts, and data may be applied to companion animal health. In the past, the field was hampered by culture-based methodologies that are often narrow in scope and have poor resolution. Molecular techniques such as quantitative polymerase chain reaction (qPCR), denaturing-gradient gel electrophoresis (DGGE), and

fluorescent in situ hybridization (FISH) began to advance the field 15 years ago. The development of high-throughput DNA sequencing techniques (e.g., 454 pyrosequencing; Illumina sequencing) dramatically changed the research landscape in regard to gastrointestinal

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microbiology. Recent use of these high-throughput techniques have allowed the characterization of the canine and feline gastrointestinal microbiota in terms of phylogeny (i.e., who is there) and functional capacity (i.e., what can they metabolize) and evaluate how it may be affected by diet, age, and disease state.¹²

It is now known that several hundred bacterial phylotypes, predominated by members of Firmicutes, Fusobacteria, Proteobacteria, Bacteroidetes, and Actinobacteria, inhabit the dog and cat gastrointestinal tract. Recent studies have revealed that the functional capacity of the gastrointestinal

microbiota in dogs and cats is quite broad and similar to that of humans and rodent models.¹³ Although these populations are quite stable over time, our laboratory has demonstrated that macronutrient profile (e.g., dietary protein: carbohydrate ratio), dietary fiber amount and type, and the form of food consumed (e.g., raw vs. extruded diets) may have dramatic effects on the

gastrointestinal microbiome of these host species.¹⁴⁻¹⁶ These dietary factors have not only been reported to impact microbial diversity and richness, gene content, and metabolic activity, but to alter host physiology and metabolism as well. Similar work in rats has shown how meat-, dairy-, and plant-based protein sources may affect gastrointestinal microbiota populations.^{17,18}

Despite all that has been learned over the past decade, the physiological relevance and long-term health implications of such changes are still not well understood. While recent studies may already allow us to feed and treat pets more effectively, continued research emphasis will be needed for years to come.

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