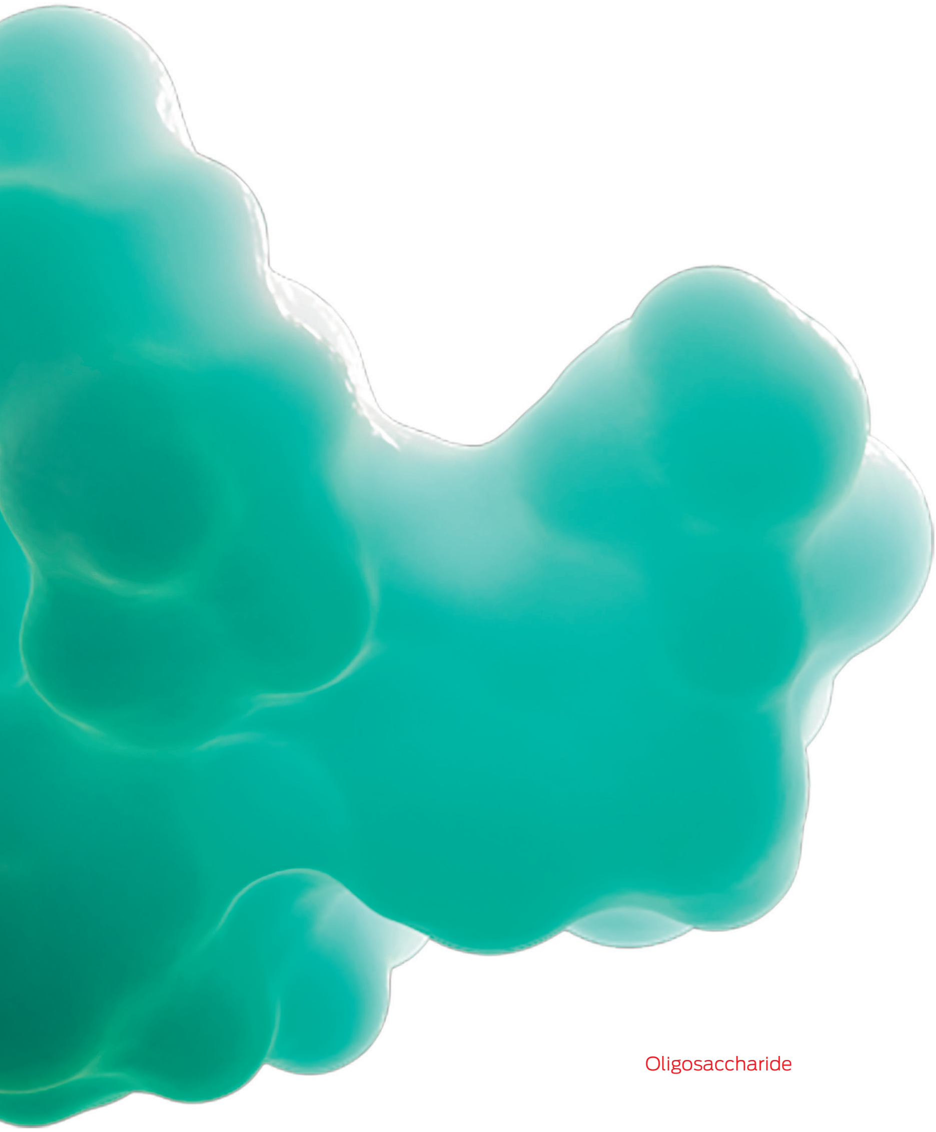
Purina Institute

Advancing Science for Pet Health

PREBIOTICS:
Effects On Gastrointestinal And Host Health

The gut microbiome plays a crucial role in host health through its impact on digestion, metabolism, and immunity.[1] The large intestine houses a diverse and complex microbial ecosystem that adapts in response to substrate availability, pH, oxygen levels, and other variables.[1-3] The majority of the bacteria in the colon are anaerobic species capable of utilizing nutrients, such as dietary fiber and protein, that escape digestion in the upper gastrointestinal tract.[2,4] While there are numerous influences on microbiome composition, nutritional intervention provides a daily opportunity to influence the health of the microbiome – and ultimately, the health of the host.



Oligosaccharide

CONTENTS

DIETARY FIBERS

Dietary fibers are generally composed of structural components and carbohydrates derived from plants and fungi.[5] They escape digestion in the upper gastrointestinal tract because the host lacks the digestive enzymes to break them down.[5,6] The physical properties of dietary fibers vary widely, and even minor variations influence the fermentability and physiological effects.[7,8]

Dietary fiber for cats? While plants (and therefore, plant fibers) are not typically present in the natural diet of cats and other carnivores, they can offer distinct physiological benefits for cats.[7,10-19]

The consumption of dietary fiber results in extensive metabolic interactions among the microorganisms in the large intestine.[1] Saccharolytic bacteria in the colon obtain carbon and energy through the fermentation of certain carbohydrates and produce end products – such as short-chain fatty acids (SCFA) – that provide benefits to the host.[4,9]

PREBIOTICS: NOT ALL DIETARY FIBERS ARE EQUAL

The concept of prebiotics was first described in 1995 by Gibson & Roberfroid[2] as "non-digestible food ingredients that beneficially influence the health of the host by stimulating the activity of one or more commensal colon bacteria." The definition has been revised since that time,[1,4,6,20,21] with the current definition defined by the International Scientific Association for Probiotics and Prebiotics (ISAPP) as "substrates selectively used by micro-organisms of the host conferring a health benefit."[21-23]

Not all dietary fibers have prebiotic effects but the majority of prebiotics are non-digestible (by the host), fermentable dietary fibers.[1,20,22] Most prebiotics are carbohydrates – more specifically, oligosaccharides.[6] To date, only inulin-type fructans (ITF) and galactans meet all of the ISAPP prebiotic criteria.[21,22,24] However, a number of substances, including non-plant sources, possess prebiotic potential and are considered "candidate prebiotics"– including human milk oligosaccharides, yeast-based substances and non-carbohydrates (e.g., polyphenols, fatty acids, herbs and some micronutrients).[20,22,24,25]

According to the International Scientific Association for Probiotics and Prebiotics (ISAPP), a substance must meet 3 criteria in order to be considered a prebiotic:[6,20-23]

- Resistant to digestion in upper GI tract
- Fermentable by microbiota
- Specifically stimulate growth and/or activity of beneficial bacteria

In addition, the Food and Agriculture Organization of the United Nations (FAO) states the substance must also be safe based on traditional studies and has to be ingested in a plausible daily amount to have the prebiotic effect.[20]

Commonly used pet food ingredients with prebiotic activity or potential

- Arabinoxylan-oligosaccharide (AXOS)
- β-glucans
- Fructans (e.g., inulin, oligofructose [OF] and fructooligosaccharides [FOS])
- Citrus pulp
- Cranberries

- Galactooligosaccharide (GOS)
- Lactulose
- Mannanoligosaccharide (MOS)
- Pectin
- Psyllium (including seed husk)
- Pumpkin
- Soybean oligosaccharides (e.g., raffinose, stachyose, verbascose)
- Spent brewers' grains
- Wheat aleurone

Fructans

Fructans were the first prebiotics identified and used as food ingredients and include inulin, oligofructose and short-chain fructooligosaccharides.[2,21] Inulin and oligofructose can be naturally found in agave, artichokes, asparagus, bananas, chicory root, garlic, onions, leeks and wheat.[1,21] They are composed of linear or branched fructose chains, usually with terminal glucose units.[6]

Figure 1:
Chicory root is approximately 55% inulin

Inulin is a long-chain carbohydrate consisting of fructose units and is most commonly extracted from chicory root,[2,6,14,21,26] which is approximately 55% inulin.[27,28]

Oligofructose **(OF)** is a shorter-chain carbohydrate composed of fructose units.[2,6,14,21] It can be extracted from plants or produced by partial enzymatic hydrolysis of inulin.[21]

Short-chain fructooligosaccharides **(scFOS)** are the shortest fructose-based chain carbohydrates in this category.[21] Although they can be extracted from plants, they are more commonly synthesized from sucrose and fructose using an enzymatic process.[21]

Galactans

Galactooligosaccharides **(GOS)** are synthetic lactose-based oligosaccharides, some of which are derived from lactulose.[6]

Other substances with prebiotic potential

Beta-glucans **(β-glucans)** are glucose-based polysaccharides that comprise major structural components of the cell wall of yeasts, fungi and some bacteria and can also be found in barley and oats.[29]

Mannanoligosaccharides **(MOS)** are derived from the cell wall of the yeast *Saccharomyces cerevisiae*.[28]

Figure 2:
Wheat aleurone

Psyllium is derived from the seeds of *Plantago ovata* and predominately consists of highly-branched arabinoxylan.[30]

Pumpkin fiber may contain rhamnogalacturonans, arabinoxylans, xyloglucans, xylogalaturonans, galacturonic acid, galactoglucomannans, and pectins.[31,32]

Wheat aleurone is the innermost layer of wheat bran and is composed of approximately 65% arabinoxylan and 29% β-glucans.[33,34]

Xylooligosaccharides **(XOS)** are polymers of xylose.[19]

Ease of handling and the ability to withstand processing and storage give prebiotics a distinct advantage in pet food manufacturing.[35]

VARIABILITY IN PREBIOTICS

The activity of a prebiotic is affected by its physicochemical structure. The degree of polymerization (DP) is based on the chain length of the fiber.[2] The original source, DP, chemical bonds, and the amount of branching of the fiber affects the ability of microorganisms to utilize it as an energy source.[1,5,21] For example, a short-chain oligosaccharide (low DP) with minimal branching will be fermented more quickly than a longer-chain (higher DP) fiber with a more complex structure. Even different prebiotics from the same prebiotic category (e.g., fructans) can have different effects based on differences in chemical structure.[21,36,37]

Factors determining variability in response to prebiotics:

- Host species- and individual-level variations in gut microbial populations[1,6,18,21]
- Structure (DP, branching) of the prebiotic[1,6,7,21]
- Base diet[21,36,41]
- Amount of prebiotic consumed[1,7,14,19,21,27,36,42-47]
- Duration of administration[18,21,46,48]

The diversity of bacterial species in the gut microbiome is associated with variability in the microbiome's ability to metabolize prebiotics.[1,6,21] Saccharolytic bacteria possess enzymes that allow them to metabolize a wide variety of carbohydrates.[1] Some "generalist" species have many enzymes that allow them to metabolize a number of complex carbohydrates, while more "specialist" species can only utilize one or a few shorter-chain carbohydrates.[1,5,6] Even bacterial species within the same genera can differ in their ability to degrade fiber sources.[1] As a result of this variation, as well as individual variation in microbiota populations, the fermentation of different prebiotics can produce different amounts and ratios of fermentation products (such as short-chain fatty acids, SCFAs) – resulting in potentially different physiological effects of the same prebiotic between hosts.[7,16,37-39]

A direct comparison of the effects of different prebiotics in dogs or cats is difficult due to wide variation in the base diets; type, duration and amount of prebiotic consumed; and methods of evaluation.[21,37,40]

COMBINING PREBIOTICS

Because they provide variable fermentation rates and prebiotic compositions, fiber blends may offer complementary or synergistic benefits beyond those provided by individual prebiotics.[20,28,46,49] Providing diverse fibers could provide metabolic support for a more diverse range of microbes – both generalists and specialists.

However, it is also possible that component prebiotics may compete for fermentation by microbiota, resulting in mixed effects or even diminished effects; therefore, as with probiotics, potential prebiotic blends should be evaluated for their efficacy in the target species.[28]

PHYSIOLOGICAL EFFECTS OF PREBIOTICS

Prebiotics can exert direct and indirect effects on gut health.

Selective enhancement of beneficial microbiota

The fermentation of prebiotic fibers is the result of complex interactions between multiple bacterial species in the colon,[9] and the microbiome population and function adapt in response to changes in the available energy sources (substrates) for the bacteria.[45,50] Prebiotics provide ample substrate and create a favorable environment for saccharolytic bacteria – such as *Bifidobacterium* and

Lactobacillus species – that are known to have health benefits for the host.[6,22,23,51,52] This activity is considered a primary function of prebiotics, and is one of the three ISAPP criteria.[22,47] Enhancing the growth of beneficial bacteria can result in enhancement of their benefits – such as increases in butyrate production as well as B vitamin production and bile acid conversion.[8,9,21-23,51]

Short-chain fatty acid production

Many benefits of prebiotics result from the effects of SCFAs produced during microbial fermentation.[22,23] The primary SCFAs formed are acetate, propionate and butyrate. SCFAs impact many molecular and cellular processes and play important roles in gut health as well as host health.[5,21,23]

SCFA generation is dependent on the available substrates, microbial composition, and intestinal transit time.[9,53] SCFAs interact directly with intestinal epithelial cells and immune cells to modify cellular processes, gene expression, and cellular differentiation, proliferation and apoptosis.[23]

Purported effects of SCFAs include:

- Energy substrate for intestinal epithelial cells (butyrate)[1,4,14,26,38,43,49,54,55]
- Increase absorptive capacity through stimulation of colonocyte proliferation[54]
- Increase expression of antimicrobial peptides (e.g., defensins)[23]
- Strengthen epithelial barrier function through induction of tight junction proteins[23]
- Modulate cellular processes in colonocytes and immune cells, including gene expression and cellular differentiation, proliferation and apoptosis[23]
- Stimulate activity of antioxidant enzymes such as glutathione S-transferases[20]
- Modulation of vagal nerve activity[55]
- Anti-inflammatory actions[5]
- Improve bioavailability of calcium and magnesium[54]

SCFAs readily diffuse through enterocytes into the bloodstream, facilitating their potential effects on glucose and lipid metabolism as well as on distant organs such as the lungs, skin, and brain.[5,6,23,55]

Reduced intraluminal pH

Fermentation of prebiotics results in the production of acetate, lactate and other acids that lower the intraluminal pH. This provides a dual benefit of a more supportive pH for butyrate-producing beneficial bacteria while creating an unfavorable environment for acid-sensitive potential pathogens.[1,2,4,6,17,56] The reduced pH affects bacterial enzyme activity,[4] stimulates mucin production,[56] and influences intestinal motility.[4]

Pathogen inhibition

While selective enhancement of beneficial bacteria is one of the three ISAPP criteria for prebiotics, some studies have demonstrated reductions in potentially pathogenic bacteria (such as *Clostridium perfringens* and *Escherichia coli*).[6,10,20,27,57-59]

Pathogen inhibition resulting from prebiotics can result from several mechanisms, including:

- Selective enhancement of beneficial microbiota that are able to utilize the available substrate and outcompete potential pathogens[16,17,20,59]
- Production of substances (such as bacteriocins and antimicrobial peptides) that are directly inhibitory to pathogens[2,6,47,49,59]
- Direct inhibition of pathogen adherence, implantation and translocation[23,41,47,60,61]
- Some oligosaccharides may structurally mimic intestinal epithelial cell receptors and serve as decoys to prevent pathogen binding[59,60]

Immunomodulation

Prebiotics may exert immunomodulatory effects.[37,47,49,62,63] Different prebiotics, as well as different levels of the same prebiotic, may exert different (or no) impacts on the immune system.[37,47,49,51,62]

The immunomodulatory effects of prebiotics are largely mediated by SCFA,[63,64] and include:

- Production of stimulatory cytokines and chemokines that influence immune cells[56]
- Induction of anti-inflammatory cytokine production coupled with inhibition of pro-inflammatory cytokine production[23]
- Production of substances that stimulate cytokine production, mononuclear cell proliferation, macrophage phagocytosis, and immunoglobulin production[23,47]
- Increase ileal IgA concentrations to enhance mucosal immunity[59]

Protein fermentation and nitrogen balance

Microbial proteolytic metabolism in the colon can produce beneficial products (such as propionate), but is also associated with the production of potentially toxic, putrefactive compounds (e.g., ammonia, phenols, thiols, biogenic amines).[21,54] Reducing protein fermentation by shifting the environment to favor saccharolytic fermentation reduces concentrations of undesirable metabolites.[24,54] An additional benefit of reducing putrefactive compounds is a potential reduction in fecal odor in dogs[26,57] and cats.[10,58] Fructans such as FOS have been associated with a shift of nitrogen elimination from urine to the colon,[13,14,65] where urease-positive microbes metabolize urea to ammonia for incorporation into bacterial proteins.[14] This may benefit patients with renal impairment.

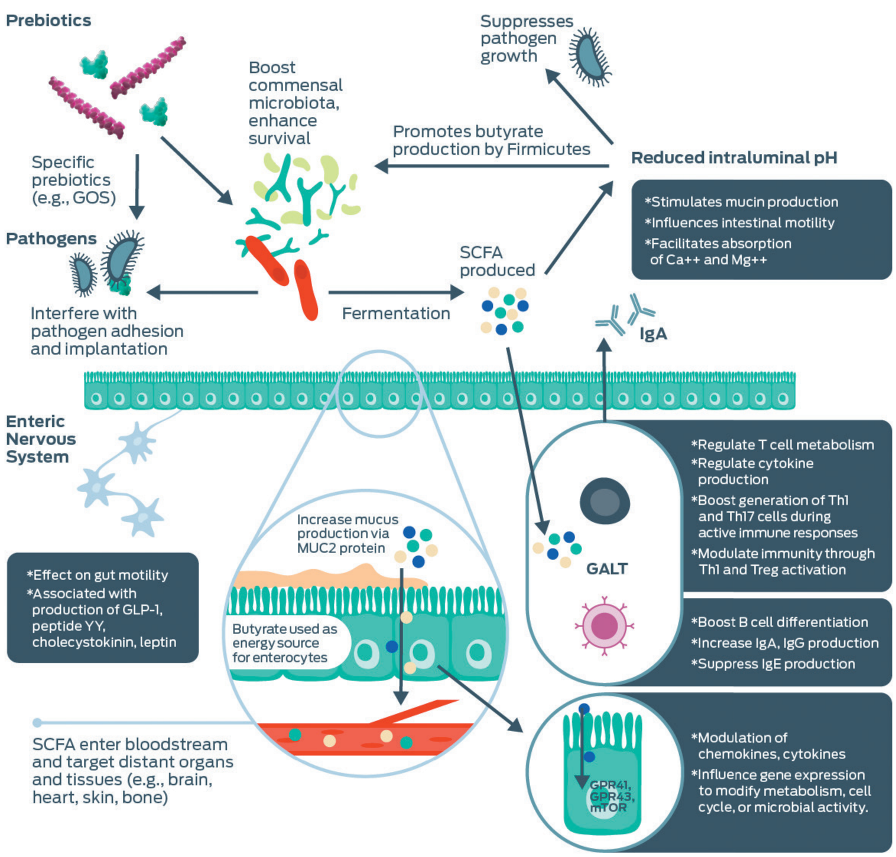


Figure 3:

Purported mechanisms of action of prebiotics. Interindividual variation in the microbiome, the base diet, and the type, inclusion level and duration of prebiotic administration may all influence the ultimate effects of the prebiotic.

Other effects

Additional purported benefits of prebiotic administration include antioxidant activities[66] and improved calcium and magnesium absorption.[4,37,67]

Adverse effects of prebiotics have been infrequently reported, and are most likely the result of osmotic effects or excessive fermentation associated with higher levels of prebiotic inclusion.[6,7,12,14,16,20] Potential adverse effects include diarrhea, bloating, abdominal discomfort, and flatulence.[20]

POTENTIAL CLINICAL BENEFITS OF PREBIOTICS

Numerous published studies have demonstrated the safety and impact of prebiotics in healthy animals, but translation of these results to diseased or dysbiotic animals is difficult and more research is needed to evaluate the clinical benefits of specific prebiotics and prebiotic blends.[40]

Preventive gastrointestinal health

Prebiotic-induced increases in intestinal villi height and absorptive capacity, enhancement of beneficial bacteria, and reductions in fecal putrefactive catabolites may facilitate intestinal health and absorptive capacity.[16,26,68,69] Reduced fecal putrefactive catabolites can lead to reduced fecal odor in dogs[26,57] and cats,[10,58] which may improve pet owner satisfaction.

Gastrointestinal conditions

Due to their antioxidant and anti-inflammatory properties, prebiotics may play a role in the management of conditions in which oxidative stress and inflammation play roles in pathogenesis (e.g., enteritis).[3,20] By selectively enhancing beneficial microbiota and directly or indirectly inhibiting pathogenic microbes, prebiotics may improve microbial balance and help mitigate dysbiosis. Through their beneficial impacts on barrier function and intestinal motility, coupled with reductions in putrefactive byproducts, prebiotics may help reduce the risk of infection.[4] Certain prebiotic fibers, such as psyllium[30] and AXOS[54] may provide anti-constipation benefits.

Prebiotic functions that may help veterinarians manage gastrointestinal conditions include:

- Antioxidant activity
- Anti-inflammatory properties
- Enhance barrier integrity
- Enhance beneficial bacteria
- Inhibit pathogens
- Reduce putrefactive byproducts
- Improve intestinal motility
- Enhance IgA production
- Immunomodulation

Synbiotic combinations

Synbiotics are combinations of prebiotics and probiotics; the beneficial prebiotic effects may be enhanced if they are used in combination with probiotics, and the presence of prebiotics may enhance the benefits of probiotic strains.[37,56,70] Complementary synbiotics are composed of prebiotic fiber(s) and probiotic(s) that each have demonstrated health benefits and function independent of each other to provide a host benefit.[24] Synergistic synbiotics contain probiotic(s) and accompanying prebiotic(s) that serve as the fermentable substrate for the probiotic to facilitate and enhance its survival and beneficial functions.[24] In synergistic synbiotics, the prebiotic and probiotic components may or may not have independent health benefits for the host.[24] Synbiotic combinations may have different effects from those of the prebiotic or probiotic administered independently, and may not always be complementary or synergistic;[71] therefore, synbiotics should be evaluated for safety and efficacy in the target species.

Metabolic support

Numerous published studies have demonstrated potential benefits of prebiotics for weight management for pets. Prebiotic fibers improve satiety and reduce voluntary food intake;[29,68,72] improve glucose homeostasis[29,38,68,73] and lipid metabolism;[29,35,73] and attenuate systemic inflammation[55] and oxidative stress.[34]

Prebiotic functions that may help with weight management include:

- Improve satiety
- Reduce voluntary food intake
- Improve glucose homeostasis
- Improve lipid metabolism
- Anti-inflammatory properties
- Attenuate oxidative stress

Immune support

The immunomodulatory actions of prebiotics have, to date, been primarily established as improved immune indices in healthy animals and vary based on the prebiotic evaluated[35,47,49,51] but may indicate opportunities for nutritional intervention to improve immune health.

Dietary supplementation with scFOS in pregnant dogs after the 35th day of gestation resulted in significantly higher IgM levels in colostrum and milk as well as a trend toward higher concentrations of anti-Bordetella IgM in the puppy's nasal secretions two weeks after vaccination.[74]

Other potential effects

Prebiotics, either through direct effects or SCFA production, may have additional beneficial impact on host health.[64,75] Potential indications include allergies,[53] colorectal neoplasia,[76] atopic dermatitis and skin health,[22,24,75] cardiovascular disease,[20,75] skeletal health,[20] and mental health/cognition.[20,22,75,77] Further research is needed to determine the benefits of prebiotics for these conditions in dogs and cats.

Nutritional interventions provide opportunities for manipulating the microbiome to create health benefits for the microbiome and the host. SCFAs play critical roles in gut and host health, and prebiotics are known enhancers of SCFA production. Prebiotics offer numerous benefits through enhancing beneficial bacteria, inhibiting potential pathogens, reducing potentially harmful microbial byproducts, and modulating immunity and inflammation.

Inulin

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