WHITE PAPER

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# Dietary biotics

Strategies to optimize pet intestinal health and wellbeing

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## Key learnings

- Intestinal gut microflora is integral to overall health and well-being in companion animals.
- Pre-, pro-, and postbiotics can support gut-related disorders by modifying the microbiome or enhancing its functions.
- Prebiotics selectively fuel beneficial gut bacteria, contributing to:
  - Pathogen agglutination
  - Enhanced microbiome diversity and immune modulation
  - Improved mineral absorption, bowel function and metabolic balance
- Probiotics promote gut health through multiple actions, including:
  - Producing short-chain fatty acids (SCFAs) and antimicrobial compounds
  - Supporting digestion via enzyme production
  - Modulating immune function
- Postbiotics provide non-living metabolites of probiotics that:
  - Provide antimicrobial compounds
  - Stimulate beneficial bacteria
  - Support digestion and immune function
- Product efficacy varies. While some biotics have validated benefits, not all products on the market are supported by scientific validation.

# Nutrition and trends in the pet food market

Over the next decade, the pet food sector is expected to experience steady growth due to increased pet ownership rates and increased concern for pet health and nutrition.

Nutrition is crucial for maintaining the health of companion animals, as malnutrition or overnutrition can lead to gastrointestinal, renal and dermatological health issues as well as obesity and diabetes. The drive to create healthier pet foods to prolong companion animals' lifespan and improve their quality of life is accelerating at pace, with an emphasis on developing novel offerings.

The increasing popularity of immune health products in humans has led to a new focus on immune health in pet products. This humanization trend is a key driver of growth in pet nutrition and will certainly accelerate even further in the coming decade.

# The role of intestinal health and the pet microbiome

Gut health and its management is an intricate and complex area governed by numerous factors including nutrition, microbiology, immunology and physiology. When gastrointestinal (GI) health is compromised, nutrient digestion and absorption are affected and susceptibility to disease is heightened. Ultimately, these can negatively impact the overall health and well-being of our companion animals.



The overall community of microorganisms in the gut is referred to as the microbiome and is recognized as a very diverse community of microbes, all of which play a pivotal role in nutritional, physiological and immune functions. Within the GI tract, there are multiple interactions between the host, intestinal environment and microbial cells in addition to dietary components. These interactions underline the critical role of the microbiome in health and well-being, although the exact way in which this is achieved is not yet fully understood.

*"When gastrointestinal (GI) health is compromised, nutrient digestion and absorption are affected and susceptibility to disease is heightened."*

The bacteria present in the microbiome can be categorized as either commensal or pathogenic.

Commensal bacteria are not disease-causing invaders but have made the host their home. Through co-evolution, these commensal bacteria have established an impressive mutualistic relationship, in which both microbes and their animal host depend on each other for survival. Microbes perform many basic physiologic and metabolic functions, as well as influencing the type and robustness of host immune responses. The immune effects of commensals are subtle because they do not cause any overt change in the health state of the host. Rather, they help maintain and regulate the host's healthy immune steady state.

Pathogenic bacteria colonize the host, but do not cause disease when the microbiome is in balance. However, they can multiply and cause disease if microbiota or host immune homeostasis are perturbed, for example, after antibiotic treatment or during intestinal inflammation. Pathogenic bacteria typically adhere to intestinal cells, which enables them to invade the cells and proliferate. Pathogenic bacteria often produce toxins that alter metabolism of the host cells and help invasion of the host, resulting in an increase in disease severity.

The makeup of the microbes within the microbiome plays a critical role in gut health, with beneficial microbes forming a protective barrier lining the gut that prevents the growth of pathogenic bacteria such as *Salmonella, Campylobacter, Clostridia* and *Escherichia,* among others. There are numerous theories on how the beneficial microbes prevent pathogen colonization. Some theories suggest that potential attachment sites on the gut cells become occupied, thereby reducing the opportunity for attachment and colonization by pathogens. Another proposed mechanism is that the intestinal microbiota secretes compounds such as short-chain fatty acids (SCFAs), organic acids, and natural antimicrobials that either inhibit the growth of, or make the environment unsuitable for, less favorable bacteria. The use of nutritional ingredients to regulate both commensal and pathogenic bacteria, thus maintaining and supporting microbiome health, is increasingly recognized as crucial to overall health and well-being.

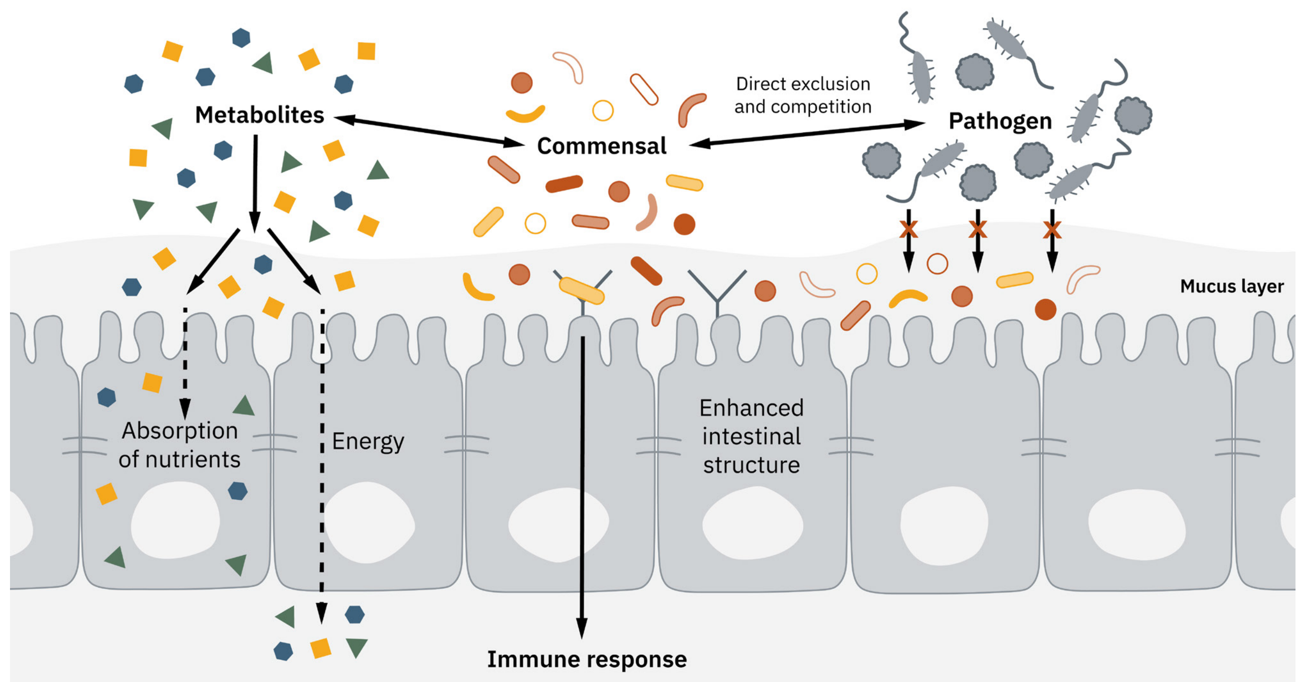



**Figure 1. Intestinal microbes influence health and well-being**

## Strengthening the resilience of the pet microbiome

Profiling and understanding the role of intestinal microbial communities is important for the development and understanding of new and existing food and dietary additives, thus allowing the manipulation of diets to improve overall health and well-being. Using techniques based on molecular sequencing technologies, difficulties associated with cultivating intestinal bacteria have been overcome, and this has provided detailed insights into the malleable nature of the microbiome. In recent years, a focus has been placed on deciphering the complex links between diet, the microbiome and intestinal health. In particular, the role of overall microbial species richness and diversity in influencing health has come under increasing scrutiny. Nutritional interventions can enhance microbiome species richness and diversity, normalizing gut function through a process of microbial repair and rehabilitation (**Figure 2**).

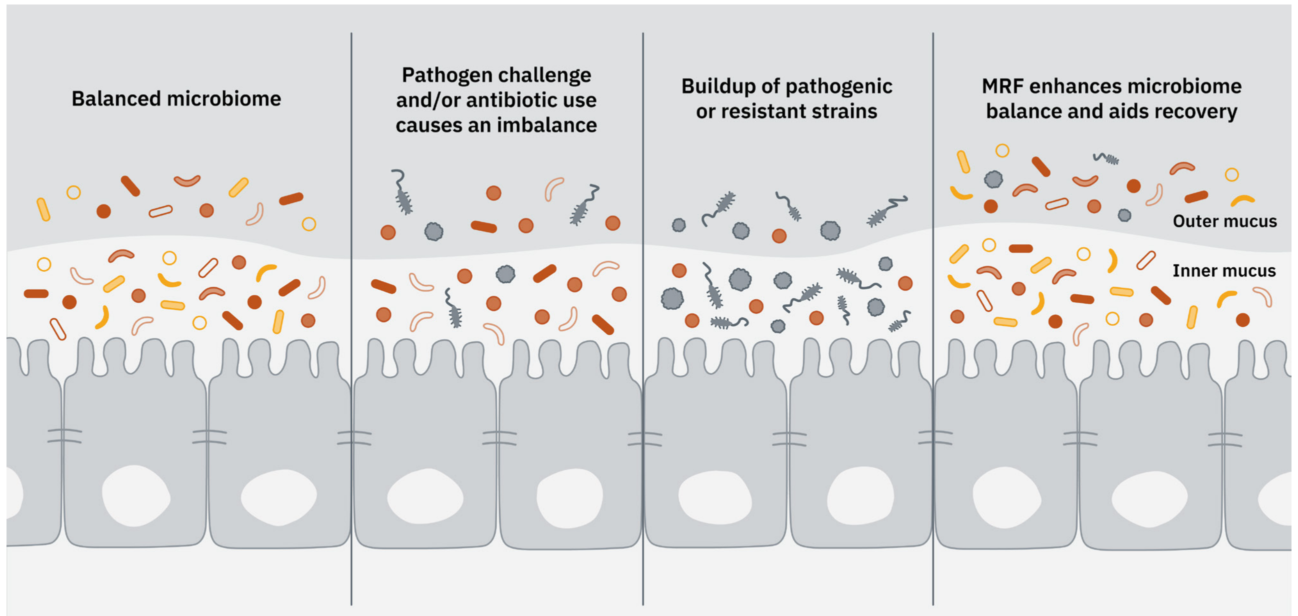



**Figure 2. Microbial diversity: The key to gut health**

## Nutritional strategies for supporting intestinal health

From a nutritional standpoint, there are many food supplements focused on stabilizing the gut microflora to aid intestinal health and decrease our companion animals' susceptibility to disease. The increased use of prebiotics and/or probiotics, via dietary supplements that focus on supporting gut health or repairing gut microflora following intestinal upset, has become progressively more popular. Broadly speaking, pre- and probiotics are the most commonly used nutritional supports for gut health. As with all aspects of nutrition, the ever-evolving role of nutritional supplementation has led to the development of newer "biotics" such as postbiotics, synbiotics and even paraprobiotics. Each can play a role in supporting intestinal function and the gut microbiome, with the goal of enhancing overall health and well-being.

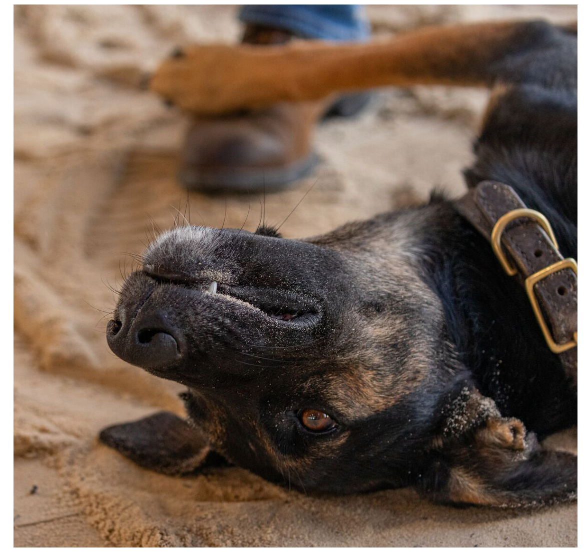
> *Each [biotic] can play a role in supporting intestinal function and the gut microbiome, with the goal of enhancing overall health and well-being.*



## Prebiotics: First line of support for pet health

The concept of prebiotics originated in the early 1990s, when they were defined as indigestible ingredients that weren't digested or absorbed by the gastrointestinal tract. In 2016, the International Scientific Association for Probiotics and Prebiotics redefined prebiotics as "a substrate that is selectively utilized by host microorganisms conferring a health benefit." This new definition of prebiotics expands its scope to include non-carbohydrates, and the site of action is not limited solely to the gastrointestinal tract, nor is its type limited to food.

In general, prebiotics transit the GI tract intact to the large intestine, where they are degraded by the intestinal microflora. The main benefit of prebiotics is their ability to stimulate the growth of beneficial bacteria that compete with other species and in doing so produce beneficial fermentation products (such as SCFAs) that are absorbed by the intestine or transported to the liver.



These metabolites can have beneficial effects such as regulating immunity, controlling pathogens, improving intestinal barrier function, increasing mineral absorption, and lowering blood lipid levels. The most abundant SCFAs in the intestine, including acetate, butyrate and propionate, can also be metabolized by beneficial bacteria, enabling maintenance of intestinal and systemic health.

The possible mechanisms by which prebiotics promote health benefits are shown in **Figure 3**. In addition, prebiotics have a protective effect not only on the gastrointestinal system but also on other parts of the body, such as the central nervous, immune and cardiovascular systems.

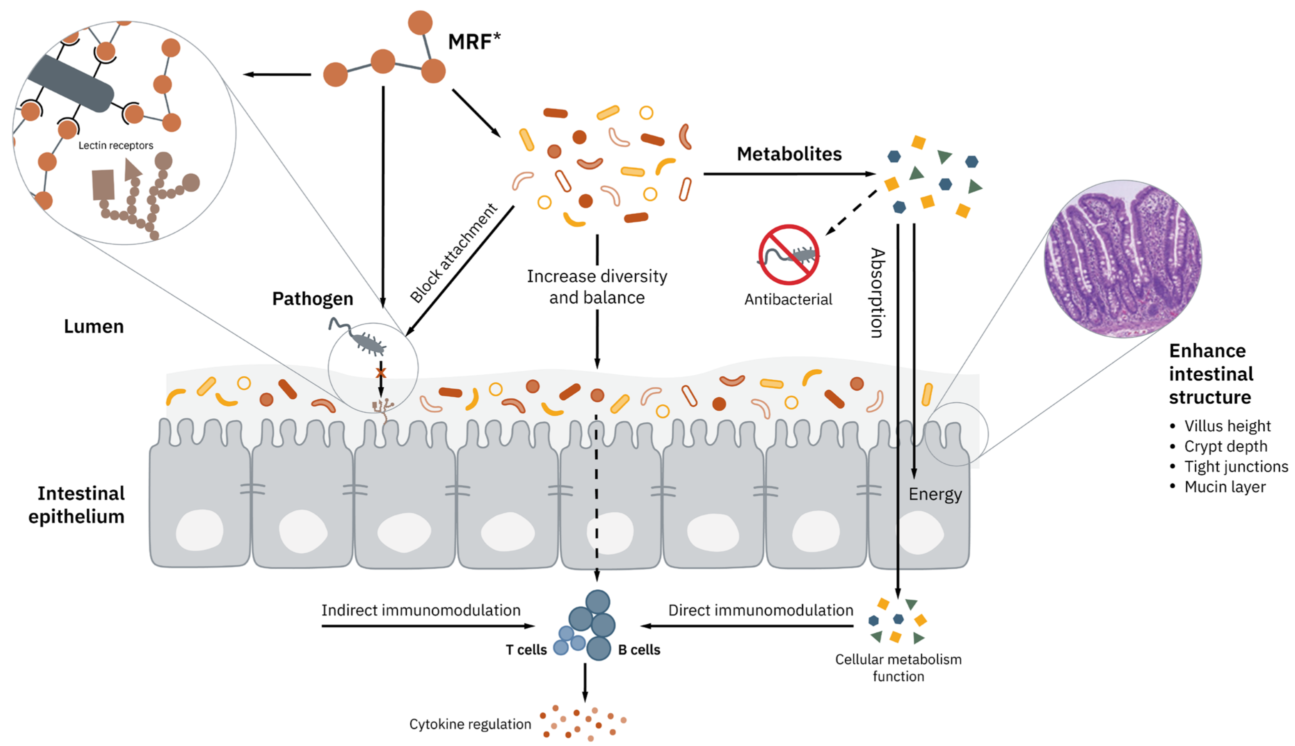



**Figure 3. Prebiotic modes of action**

**MRF: Mannan-rich fraction*

# Understanding different types of prebiotics

Previous studies have considered prebiotics to be oligosaccharide carbohydrates, including mannan-oligosaccharides (MOS), xylo-oligosaccharides (XOS), galacto-oligosaccharides (GOS) and fructose-oligosaccharides (FOS). Newer additions to this class include polysaccharides, polyphenols and polypeptide polymers.

With respect to inclusion in the diet, prebiotics are often described as being "food for bacteria." The goal when supplementing diets with prebiotics is to provide components that will not only increase the populations of good bacteria in the gut but also directly control pathogenic species that may take hold in the GI tract.

**Table 1** highlights some of the main differences between materials that have been classically considered prebiotics and illustrates that not all prebiotics are the same. Moreover, within each group (MOS, XOS, FOS, etc.) one can find differences in how individual products act in the body.

**Table 1. Source-dependent prebiotic differences and impacts**

| | Inulin | FOS | MOS | MRF |
|---|---|---|---|---|
| Developed through nutrigenomic studies | X | X | X | ✓ |
| Branched structure | X | ✓ | ✓ | ✓✓ |
| Heat stability | X | X | ✓ | ✓✓ |
| Positive microbiome impact | ✓ | ✓ | ✓ | ✓✓ |
| *Salmonella* and *E. coli* agglutination | X | X | ✓ | ✓✓ |
| Broad-spectrum pathogen control | ✓ | ✓ | ✓ | ✓✓ |
| Decreases gut inflammation | ✓ | ✓ | ✓ | ✓✓ |
| Potential to negatively trigger immune response | ✓ | ✓ | X | X |
| Potential to enhance vaccine response | ? | ? | ✓ | ✓✓ |
| Reduces leaky gut | ✓ | ? | ✓ | ✓✓ |

*Fructose-oligosaccharides (FOS), mannan-oligosaccharides (MOS), mannan-rich fraction (MRF)*

# Prebiotics for managing harmful pathogens

Bacterial adherence to host tissue is an important initial step enabling gastrointestinal tract colonization and, in the case of pathogens, infection. Adherence typically involves the interaction of complementary molecules on the surface of a bacterium with those of the host epithelium.

Historically, the first adherence specificity recognized in intestinal bacteria involved binding via mannose-selective receptors. Almost all isolates of *E. coli,* as well as other members of the Enterobacteriaceae, such as *Enterobacter, Klebsiella, Shigella* and *Salmonella,* attach to mannose receptors by means of type 1 fimbriae.

Attachment of type 1 fimbriae to D-mannose receptors can be blocked by mannose-containing receptor analogs that act like decoy molecules, thereby stopping pathogen attachment to the intestine. From a nutritional standpoint, there are many food supplements focused on pathogen adhesion and GI tract exclusion, with the most commonly used being prebiotics such as yeast cell wall mannan oligosaccharides (MOS). These are complex mannose-containing preparations that are linked to a protein group.

The use of MOS to protect and enhance gastrointestinal health stemmed from research that focused on the ability of mannose, the pure single unit of the complex sugar in MOS, to control and prevent the risk of *Salmonella* colonization in the intestinal tract. Subsequently, distinct forms of mannose-type sugars were found to interact differently with type 1 fimbriae, and it was noted that the α-1,3 and α-1,6 branched mannans present in the cell wall of *Saccharomyces cerevisiae* were particularly effective. Based on the in vitro findings, applied research trials determined that the inhibition and reduction of *Salmonella* colonization could be controlled in vivo. Further research has demonstrated the efficacy of such control strategies in a wide range of species, including companion animals.



Within the cell wall of *Saccharomyces cerevisiae* there are two main locations where MOS is found: attached to cell wall proteins as part of –O and –N glycosyl groups, or as components of larger α-D-mannanose polysaccharides. These larger mannose-containing polysaccharides consist of α-(1,2)- and α-(1,3)-D-mannose branches, which are attached to extended α-(1,6)-D-mannose chains. Yeast MOS, given their capability to bind type 1 fimbriae on the surface of bacterial membranes, act as a front-line defense mechanism through their pathogen adhesion capabilities (**Figure 4**).

MOS are widely used in animal nutrition, given their well-documented ability to bind and limit the colonization of gut pathogens. They have proven to be an effective solution for antibiotic-free livestock diets, as well as providing support for immunity and digestion, leading to notable improvements in health and well-being.

> ***"The main benefit of prebiotics is their ability to stimulate the growth of beneficial bacteria that compete with other species and in doing so produce beneficial fermentation products."***

As first-generation variants, most commercially available MOS products are derived from the cell wall of the yeast *Saccharomyces cerevisiae.* Further research into yeast mannans has focused on fractionation of the yeast cell wall and the isolation of a mannose-rich fraction (MRF). This "second-generation" product can best be described as an enhanced MOS-type product and has been demonstrated to have capabilities beyond simple bacterial adherence and agglutination. MRF has been documented to have enhanced microbiome-modulating capabilities, with the ability to increase the richness and diversity of the microbiome. MOS products, which have been used widely in companion animal diets over the past 20 years, are now being superseded by the next generation, MRF products.

## MOS and MRF adsorb pathogenic bacteria

Agglutination: Front-line defense

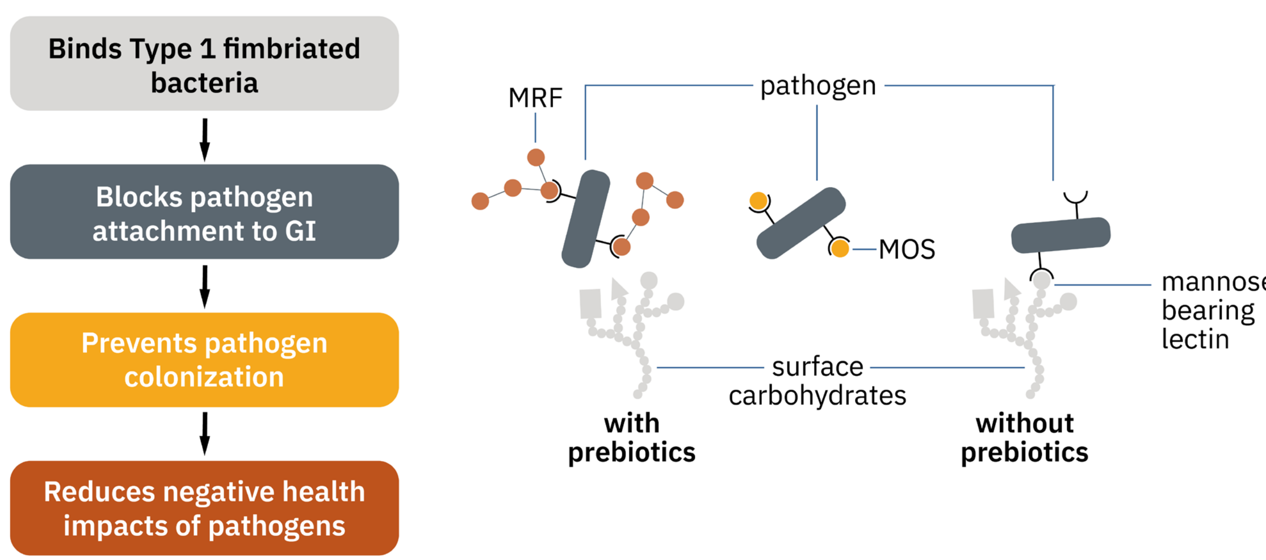



**Figure 4. Yeast mannan-oligosaccharides adsorb pathogenic bacteria**

Salmonellosis, as a disease, requires efficient overall controls, including dietary measures. The variable nature of *Salmonella* serotype prevalence is well documented, and in the highly regulated environment of pathogen control, information on the occurrence of individual *Salmonella* serotypes is readily available.

> ***MRF has been documented to have enhanced microbiome-modulating capabilities, with the ability to increase the richness and diversity of the microbiome.***

The heat map below (**Figure 5**) presents recent data with respect to *Salmonella* serotypes isolated from dogs, with green representing low prevalence and red indicating high prevalence. These heat maps highlight some interesting features associated with *Salmonella* occurrence in dogs. Firstly, one can appreciate the highly variable nature of serotype recovery between 2021 and 2022. Of more interest, however, are the quite striking year-on-year changes in *Salmonella* serotype prevalence. From a pathogen control viewpoint, this presents a challenge, in that any *Salmonella* control mechanism needs to be "broad spectrum" to account for not only the variable nature but also the temporal changes in *Salmonella* abundance.

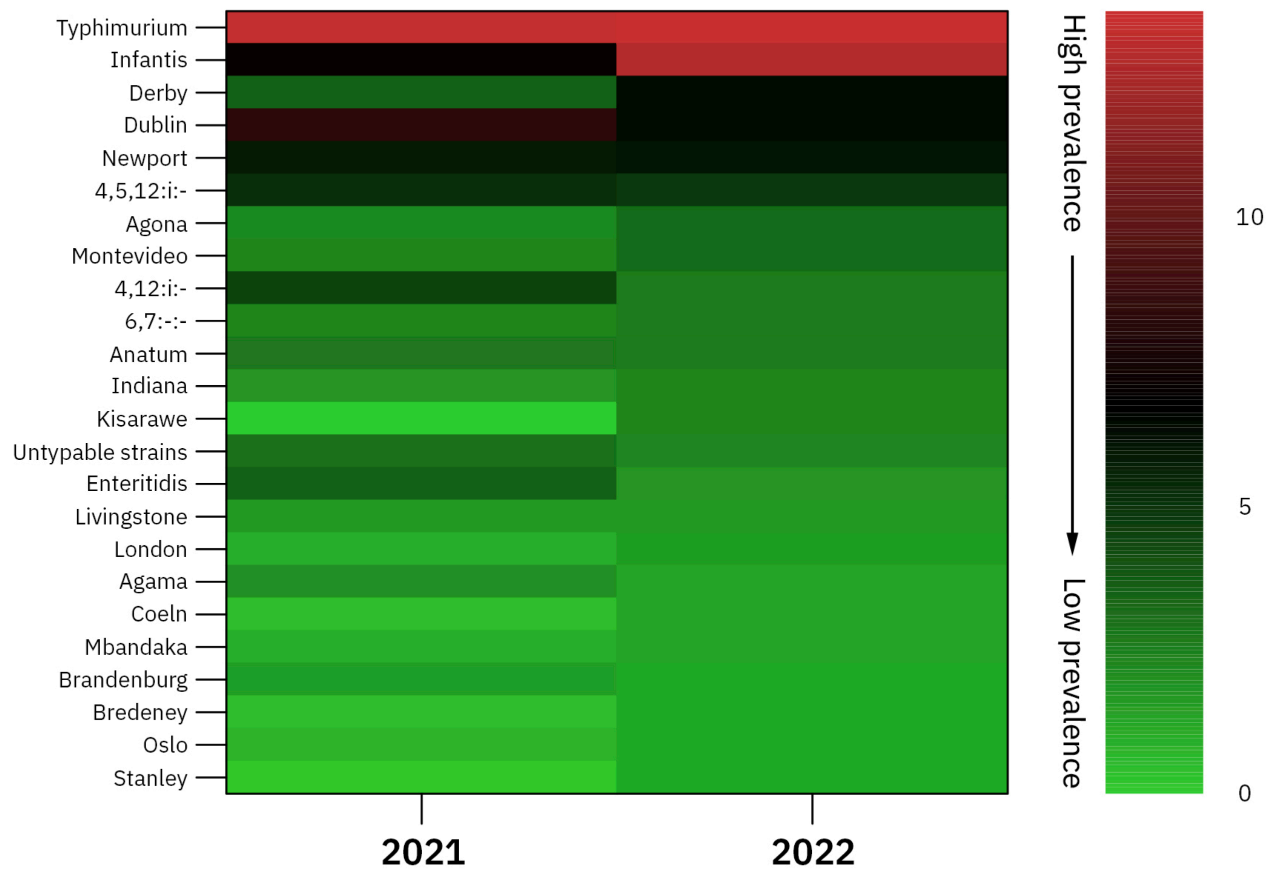



**Figure 5. The variable nature of Salmonella serovars isolated from dogs (DEFRA, UK 2021-22, adapted)**

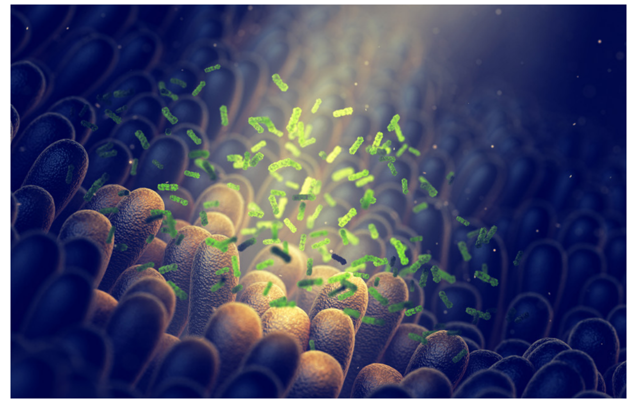
Long-term adherence and agglutination studies have demonstrated the ability of MRF to adhere to and agglutinate, or control, a broad spectrum of *Salmonella* and *Escherichia* isolates. In controlled studies, a reduction in the prevalence and concentration of different serovars of *Salmonella* as well as *E. coli* has been reported with the use of MRF in many species, including companion animals such as dogs. As such, MRF represents an exceptional control mechanism for pathogens with type 1 fimbriae.

Given the increasing popularity of gut microflora modifiers in companion animal diets, yeast mannan represents a technology that has become a critical part of the non-pharmaceutical pathogen control technologies that are available.



## The role of prebiotics in microbiome resilience

The microbiome is central to controlling and mitigating many illness states, and strengthening its resilience is increasingly recognized as important for health and well-being. With respect to prebiotic use in strengthening microbiome resilience, one of the more interesting capabilities of MRF is its ability to increase microbiome species richness and diversity. In doing so, MRF has been noted to reduce the risk of pathogen growth and enhance the colonization resistance of the GI tract, thereby significantly lowering the likelihood of intestinal pathogen colonization.



While the effects of MRF supplementation on health and well-being have been studied comprehensively, newer studies have focused on the effects on the overall bacterial community of the gut, and such work has shown that supplementation with MRF can significantly alter the intestinal microbiome. One specific study, which focused on multiple trials, identified consistent alterations in the cecal microbiota of monogastric species, demonstrating increases in the phylum Bacteroidetes and decreases in the phylum Firmicutes (**Figure 6**). These changes represent not only better microbial balance within the GI tract, but also increased species richness or diversity.

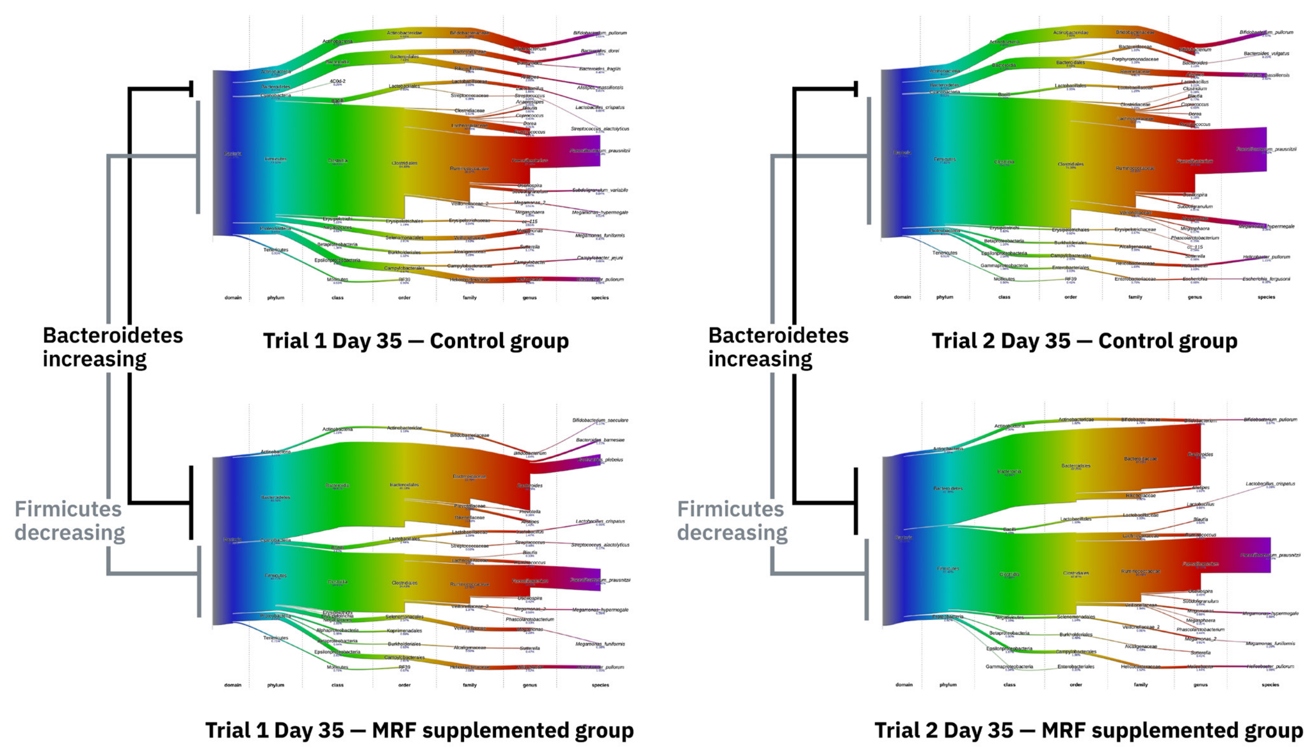



**Figure 6. MRF consistently alters cecal microbiome diversity**

Of particular interest in this study was the capability of MRF to consistently enhance microbial balance and diversity. The prebiotic stimulatory impacts by MRF on the growth of Bacteroidetes have been demonstrated in multiple species. These studies have also demonstrated a consistent reduction in the overall prevalence of a specific bacterial grouping (phylum) known as Proteobacteria, which includes such well-known pathogens as *Escherichia* and *Salmonella*.

Studies in companion animals have also demonstrated the ability of MRF to beneficially modulate the microbiome, with a recent study in cats further reaffirming this capability (**Figure 7**).

The data generated using DNA-sequencing technologies to analyze the microbiome has allowed us not only to map the microbiome but also to search for other disease-causing pathogens and determine the impact of MRF on them. Multiple additional studies in numerous species have focused on the microbiome-potentiating effects of MRF and noted its ability to reduce the prevalence of many pathogens with resonance for intestinal health, including *Campylobacter, Helicobacter, Clostridium* and *Listeria*. Conversely, many of these studies also demonstrate the ability of MRF to promote the growth of beneficial bacterial groups such as Bifidobacteria and *Lactobacillus*.

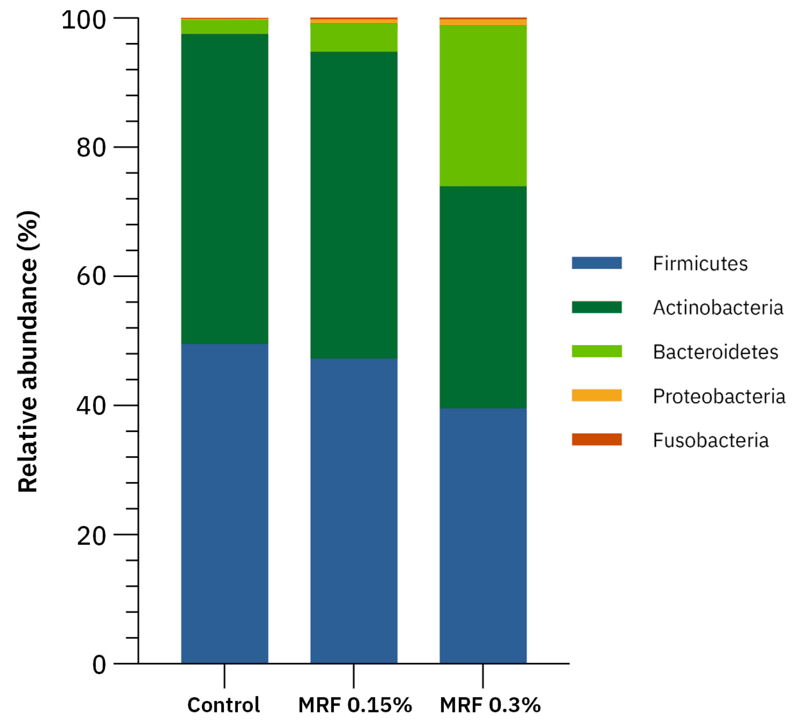



**Figure 7. MRF increases microbial diversity and enhances microbial balance in cats**

In essence, the use of MRF can aid in strengthening the resilience of the gut microflora by increasing overall species richness, reducing pathogen load, increasing beneficial bacterial populations, and enhancing the gut's resistance to pathogen colonization.

## Prebiotics for immune system modulation

The health of our companion animals is ultimately dependent on three lines of immune defense, which fall under two broad categories:

- **Innate immunity:** The nonspecific defense mechanisms an animal is born with make up the first and second lines of defense. The first line of defense is multilayered and includes physical barriers such as skin; intestinal mucus layer; and the microbiome. The second line of defense, which is also a non-specific innate response, involves white blood cells and other immune system components, such as inflammation, fever and antimicrobial proteins.
- **Acquired immunity:** Highly specific cellular and molecular responses to pathogens continually develop during the lifetime of an animal, forming the third line of defense. There are numerous components to this acquired immune response, including lymphocytes, B cells and T cells. B cells produce an antibody response and T cells produce a cell-mediated immune response.

Together, this complex network of systems acts in concert to protect and maintain companion animal health, with pre- and probiotics having multiple interconnected roles to play in supporting and strengthening overall immunity.

With respect to prebiotics such as MRF and their role in immune strengthening, numerous beneficial impacts on the innate response have been noted, leading to enhancements of the first and second lines of defense. For instance, improvements in gut structure have been shown, with villus height, crypt depth and villus surface area being positively impacted.

Intestinal barrier integrity has also been shown to be beneficially supported by MRF, with the expression of tight junction proteins critical for barrier function found to be increased in both the presence and absence of *Salmonella*.



The intestinal mucus layer acts as a barrier for the epithelium against pathogens, supporting favorable bacteria and providing an optimal environment for enzyme activity and nutrient absorption. Numerous studies have demonstrated the ability of MRF to enhance mucus layer thickness and also to effect changes in mucin production.

The role of MRF in modulating the microbiome is a pivotal component of the innate immune response. Through enhancing microbial species richness, pathogen colonization of the GI tract becomes more limited. Use of MRF has been noted in multiple studies and species to enhance the cecal levels of SCFAs including butyrate and propionate, both of which can play a vital role in positively influencing the immune, inflammatory and stress responses. These SCFAs may also play a role in limiting and controlling the growth of pathogens such as *Campylobacter* and *Helicobacter,* with pathogen abundance decreasing following mannan prebiotic inclusion.

> ***Through enhancing microbial species richness, pathogen colonization of the GI tract becomes more limited.***

Additional work has demonstrated the anti-inflammatory effects of MRF, with reductions noted in the expression levels of pro-inflammatory cytokines both *in vitro* and *in vivo.* Moreover, IL-10, which is anti-inflammatory and capable of stimulating B cell-produced antibodies, can also be favorably affected.

Prebiotics also have a role to play in modulating the acquired immune response, the third line of defense. MRF, for instance, has been noted to stimulate milk IgG concentrations in addition to increasing the birth and weaning weights of young animals, an attribute important to survivability in early life.

Dietary supplementation with MRF has also been found to enhance vaccine response, with increased titers being observed.

## Probiotics: Enhancing gut health in pets

Probiotics are live bacteria and yeasts that have beneficial intestinal health effects. By providing specific strains of bacteria to the intestine, we can direct the gut microbial populations toward species and strains that are known to improve health and/or well-being. These augmented beneficial populations then outcompete the bad bacteria for food and colonization sites while also producing metabolites that enhance gut barrier function and immune activity and reduce gut inflammation (**Figure 8**).

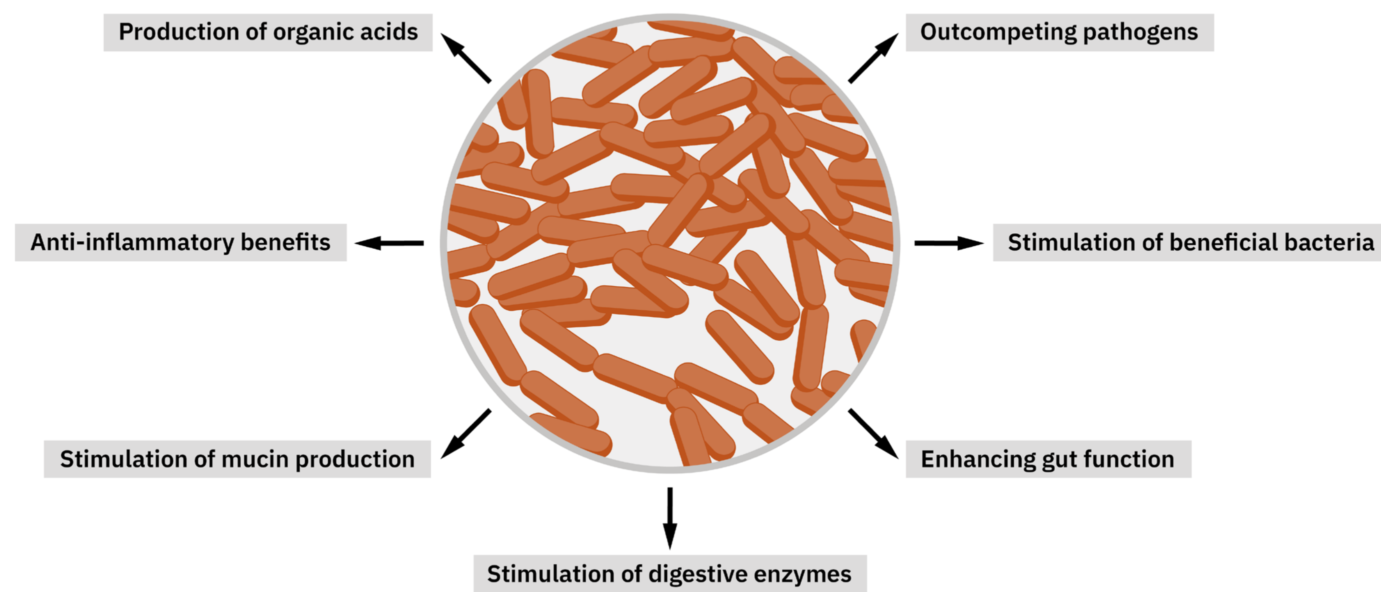



**Figure 8. Beneficial impacts of probiotics on intestinal health**

The practical application of probiotics is multifaceted, their use being noted to help establish a healthy gut microflora and prevent establishment of pathogenic bacteria immediately following birth. Their use in re-establishing beneficial microflora depleted by antibiotics and preventing re-infection by pathogens is also well documented. Additionally, probiotics can treat or prevent diarrhea through suppression and exclusion of pathogenic bacteria such as *E. coli* and *Salmonella,* which increases the host animal's resistance to these bacteria. This can occur either by direct antagonism or by stimulating an immune response via increased phagocytic activity and/or elevation of secretory IgA levels.

## Exploring the various types of probiotics

A variety of organisms that comprise normal intestinal microflora have been cultivated and used as probiotics. These include non-pathogenic lactic-acid-producing bacteria such as *Lactobacillus* and *Bifidobacterium* species. In addition, non-pathogenic yeasts such as *Saccharomyces* spp. are used as probiotics. All of those currently in use are validated for their safety and efficacy. Considerable research into the development of next-generation probiotics is ongoing; these include members of *Akkermansia, Faecalibacterium, Prevotella* and *Propionibacterium,* among others. Some examples of selected probiotic strains and their health-promoting benefits are outlined in **Table 2**, which is by no means exhaustive.

**Table 2. Selected probiotics and their health applications**

| Probiotic strain | Function |
|---|---|
| *Saccharomyces cerevisiae* | • **Stimulates microbial digestion –** enhances nutrient digestibility and bioavailability<br>• Supports the normal microbiota of the gut<br>• Helps inhibit the pathogenicity of diarrheal infections |
| *Bacillus subtilis* | • **Supports beneficial bacteria –** promotes growth of good bacteria that reduce the bad bacteria<br>• Supports short-chain fatty acid production<br>• Improves immunity and disease resistance |
| *Bacillus licheniformis* | • **Supports beneficial bacteria –** promotes growth of good bacteria that reduce the bad bacteria<br>• Supports short-chain fatty acid production<br>• Modulates immune response, has antimicrobial properties, stimulates enzyme secretion, improves microbial diversity<br>• Associated with hepatoprotection and cardio protection |
| *Lactobacillus acidophilus* | • **Aids the digestive process –** increases microbial populations that support nutrient digestion<br>• Reduces cytokines to relieve inflammatory bowel disease, modulates immunity, lowers cholesterol and relieves diarrhea |
| *Enterococcus faecium* | • **Aids the digestive process –** increases microbial populations that support nutrient digestion<br>• Lowers cholesterol, supports the immune and gastrointestinal systems, helps alleviate respiratory allergies |
| *Bifidobacteria* | • **Supports beneficial bacteria –** promotes growth of good bacteria that reduce the bad bacteria<br>• Produces exopolysaccharides with noted antioxidant, anticancer, antibacterial and immunological activities. |

Probiotics that retain high viability during intestinal tract transit remain unaffected by the acidic environment of the stomach and retain the ability to colonize the gut. This ensures optimal efficacy and function.



## How probiotics support pet gut health

The use of probiotics administers billions of live, beneficial microorganisms that boost the gut's normal microflora. This improves various aspects of health and growth, including:

- aiding overall digestion
- breaking down complex carbohydrates and other indigestible substances
- promoting the synthesis and absorption of vitamins and minerals
- stimulating both nonspecific and certain specific host defense mechanisms of the immune system

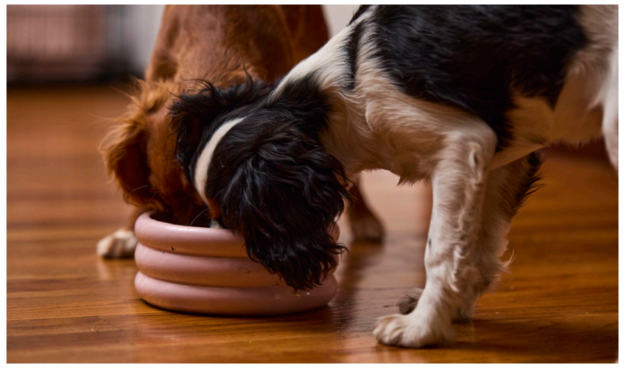
The beneficial effects of probiotics on host health can be local or systemic in nature. Probiotics have been shown to be beneficial with respect to disease states including IBD, diarrhea, type 2 diabetes and atopic dermatitis. Notably, these impacts can be strain-specific, and not all strains of a specific microbial species will have the same benefit.

Probiotics have been proposed to have multiple mechanisms of action. For instance, they can confer benefits to host health through interacting with the resident microbiota or by stimulating host cells. They have been noted for their health-promoting benefits through optimizing gut microfloral balance and are commonly used after an acute or chronic change in normal gut flora, such as that typically induced by antibiotic administration. There are many hypothesized mechanisms of the ability of probiotics to influence microbiome balance, including competitive inhibition with pathogenic bacteria, promotion of gut barrier activities, and immunomodulation.

***"There are many hypothesized mechanisms of the ability of probiotics to influence microbiome balance, including competitive inhibition with pathogenic bacteria, promotion of gut barrier activities, and immunomodulation."***

One of the most well-studied mechanisms by which probiotics help to restore the predominance of beneficial flora over pathogenic bacteria in the GI tract is through stimulation of mucus production and support of the mucus barrier. Sticky, hair-like structures (pili) extend from the surface of some probiotics, allowing them to attach to intestinal mucus and epithelial cells. The probiotic then secretes specific proteins which stimulate mucus production by epithelial cells. Increased intestinal secretion of defensins and antibodies associated with normal mucosal barrier function has also been observed with other probiotic strains.

Probiotics can inhibit the colonization of the GI tract with pathogens via colonization resistance. The well-documented production of antimicrobial molecules, such as bacteriocins and organic acids (e.g., SCFAs), by probiotic strains is one of the main mechanisms by which this occurs. With respect to the production of SCFAs such as butyrate, strains belonging to the *Lactobacillus* and *Bifidobacterium* genera do not produce butyrate per se but can stimulate its production by other bacterial groups through a process known as cross-feeding.



Additionally, the beneficial effects of probiotics can be imparted, at least in part, by the induction of host defense peptides (HDPs). Some probiotic strains of *Lactobacillus* and *Bifidobacterium* can stimulate the production of host-derived HDPs in humans and animals. HDPs are antimicrobial peptides and are ubiquitously expressed in epithelial cells and phagocytes. Given their antimicrobial and immunomodulatory activities, they are an important component of the host immune defense response.

In addition to the control and inhibition of pathogens, probiotics contribute to the integrity of epithelial barrier function. This is integral to intestinal homeostasis as the first physical barrier against the external environment and is largely maintained by the mucus layer and tight junctions which link adjacent epithelial cells.

Studies have demonstrated that *Lactobacillus* and *Bifidobacterium* directly or indirectly promote the production of mucins and tight-junction proteins.

Several beneficial effects of probiotics on the host intestinal mucosal defense system have been identified. Their ability to modulate the immune system is one of the most beneficial effects with respect to promoting health. Probiotics have also been found to enhance innate immunity and modulate pathogen-induced inflammation via toll-like receptor-regulated signaling pathways. Their immunomodulatory capabilities extend to stimulation of T-cell differentiation, regulation of pro- and anti-inflammatory cytokine profiles, and induction of secretory IgA production. Furthermore, some probiotic strains, such as *Lactobacillus* spp., have been shown to produce enzymes like β-galactosidase and bile salt hydrolase, which are involved in lactose digestion and bile acid metabolism respectively.

While the mechanisms of action of probiotics are diverse, it is noteworthy that they are heterogeneous and strain specific.

## Beyond prebiotics and probiotics: The future of biotics

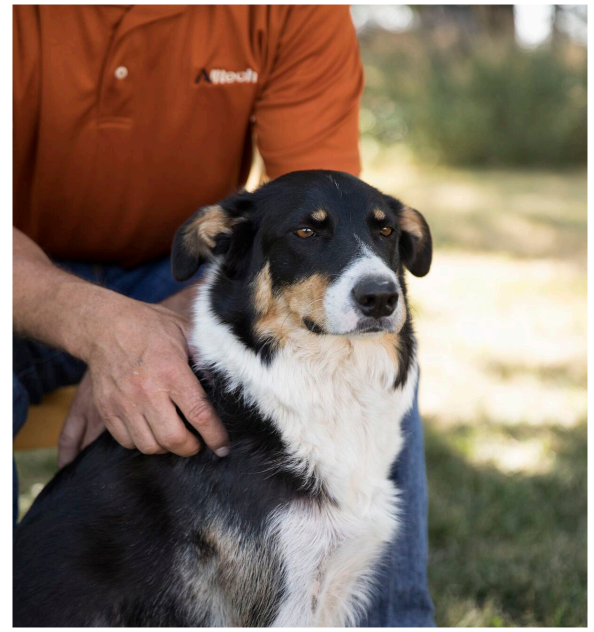
While pre- and probiotics are amongst the most common nutritional supports for gut health, newer biotics such as synbiotics, postbiotics and even paraprobiotics have been developed. Each of the biotics can have a role to play in supporting intestinal function, but some confusion has been created by the ever-evolving biotics landscape.

For instance, advances in our understanding of pre- and probiotic function and action have enabled us to utilize them synergistically. Synbiotics, which are mixtures of pre- and probiotics that have been specifically formulated to act in concert, have begun to be successfully applied as nutritional aids. In the case of synbiotics, the prebiotic component aids in stimulating the probiotic strain and in doing so further enhances effectiveness, benefiting gut health, metabolism and immune function.

Postbiotics have been recently defined as “a preparation of inanimate microorganisms and/or their components that confers a health benefit on the host.” This makes a clear distinction between probiotics and postbiotics, in that while probiotics consist of live microorganisms, postbiotics are the non-living organisms created by the fermentation process. In general, *Lactobacillus, Bifidobacterium, Bacillus,* or yeasts such as *Saccharomyces,* with proven probiotic properties, are used to obtain postbiotics. During their growth phase, probiotic microorganisms produce metabolites that play a key role in both regulating the development of their own cells and encouraging the growth of other beneficial organisms, in addition to inhibiting competing organisms which may be pathogenic to the host. Postbiotics, along with dead microbial cells, can contain the metabolites/CFS (cell-free supernatants) and soluble factors (products or metabolic byproducts) secreted while they were alive. These can include SCFAs (butyrate, propionate), proteins, vitamins, carbohydrates (such as teichoic acids and galactose-rich polysaccharides), and complex molecules such as lipoteichoic acids or peptidoglycan-derived muropeptides.



## Postbiotics: New frontiers in intestinal health for pets



A distinct feature of postbiotics, when compared to probiotics, is that rather than introducing new organisms to the gastrointestinal microbiota, postbiotics moderate the indigenous beneficial microbiota, in addition to supporting and stabilizing host homeostasis. They have been found to aid in the maintenance of a healthy digestive system and, in addition, support immune health (**Figure 9**).

The most important postbiotics are organic acids, short-chain fatty acids (SCFA) and bacteriocins. Their modes of action may be direct, such as through interaction with the host cells, or indirect, such as by promotion of the growth of microbes beneficial to health, in addition to inhibition of the development of pathogenic strains. To date, bacteriocins are the most studied antimicrobial components of postbiotic with activity against various pathogens such as *Listeria, Salmonella* and *E. coli.*

The antibacterial action of cell-free supernatants from *Lactobacillus* and *Bifidobacterium* have been shown to have antibacterial properties against the invasion of enteroinvasive *E. coli.* Bifidocins, produced by strains of bifidobacteria, have a wide spectrum of bactericidal action against both gram-positive and gram-negative bacteria as well as certain yeasts. The stimulatory capabilities of additional cellular components such as exopolysaccharides (EPS) from *Bifidobacterium bifidum* toward lactobacilli and other beneficial bacteria have been demonstrated. In contrast, the inhibitory impacts of EPS on pathogenic enterobacteria have also been shown, highlighting the dual capabilities of these important postbiotics. Reuterin, a well-known antibacterial metabolite produced by *Lactobacillus reuteri,* inhibits pathogenic gut bacteria by oxidizing thiol groups and creating stress for the organisms. Teichoic acids, a cellular component of *Lactobacillus* strains, have exhibited inhibitory effects on biofilm formation of oral or enteric pathogens including *Streptococcus mutans, Staphylococcus aureus,* and *Enterococcus faecalis.*

Postbiotics can also compete with pathogens for intestinal adhesion sites if the adhesins (e.g., fimbriae and lectins) in postbiotics retain functionality after processing. Lectins extracted from *Lactobacilli* are prospective bioactive components capable of preventing gastrointestinal and urogenital infections caused by a wide range of pathogens, involving uropathogenic *E. coli* and *Salmonella* species.

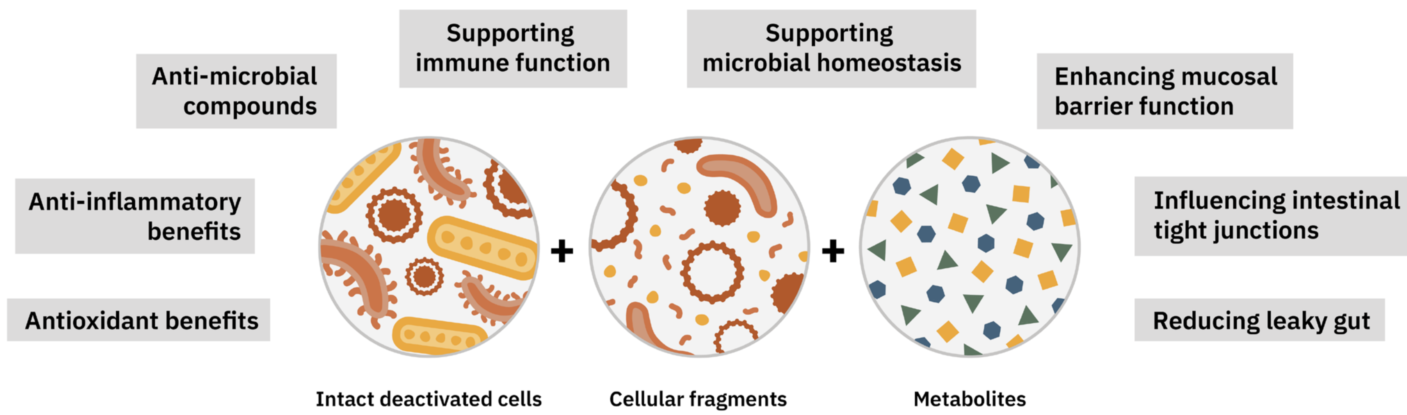



**Figure 9. Postbiotic mode of action**

> *A substantial body of evidence suggests the effectiveness of postbiotics in alleviating the signs and symptoms of inflammatory gastrointestinal diseases.*

SCFAs, important beneficial postbiotics, are known for their anti-inflammatory and antioxidant properties. Several inflammatory disorders, including inflammatory bowel disease (IBD), arthritis, gastritis, atherosclerosis and chronic inflammation, are associated with the adverse side effects of inflammation. A substantial body of evidence suggests the effectiveness of postbiotics in alleviating the signs and symptoms of inflammatory gastrointestinal diseases. Postbiotics can be effective against inflammation by modulating the production of pro-inflammatory proteins. Both anti-inflammatory and immunostimulatory effects have been demonstrated, suggesting heterogeneity of both the structure and composition of postbiotics.

An important consideration, however, is that the impact of postbiotics on the inflammatory response is dependent on the bacterial strain used, downstream processing, and type of postbiotic preparation (e.g., cell-free supernatant).

Postbiotics have also been shown to enhance mucosal barrier function, influencing intestinal tight junctions and reducing leaky gut.

A consideration perhaps when choosing postbiotics over pre- or probiotics is whether the peptides and metabolites can transit the acidic conditions of the stomach, thus reaching the site of impact, the lower intestinal tract, in active form.

Interestingly, postbiotics can be produced within the GI tract through their stimulation by pre- and postbiotics that can be included in the diet. A great example of this is the stimulation of butyrate production in the GI tract by MRF, in essence an example of a prebiotic stimulating the production of a postbiotic.

## Choosing the right gut health solution for companion animals

One of the most common questions is, “Given the range of biotics available, which one should I choose for my pet’s health?”

In short, while all the biotics can have individual roles to play in optimizing health and well-being, they can also act synergistically and support one another. Prebiotics that act as a front-line defense, mopping up pathogens and stimulating the growth of beneficial gut microbes, can also act as fuel for probiotics. Probiotics, in turn, can stimulate the growth of additional species and strains that are known to improve health, and they can also have beneficial immunomodulatory properties.

Postbiotics, being distinct from both pre- and probiotics, can also play a role in companion animal health by further stimulating growth of beneficial bacteria, in addition to supporting digestive and immune functions.

Ultimately, the answer to the question above is almost always another question: “What is the desired outcome?” Once this is determined, the advice is to look at all biotics and consider their benefits. Critically, the synergistic effects of combining pre-, pro- and postbiotics needs to be considered with respect to the overall accumulated benefits they can impart on our pet’s health and well-being. In essence, different biotics can act as individual layers of protection, but when utilized synergistically, the individual layers provide a strengthened defense against poor intestinal health, as well as supporting overall well-being.



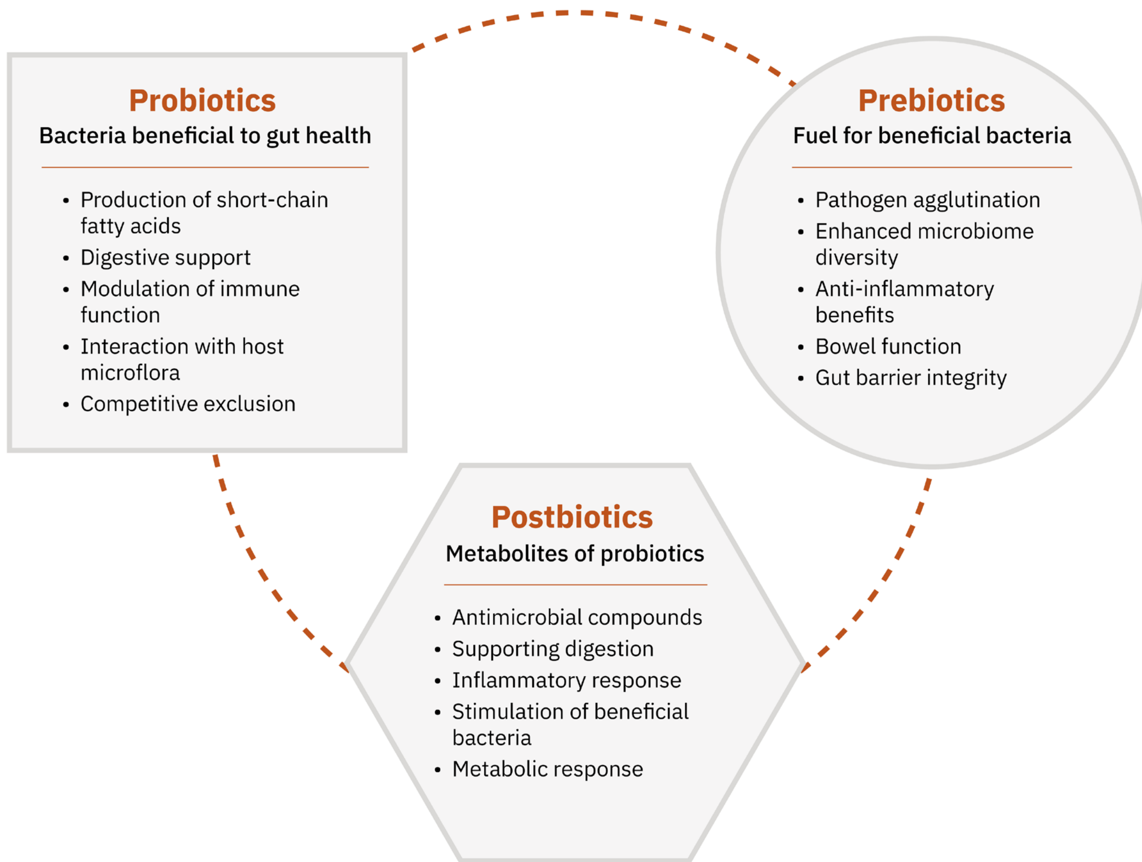



**Figure 10. Modes of action for pre-, pro- and postbiotics**

## Summary

> *Interventions with prebiotics, probiotics, and post biotics are promising biotic tools to actively shape and maintain a health-promoting gut microbiota.*

While gut health and its management is an intricate and complex area governed by numerous factors, dietary supplements that focus on supporting gut health or enhancing the gut microflora have become progressively more popular. Distinct dietary components play key roles in shaping the gut microbiota and supporting a diverse microbial ecosystem, whereas inter- individual differences in the microbial composition results in different responses to the same diets. Several factors can disrupt the normal composition of the gut microbiota, leading to a state of dysbiosis. Frequently such imbalances are manifested by a decreased prevalence of beneficial bacteria and an increase in potentially harmful species. Interventions with prebiotics, probiotics, and post biotics are promising biotic tools to actively shape and maintain a health-promoting gut microbiota.

Prebiotics particularly MRF work by; binding harmful bacteria and preventing GI colonisation, stimulate the growth of beneficial bacteria that compete with other species and in doing so produce beneficial fermentation products (such as SCFAs), improve GI barrier function and modulate the immune system. Probiotics, depending on the strain used, act by; interacting with the gut microbial community to out compete bad bacteria, produce fermentation acids, slightly lower the GIT pH for favourable bacterial growth and support digestion. Post biotics are independent of cell viability; they are known to promote commensal bacterial growth and play a role in combatting GI tract inflammation. Maintaining a balanced microbial gut ecosystem has enormous potential for preventive and therapeutic approaches to support general pet health and wellness.

## About Alltech


Founded in 1980 by Irish scientist Dr. Pearse Lyons, Alltech delivers smarter, more sustainable solutions for agriculture. Our portfolio of products and services improves the health and performance of animals and plants, resulting in better nutrition for all and a decreased environmental impact.

We produce specialty ingredients, premixes, feed and supplements for animal nutrition and biological solutions that enhance soil and crop health, supported by an unmatched platform of services delivered by world-leading experts.

For decades, we have partnered with pet food manufacturers to deliver high-quality, science-backed solutions that enhance pet health and well-being. Our expertise in animal nutrition, commitment to quality and focus on innovation ensures that pets receive the best possible nutrition. With a strong foundation in research and an unwavering dedication to our customers, Alltech is helping families enjoy healthier, happier pets.

Strengthened by more than 40 years of scientific research, Alltech carries forward a legacy of innovation and an entrepreneurial culture. As a private, family-owned company, we adapt quickly to customer needs and focus on advanced innovation.

We believe agriculture has the greatest potential to positively shape the future of our planet. Our more than 5,000 talented team members worldwide share our purpose of Working Together for a Planet of Plenty®. Together, we can provide nutrition for all, revitalize local economies, and replenish the planet's natural resources.

Headquartered in Kentucky, USA, Alltech serves customers in more than 140 countries, has five bioscience centers, and operates more than 75 manufacturing facilities across the globe.

## References

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