



# Prebiotic-driven Gut Microbiota Dynamics: Enhancing Canine Health via Pet Food Formulation

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## ABSTRACT

Expanding our understanding of canine nutrition is paramount for ensuring the well-being of our beloved companions. In recent years, there has been a paradigm shift towards recognizing the intricate interplay between diet and health outcomes in dogs. This shift has led to a surge of interest in exploring novel dietary interventions, with a focus on ingredients that can positively modulate the gut microbiota and enhance immune function. Prebiotics, defined as non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, have emerged as key players in this domain. These substances, often found in fiber-rich foods such as fruits, vegetables, and whole grains, serve as fuel for beneficial gut bacteria, promoting their proliferation and diversity. By fostering a healthy gut microbiota, prebiotics contribute to improved nutrient absorption, reduced inflammation, and enhanced immune response in dogs. To fully realize the potential of prebiotics and symbiotics in canine nutrition, collaboration between veterinarians, animal nutritionists, and researchers is essential. By pooling expertise from diverse fields, we can design robust studies to investigate the efficacy of these interventions across different dog breeds, ages, and health conditions. Additionally, ongoing monitoring and surveillance are necessary to ensure the safety and efficacy of prebiotic and symbiotic products in the market. Through interdisciplinary efforts, we can harness the transformative power of advanced nutrition to enhance the lives of dogs and strengthen the human-animal bond for generations to come.

**KEYWORDS:** Gut health, nutrition, pet food, prebiotics

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## 1. INTRODUCTION

Prebiotic-enriched pet foods offer promise in managing inflammatory bowel disease (IBD) in dogs by modulating gut microbiota dynamics, providing a pathway to alleviate symptoms and enhance overall well-being (Cai et al., 2020; So et al., 2018).

Dogs with IBD face inflammation. Recent research shows stem cells and diet help, easing symptoms and modulating immunity (Cristóbal et al., 2021; An et al., 2020). Additionally, fecal microbiota transplantation (FMT) emerges as a novel approach, addressing the dysbiosis implicated in canine IBD pathogenesis (Niina et al., 2021; Chaitman and Gaschen, 2021). Recent advancements in regenerative medicine, specifically using mesenchymal stem cells (MSCs), show promise for managing canine inflammatory bowel disease (IBD) by providing immunomodulation and tissue repair (Pérez-Merino et al., 2015; Kang and Park, 2020). Furthermore, the integration of dietary interventions, such as dietary fiber supplementation, underscores the importance of gut microbiota modulation in ameliorating canine IBD (Cai et al., 2020; So et al., 2018). Allogeneic mesenchymal stem cell (MSC) transplantation is a novel IBD therapy for dogs, proving sustained efficacy and potential corticosteroid dose reduction (Cristóbal et al., 2021). Notably, pre-treatment of canine adipose tissue derived MSCs with pro-inflammatory cytokines enhances their immunomodulatory properties, offering a promising avenue for enhancing therapeutic outcomes (Song et al., 2019; Dias et al., 2019). Fiber, like oligosaccharides and polysaccharides, helps manage canine IBD by promoting beneficial gut bacteria (Cai et al., 2020; So et al., 2018). Fecal microbiota transplantation (FMT) emerges as a promising therapeutic strategy for canine IBD, addressing the dysbiosis implicated in disease pathogenesis and fostering restoration of a balanced gut microbiome (Niina et al., 2021; Chaitman and Gaschen, 2021). However, further research is warranted to elucidate the long-term efficacy and safety of FMT in managing canine gastrointestinal disorders (Froebel et al., 2020; Gal, 2021). The integration of innovative therapeutic modalities, including mesenchymal stem cell transplantation (Koh et al., 2016), dietary interventions, and fecal microbiota transplantation (Mendis et al., 2017), heralds a new era in the management of canine inflammatory bowel disease (Xiao and Bai, 2019).

A balanced intake of macronutrients, micronutrients, and moisture is essential throughout a dog's life to ensure optimal health and longevity (Kumar et al., 2023). Tailored canine diets prevent health issues caused by nutritional imbalances or deficiencies. (Welsch, 1996). During this period, the concept of a balanced diet emerged, stressing the provision of essential nutrients in appropriate proportions

to support overall health (Welsch, 1996). In response to these challenges, nutrition science evolved to embrace new paradigms and innovative approaches aimed at optimizing health and well-being across the lifespan (Milner, 2000). Optimized nutrition tailors diets to maximize well-being and minimize disease risk across lifespans (Milner, 2000). Among the various functional food components, prebiotics and probiotics have emerged as key players in promoting gut health and immune function across different species (Roberfroid, 2007; Marcinakova et al., 2006).

Prebiotics, which provide non-digestible fibers that serve as substrates for beneficial gut bacteria, and probiotics, which introduce beneficial microorganisms into the gut, have shown considerable promise in enhancing gastrointestinal health and immune function in both humans and animals (Kumar et al., 2023). Synbiotics, pairing prebiotics with probiotics, optimize gut microbiota and health synergistically (Tzortzis et al., 2003). While studies show dietary interventions improve dog gut health and immune function, further research is needed to understand mechanisms and optimal dosing. (Saavedra and Tschernia, 2002; Diez et al., 1998; Swanson et al., 2002a; Propst et al., 2003; Pasupathy et al., 2001; Benyacoub et al., 2003; Biagi et al., 2007). These considerations, this review aims to delve into the intricate realm of canine nutrition, with a particular emphasis on the emerging role of prebiotics.

## 2. GAPS IN PREBIOTIC RESEARCH FOR CANINE NUTRITION

**Optimization of Prebiotic Sources and Dosages:** Despite the recognized potential of prebiotics like inulin and oligofructose in modulating the gut microbiome and enhancing immune function in canines, there exists a paucity of studies elucidating the optimal sources and concentrations of prebiotics across diverse canine cohorts. Systematic investigations are warranted to delineate the most efficacious prebiotic formulations and dosage regimens to maximize therapeutic benefits while mitigating potential untoward effects (Kumar et al., 2023). **Longitudinal Assessment of Gut Microbiota Dynamics:** While short-term inquiries have demonstrated the ability of prebiotics to influence the composition of the canine gut microbiota, a dearth of longitudinal analyses impedes our comprehension of sustained alterations in microbial diversity, resilience, and metabolic activity consequent to prebiotic supplementation. Longitudinal endeavors are indispensable to discern the enduring impact of prebiotics on the gut microbiota across varying life stages and health paradigms in canines. **Elucidation of Host-Prebiotic Immunomodulatory Interactions:** The precise mechanistic underpinnings of prebiotic-mediated immunomodulation in canines remain enigmatic. There is a compelling need for further

investigations aimed at elucidating the molecular pathways through which prebiotics interface with the host immune milieu, potentiating immune enhancement and attenuating inflammatory and immune-mediated maladies in canines. Deciphering effects on gastrointestinal homeostasis: while prebiotics have been implicated in bolstering gastrointestinal health and curtailing the incidence of gastrointestinal ailments in canines, the specific mechanisms governing these phenomena remain nebulous. In-depth inquiries probing the impact of prebiotics on intestinal barrier integrity, mucosal immune reactivity, and gut inflammation in canines are imperative to unravel their role in fostering gastrointestinal equilibrium. Interindividual Variability in Responsiveness: Canines exhibit considerable heterogeneity in their responsiveness to prebiotic supplementation, influenced by a myriad of factors including age, breed, diet, and baseline gut microbiota composition. Rigorous investigations aimed at identifying biomarkers and prognostic indices of individual susceptibility to prebiotic interventions are essential for tailoring personalized dietary recommendations tailored to the idiosyncratic requirements of each canine. Comprehensive safety and tolerability assessments: despite the generally recognized safety profile of prebiotics in canines, comprehensive evaluations pertaining to their long-term safety and tolerability, particularly at escalated dosages or in vulnerable subpopulations, remain scant. Thorough safety appraisals encompassing toxicological appraisals and clinical trials are indispensable to delineate potential adverse outcomes associated with prebiotic supplementation in canines, thereby furnishing evidence-based guidelines for safe usage across diverse canine cohorts.

Addressing these pivotal research lacunae through methodologically rigorous investigations and translational endeavors will engender a more nuanced understanding of the multifaceted role of prebiotics in canine nutrition and health.

### 3. UNDERSTANDING THE RATIONALE BEHIND FUNCTIONAL FOODS

Functional foods are increasingly recognized for their potential to not only provide essential nutrients for optimal health but also to modulate various bodily functions, contributing to overall well-being. This concept has gained popularity in recent years, leading to the emergence of the term “functional food.” Functional foods are defined as foods that go beyond basic nutrition, offering additional physiological benefits such as disease prevention or enhanced physiological functions (Milner, 2000; López-Varela et al., 2002). While all foods serve functional roles by providing taste, aroma, or nutrition, the term “functional food” now denotes foods that offer specific health benefits beyond basic nutrition (Kwak and Jukes, 2001).

The classification of functional foods is broad and encompasses a range of foods with diverse components that impact various bodily functions relevant to health and disease prevention (Roberfroid, 2007). These foods contain one or more components in adequate concentrations that positively influence cellular or physiological functions (Roberfroid, 1996). The primary role of functional foods is often in reducing the risk of diseases rather than preventing them entirely (Diplock et al., 1999). Thus, regular consumption of functional foods as part of a balanced diet can significantly lower the likelihood of developing certain diseases (Kumar et al., 2023; and Kumar and Goswami, 2024). Functional foods can take various forms, including natural foods, foods with added or removed components, or foods with modified bioavailability of certain components (Roberfroid, 2007). The Institute of Medicine of the National Academy of Sciences specifically defines functional foods as those in which the concentrations of one or more ingredients have been altered to enhance their contribution to a healthy diet (IOM/NAC, 1994). Ultimately, the scientific community aims to develop functional foods that improve quality of life (Danone Vitapole, 2000).

### 4. FUNCTIONAL FOODS: AN OVERVIEW

Defining functional foods can vary depending on sources, but it generally refers to processed foods with added ingredients aimed at aiding specific bodily functions while providing nutrition. The Institute of Medicine’s Food and Nutrition Board describes functional foods as those that offer health benefits beyond their basic nutritional content. The International Food Information Council (IFIC) defines functional foods as those that go beyond basic nutrition to provide additional health benefits. These benefits should be achievable by consuming normal amounts of the food as part of a regular diet. Functional foods are primarily used to enhance physiological functions, potentially aiding in disease prevention or treatment (Kumar et al., 2023 & Kumar et al., 2024). Various methods, such as adding or removing components or utilizing food processing techniques and genetic engineering, can be employed to create functional foods. Among the myriad of functional foods, significant components added to canine diets include prebiotics, probiotics, and their combination known as synbiotics.

### 5. EXPLORING THE ROLE OF PREBIOTICS IN ANIMAL NUTRITION

Prebiotics are food components that resist degradation in the small intestine and can positively influence the host by selectively promoting the growth and/or activity of specific bacteria (Schrezenmeir and de Vrese, 2001; Marteau and Boutron-Ruault, 2002). Interest in the potential

effects of prebiotics in animal nutrition emerged in the 1980s, leading to extensive research into their utilization in animal feed and pet food (Verdonk et al., 2005). These fermentable carbohydrates serve as nourishment for beneficial gut microflora. Gibson and Roberfroid (1995) defined prebiotics as “non-digestible food components that exert beneficial effects on the host by selectively stimulating the growth and/or activity of specific bacteria in the colon, thereby improving host health.” Prebiotics encompass a variety of substances, including starches, dietary fibers, non-absorbable sugars, sugar alcohols, and oligosaccharides with oligosaccharides receiving particular attention due to their numerous health benefits (Tomomatsu, 1994). Oligosaccharides are short-chain polysaccharides composed of three to ten simple sugars, naturally occurring in various fruits and vegetables such as banana, chicory, garlic, onions, milk, honey, and artichokes. There’s a growing interest in incorporating prebiotics into animal feed, particularly for young pigs, poultry, and pet dogs, to enhance gut health or alleviate health issues. Common prebiotics used in pet nutrition include fructo-oligosaccharides (FOS) and other short-chain FOS, typically comprising short-chain oligosaccharides made of D-fructose and D-glucose, containing three to five monosaccharide units. Other potential prebiotics include inulins or fructans, isomalto-oligosaccharides, lactilol, lactosucrose, lactulose, pyrodextrins, transgalactooligosaccharides, xylo-oligosaccharides, and mannan-oligosaccharides (Kumar et al., 2024).

Natural sources of prebiotics mainly include soy-oligosaccharides and chicory inulin-fructan. Soy oligosaccharides, found in soybeans and other legumes, primarily consist of raffinose and stachyose. Raffinose comprises D-galactose, D-glucose, and D-fructose molecules, while stachyose comprises two D-galactose, one D-glucose, and one D-fructose molecule. Inulin-type fructans, non-digestible carbohydrates widely present in various plant-based feed and food ingredients, are extensively studied prebiotics in domesticated animals (Flickinger et al., 2003a).

## 6. CONCEPT BEHIND PREBIOTICS

The concept behind prebiotics involves categorizing them based on their molecular length, which includes mono-, di-, oligo-, or polysaccharides. Oligosaccharides are characterized as glycosides containing a limited number (<10) of hexose or pentose units and are found naturally in various feedstuffs or synthesized commercially (Iji and Tivey, 1998). The response to synthetic and natural oligosaccharides may differ due to variations in concentration levels in the diet. These prebiotics primarily comprise non-digestible oligosaccharides, with major examples being inulin and

oligofructose (Delzenne and Roberfroid, 1994 and kumar et al., 2023). Inulin and oligofructose, both linear  $\beta$  (2–1) fructans, are resistant to enzymatic hydrolysis in the small intestine due to the  $\beta$  configuration of the anomeric C2 in their fructose monomers, rendering them non-digestible (Roberfroid and Delzenne, 1998; Roberfroid and Slavin, 2000). Carbohydrates reaching the caecum serve as potential substrates for fermentation by the gut microbiota, with evidence supporting fermentation of identified prebiotics in the lower digestive tract. Fermentation patterns vary depending on the oligosaccharide structure; fructans are extensively fermented, while arabinoxylan and xylooligosaccharides show differential fermentation rates. Bifidobacteria and Bacteroides utilize carbohydrates based on their degree of polymerization, indicating metabolic collaboration among species (Molis et al., 1996; Alles et al., 1996).

Inulin and oligofructose fermentation in the large bowel results in the production of lactate and short-chain fatty acids, particularly acetate, as fermentation end-products (McBain and Macfarlane, 1997). Human studies have demonstrated significant changes in fecal flora composition following inulin and oligofructose consumption, confirming their prebiotic effects (Buddington et al., 1996; Gibson, 2000; Gibson et al., 1995; Kleessen et al., 1997; Roberfroid et al., 1998; Van Loo et al., 1998). Ideal prebiotics should exhibit characteristics such as resistance to host enzyme hydrolysis, selective enrichment of beneficial bacteria, alteration of intestinal microbiota and activities, positive influence on hindgut health, and beneficial effects on host health or well-being (Guarner, 2006).

## 7. PREBIOTICS AN UPDATE

Gibson and co-authors (2004) recently revisited their initial prebiotic concept considering extensive research from the past decade, focusing on three key elements of their definition: 1) resistance to digestion, 2) fermentation by the large intestinal microflora, and 3) selective impact on flora conducive to health. Their updated definition states: “A prebiotic is a selectively fermented ingredient that induces specific alterations, both in the composition and/or activity, within the gastrointestinal microflora, leading to benefits for host well-being and health.” Consequently, a prebiotic substrate should be readily available to certain bacterial groups, such as lactobacilli and bifidobacteria (considered indicative organisms for intestinal health), while being less accessible to potentially harmful bacteria like toxin-producing Clostridia, proteolytic Bacteroides, and toxogenic Escherichia coli. This selective availability fosters a “healthier” microbiota composition, with bifidobacteria and/or lactobacilli prevailing in the intestine and potentially exerting health-promoting effects. According to Gibson

et al. (2004) and Roberfroid (2005), inulin-type fructans, galactooligosaccharides, and lactulose are the only established prebiotics thus far. However, lactulose is regarded more as a therapeutic agent than a dietary component (Venter, 2007).

## 8. EFFECT OF PREBIOTICS ON FOOD INTAKE AND DIGESTIBILITY

Over the past two decades, research has highlighted the significant role of the gut in producing various peptides influencing metabolic and physiological processes, establishing a connection between the gut and the brain (Wynne et al., 2005). Among these, glucagon-like peptide-1 (GLP-1) and ghrelin have emerged as key regulators of appetite, with GLP-1 promoting satiety and ghrelin stimulating hunger (Drucker, 2002; Kumar et al., 2024). Recent experimental evidence suggests that inulin-type fructans can modulate the production of GLP-1 and ghrelin, thereby influencing appetite regulation and food intake (Roberfroid, 2007). Feeding prebiotics like inulin or oligofructose to broilers has been associated with improved zootechnical performance, possibly linked to enhanced absorptive capacity of the chicken gastrointestinal tract due to increased length of the small intestine and colon (Yusrizal and Chen, 2003). Similarly, dietary supplementation of chicory oligofructose and inulin has been shown to enhance feed efficiency and performance in layers, likely through improved utilization of dietary nutrients via absorption and metabolism (Chen et al., 2005). Prebiotic supplementation in pigs has also been linked to increased absorptive capacity of the intestinal tract, leading to improved feed conversion and faster growth, possibly mediated by improvements in intestinal architecture (Shim, 2005).

In contrast, studies on dogs have shown mixed results regarding the effects of prebiotic supplementation on food intake and digestibility. While some studies reported no significant impact on food intake upon supplementation with inulin or oligofructose (Verlinden et al., 2006; Propst et al., 2003), others noted lower food intake and decreased total-tract digestibility with certain prebiotics (Swanson et al., 2002b). The effects of prebiotics on nutrient digestibility in dogs appear to vary depending on factors such as the type and level of prebiotic supplementation (Strickling et al., 2000; Flickinger et al., 2003b). Furthermore, prebiotics have been shown to influence mineral bioavailability and absorption, with inulin-type fructans notably enhancing the absorption of minerals like calcium and magnesium (Cashman, 2003). Studies in animals have demonstrated increased mineral absorption and bone mineral content following prebiotic supplementation, suggesting a potential role in promoting bone health (Weaver, 2005; Coudray et al., 2003). However, the effects of prebiotics on mineral absorption and retention may vary depending on the specific

prebiotic type and dosage, as well as individual factors such as age and health status (Scholz-Ahrens et al., 2002).

## 9. MECHANISMS OF ACTION OF PREBIOTIC ON MINERAL METABOLISM

Various mechanisms underlie the action of prebiotics on mineral metabolism, including increased solubility of minerals due to enhanced production of short-chain fatty acids (SCFAs) by bacteria, promotion of enterocyte proliferation through bacterial fermentation products, and upregulation of calcium binding proteins (Coudray et al., 2003; Scholz-Ahrens and Schrezenmeir, 2002; Cashman, 2003). These effects result in decreased pH in the intestinal contents, leading to increased ionized mineral concentration and improved absorption (Levrat et al., 1993). Butyrate, a byproduct of fermentation, plays a role in stimulating calcium absorption pathways (Maiyar and Norman, 1992). Evidence suggests that inulin-type fructans exert their effects via microbial flora modulation, as changes in mucus production were observed only in animals with innate flora (Kleessen et al., 2003). These fructans likely contribute to calcium uptake stimulation by enterocytes and phytic acid degradation by probiotic strains containing phytase (Gilman and Cashman, 2006; Lan et al., 2002). Overall, these mechanisms promote calcium absorption by favoring a healthier gut environment.

Regarding hindgut health, prebiotics exhibit stool bulking effects and alter faecal characteristics, reflecting hindgut fermentation status in canines and humans (Kleessen et al., 2003). Dietary prebiotics enhance intestinal mucosal health via gut flora modulation, evidenced by increased villi and deeper crypts in bacteria-associated rats (Kleessen et al., 2003). Moreover, prebiotic supplementation improves the colonic epithelial mucus layer, contributing to a stabilized mucosa and enhanced absorptive function (Kleessen et al., 2003). Such effects help prevent gastrointestinal infections and oxidative damage to enterocytes, promoting effective gut function and absorption processes (Ito et al., 2003).

## 10. CONCEPT OF BALANCED COLONIC MICROFLORA AND PREBIOTICS

The colonic microflora's composition is crucial for maintaining colon and overall body health, aiming for a "balanced microflora" dominated by beneficial bacteria like lactobacilli and bifidobacteria to regulate potentially harmful microorganisms' proliferation (Gibson and Roberfroid, 1995). However, the presence of some potentially harmful bacteria may still have a role in the complex ecosystem of the colonic microflora, provided they remain in small numbers compared to beneficial species (Gibson and Roberfroid, 1995). Advanced molecular techniques allow for a deeper exploration of the gut microflora's composition, enabling

the development of dietary strategies like prebiotics to modulate microbiota composition, promoting colonic health and indirectly benefiting the host's overall well-being and disease resistance (Roberfroid, 1998). The concept of prebiotics involves selectively fermentable ingredients that induce specific changes in the gastrointestinal microflora, conferring health benefits to the host (Gibson et al., 2004). Fermentation of prebiotics targets health-promoting bacteria like lactobacilli and bifidobacteria, aiming to enhance host well-being (Roberfroid, 2007).

## 11. EFFECT OF PREBIOTICS ON GASTROINTESTINAL MICROFLORA

Published data on the intestinal microbiota of dogs and cats are limited compared to other monogastric animals, but they suggest a complex colonic bacterial population with diverse species and significant fermentation activity (Balish et al., 1977; Davis et al., 1977). This microbial population, rich in anaerobic bacteria, plays a crucial role in various physiological functions and can influence the development of diseases by metabolizing ingested or endogenous compounds (Mitsuoka, 1990). Dietary ingredients, such as protein source and level, can alter the composition of colonic microbiota and potentially influence the occurrence of pathogens (Amtsberg et al., 1980; Hussein et al., 1999). The large bowel and its symbiotic microbial ecosystem are targets for functional food development, with increasing interest in probiotics, prebiotics, and synbiotics. Inulin-type fructans, like inulin and oligofructose, stand out as unique functional food components due to their physiological and nutritional effects on gastrointestinal functions (Roberfroid, 2007). These prebiotics selectively stimulate the growth of beneficial bacteria such as bifidobacteria and lactobacilli while suppressing the growth of proteolytic bacteria like *Clostridium perfringens* (Gibson et al., 1995). Studies have shown that inulin-type fructans, particularly chicory inulin and oligofructose, promote the growth of bifidobacteria in both humans and dogs (Roberfroid et al., 1998; Menne et al., 2000; Swanson et al., 2002a). Additionally, fructans have been associated with decreased faecal odour components. FOS supplementation in animals has garnered interest due to its potential to modulate colonic bacterial populations and improve fermentation end-product potential for host health (Flickinger and Fahey, 2002). Dogs supplemented with FOS have shown lower concentrations of *Clostridium perfringens* and higher concentrations of beneficial bacteria like Bifidobacteria and lactobacilli (Propst et al., 2003; Swanson et al., 2002a). Similarly, oligofructose supplementation increased bifidobacteria concentrations in dog faeces. The immunological effects observed with probiotics may also be demonstrated with prebiotics, leading to similar clinical benefits (Saavedra and Tschernia, 2002).

## 12. GLUCOSE METABOLISM

The water-soluble extract of chicory has been shown to reduce glucose uptake from the perfused jejunum in rats (Kim and Shin, 1996). However, the precise impact of prebiotics on glycemia and insulinemia remains unclear, with some data presenting contradictory results, suggesting that these effects may vary depending on physiological (fasting versus post-prandial state) or disease (diabetes) conditions. For instance, in rats fed oligofructose at a dose of 10% in their diet for 30 days, postprandial glycemia decreased by 17% and insulinemia by 26% respectively. Similarly, rats fed 10% synthetic fructan for 3 months exhibited a reduced glycemic response to saccharose or maltose, possibly due to a reduction in disaccharidase activity in the gastrointestinal tract (Oku et al., 1984).

In streptozotocin-treated rats (diabetic), consumption of a diet containing 20% oligofructose for 2 months led to a decrease in post-prandial glycemia, despite no modification of the glycemic or insulinemic response to a saccharose or maltose load. Moreover, chronic ingestion of synthetic fructan (SFr) at 20 g day<sup>-1</sup> for 4 weeks did not alter fasting plasma glucose and insulin levels in healthy human volunteers, although it did reduce basal hepatic glucose production (Luo et al., 1996). Similarly, when 10 g of artichoke inulin was added to a 50 g wheat-starch meal in healthy human subjects, the blood glycemic response was attenuated, despite no apparent interference by inulin on starch absorption (Rumessen et al., 1990). Studies in rats fed with 10% and 20% SFr in their diet for 6 weeks demonstrated a dose-dependent effect on mouth-to-anus transit time, with a 25% and 50% reduction, respectively (Oku et al., 1984; Tokunga et al., 1986). This suggests that, like other dietary fibers, inulin and oligofructose may influence macronutrient absorption, particularly carbohydrates, by delaying gastric emptying and/or shortening small-intestinal transit time. Lower fasting glycemia has been observed in normal subjects fed with SFr (Luo et al., 1996). The reduced hepatic gluconeogenesis induced by prebiotic intake may be mediated by short-chain carboxylic acids, especially propionate. Propionate, when given in the diet of rats for 4 weeks, reduced fasting blood glucose levels (Boillot et al., 1995). It also inhibited gluconeogenesis in isolated hepatocytes, possibly via its metabolic conversion into methyl malonyl-coenzyme A (CoA) and succinyl-CoA, both of which are specific inhibitors of pyruvate carboxylase (Baird et al., 1980). Furthermore, propionate enhanced glucose utilization by depleting hepatic citrate, an allosteric inhibitor of phosphofructokinase. Additionally, propionate may indirectly influence hepatic glucose metabolism by reducing plasma fatty acid concentration, a factor closely related to gluconeogenesis (Lee et al., 1996).

### 13. LIPID METABOLISM

Interest in the food industry revolves around the development of functional foods aimed at modulating blood lipids, including cholesterol and triglycerides (Venter, 2007). Inulin-type fructans have been noted to influence lipid metabolism by affecting triglyceridemic and cholesterol, as well as the distribution of lipids among different lipoproteins in a manner conducive to health (Delzenne et al., 2002). Animal studies have consistently shown that inulin-type fructans primarily decrease triglyceridemic, with variable effects on cholesterol across different studies. These reductions are associated with a decrease in the number of VLDL particles, while human trials have primarily demonstrated a reduction in triglyceridemic, with modest effects on cholesterol under both normal and slightly hypertriglyceridemic conditions. In rodent models, adding inulin-type fructans to the diet reduces liver lipogenesis by suppressing the expression of genes encoding lipogenic enzymes. Dogs also exhibit a similar mechanism, with evidence suggesting reduced hepatic lipogenesis with inulin intake (Letexier et al., 2003). Additionally, propionate, a gluconeogenic compound, has been shown to inhibit cholesterol synthesis, leading to the hypothesis that substrates reducing the acetate: propionate ratio may lower serum lipids and potentially reduce cardiovascular disease risk (Wong et al., 2006).

Several mechanisms have been proposed to explain the lipid-lowering effects of prebiotics, including modulation of glucose or insulin concentrations, production of short-chain fatty acids (SCFA) in the colon, and the precipitation and excretion of bile acids, necessitating cholesterol utilization for bile acid synthesis (Pedersen et al., 1997; Roberfroid and Slavin, 2000; Delzenne and Williams, 2002). Animal studies have highlighted inhibition of hepatic fatty acid synthesis as a key mechanism for the triglyceride-lowering effects of inulin and fructooligosaccharides (Venter, 2007).

While animal models consistently demonstrate lipid-lowering effects of inulin and fructooligosaccharides, human studies have produced conflicting results. Some studies showed no significant effect on lipid metabolism, while others reported reductions in total serum cholesterol and LDL cholesterol. Moreover, supplementation with prebiotics like MOS has been shown to reduce plasma triglyceride levels in dogs fed homemade diets.

### 14. UREA METABOLISM

Feeding rats a diet enriched with inulin and oligofructose (10%) for a short duration resulted in reduced uremia, observed in both normal and nephrectomies rats (Delzenne et al., 1995; Younes et al., 1997). In rats, dietary inulin notably increased fecal nitrogen excretion while decreasing

renal nitrogen excretion (Younes et al., 1995). Inulin and oligofructose act as energy sources for intestinal bacteria, which require nitrogen for protein synthesis during their growth phase. However, their impact on protein digestibility in the small intestine appears minimal (Levrat et al., 1993). When fermentable carbohydrate intake is high, the available ammonia for bacterial growth may become insufficient, leading to the utilization of blood urea as a nitrogen source for bacterial protein synthesis in the caecum (Younes et al., 1995). Additionally, propionate, a key fermentation product of inulin-type fructans, can inhibit ureagenesis in the liver in the presence of ammonia and amino acids. Chicory root extract has also been shown to inhibit xanthine oxidase, an enzyme involved in uric acid synthesis from purines, potentially offering relief from conditions like gout (Lupton and Marchand, 1989).

Dietary fibers and prebiotics, such as lactulose, have a longstanding association with reduced blood NH<sub>3</sub> and serum urea levels. These effects are linked to increased colonic biomass growth, nitrogen fixation by colonic bacteria, acidification of the colon, and conversion of diffusible NH<sub>3</sub> into less diffusible NH<sub>4</sub><sup>+</sup> ions. Inulin and oligofructose have been reported to increase fecal nitrogen elimination, supporting these systemic effects (Bouhnik et al., 1996; Gibson et al., 1995).

### 15. EFFECT OF PREBIOTICS ON IMMUNE SYSTEM

The immune system functions as the body's defense mechanism against external threats like bacteria, viruses, parasites, as well as internal dangers such as malignant or autoreactive cells. It safeguards the body by preventing invasion from pathogenic organisms. The immune system comprises two main branches: the innate or nonspecific immune system, consisting of inborn components, and the acquired or specific immune system, which adapts over time. Outlines the components and cells involved in these immune system branches (Delves and Roitt, 2000).

The innate immune system offers immediate immunity against invading organisms without prior exposure to antigens. It includes physical barriers like the skin and mucous membranes, as well as cell-mediated barriers such as phagocytic cells, inflammatory cells, dendritic cells, and natural killer cells, along with soluble mediators like cytokines, complement, and acute-phase proteins. This arm of immunity provides early defense while lymphocytes are activated, a process that typically takes several days (Delves and Roitt, 2000). Upon challenge, the innate immune system can activate the acquired immune system, which develops throughout an individual's life. Lymphocytes play a crucial role in this system, modulating other immune cells'

function and directly targeting infected cells (Delves and Roitt, 2000).

The acquired immune system relies on two primary cell types: T-lymphocytes and B-lymphocytes, enabling specific recognition and response to invaders. B-lymphocytes produce antibodies tailored to specific antigens, contributing to the immune system's memory. T-lymphocytes differentiate into various functional cell types with specific cytokine patterns, including T-helper (Th), T-suppressor cells (Ts), cytotoxic (CTL) cells, and regulatory T-cells (Treg). Th cells further divide into Th1 and Th2 subsets, which respectively mediate immunity to intracellular and extracellular pathogens by secreting specific cytokines (Mowat, 2003).

The gut hosts the largest immune organ, the gut-associated lymphoid tissue (GALT), where continuous exposure to various antigens occurs. GALT comprises approximately 60% of the body's lymphocytes, organized into inductive (e.g., Peyer's patches) and effector sites (e.g., lamina propria, intraepithelial lymphocytes), forming a specialized immune network (Iijima et al., 2001).

## 16. ROLE OF PREBIOTICS IN BODY DEFENSE MECHANISM

The body's defense mechanisms are diverse, involving various organs and mechanisms to combat potential threats. Functional food science aims to identify components that can positively influence these defense functions to strengthen, restore, or rebalance them. While essential nutrients have been studied for their beneficial role in immune function, the impact of non-essential food constituents, such as phytochemicals, microbial products, and prebiotics, has not been extensively explored (Calder et al., 2002). Among these constituents, inulin-type fructans have shown promise in positively affecting gastrointestinal functions by modulating mucosal structure, composition, and microbial activity. They also exert beneficial effects on the immune system, particularly intestinal immune functions by targeting gut-associated lymphoid tissue, including Peyer's patches, potentially reducing the risk of gastrointestinal diseases (Guarner, 2005; Watzl et al., 2005).

The composition of the intestinal microflora, crucial for proper immune function, is influenced by dietary components, including prebiotics. In addition to altering microflora composition, prebiotic metabolites may impact the gut-associated lymphoid tissue (GALT), potentially modulating both systemic and local immune responses. Although systematic studies assessing lymphocyte activity or other immune function tests have not been conducted, lactulose administration has been shown to elevate serum glutamine levels, possibly by sparing glutamine as a substrate for the colonic mucosa through increased short-chain fatty

acid production. This effect may have implications for immune function, particularly in scenarios where SCFA production is increased.

## 17. EFFECTS OF PREBIOTICS ON IMMUNE RESPONSE

Numerous studies suggest that the consumption of prebiotics, such as inulin and oligofructose, leads to an increase in beneficial lactic acid bacteria (Buddington et al., 1996; Kleesen et al., 1997; Menne et al., 2000). Research conducted with recognized prebiotics, including oligofructose, has demonstrated an increase in lymphocyte and/or leukocyte numbers in the gut-associated lymphoid tissue (GALT) and peripheral blood (Pierre et al., 1997; Field et al., 1999; Kaufhold et al., 2000). Moreover, lactulose supplementation has been associated with elevated IgA secretion or IgA+ cell counts in the GALT, a decrease in the CD4+/CD8+ ratio in the spleen, and enhanced phagocytic function of intraperitoneal macrophages. Studies in dogs have shown that dietary supplementation of prebiotics positively influences humoral immunity and immunoglobulin status (Pawar et al., 2008). Furthermore, cell-mediated immune responses, such as delayed-type hypersensitivity (DTH) responses, have been enhanced in dogs fed diets supplemented with prebiotics (Pawar et al., 2008). The alteration of the gastrointestinal microflora due to prebiotic supplementation is believed to impact systemic immunological functions, particularly B- and T-lymphocytes (Savage et al., 1996).

Fermentable fibers and prebiotics have been shown to alter the proportion and response of T-cells in GALT, with higher proportions of CD8+ T-cells and CD4+/CD8+ ratios observed in response to high fermentable fiber diets (Field et al., 1999). Oligosaccharides containing mannose have been found to stimulate the immune system by triggering the secretion of mannose-binding protein, which can initiate the complement cascade (Newman, 1994). The effectiveness of prebiotics as immunomodulators in various immune compartments, including GALT, secondary lymphoid tissues, and peripheral circulation, has been extensively reviewed (Schley and Field, 2002). The presence of food in the small intestine is crucial for the development and function of GALT, and dietary components interact closely with the intestinal immune system (Ruthlein et al., 1992). Animal studies suggest that non-digestible constituents, including prebiotics, exert specific immunological effects in the GALT (Field et al., 1999).

While some studies, such as one with dogs supplemented with a low dose of oligofructose, did not find significant effects on bifidobacteria numbers or immunological markers, others support the hypothesis that changes in bifidobacteria

induced by prebiotics supplementation are linked to alterations in immunological functions (Swanson et al., 2002). Additionally, prebiotics may promote an increase in bacterial cell wall components and DNA, stimulating mucosal immune cells.

In summary, short-chain fatty acids (SCFA) produced from prebiotic fermentation may influence immune cells within the GALT, activating them via SCFA receptors and explaining differences in systemic and local immune effects observed in prebiotic-supplemented animals (Bach Knudsen et al., 2003; Field et al., 1999).

## 18. CHALLENGES

### 18.1. Precision nutrition for dogs

Bridging the Gap Between Research and Practice: Achieving precision nutrition tailored to individual canine needs necessitates a comprehensive understanding of the complex interplay between genetic predispositions, environmental factors, and dietary interventions. Translating cutting-edge research findings into practical dietary recommendations poses challenges due to the inherent variability in canine populations and the limited availability of personalized nutrition tools in veterinary practice. Overcoming these challenges requires bridging the gap between researchers and practitioners through interdisciplinary collaboration and the development of user-friendly decision-support tools.

### 18.2. Validating functional food efficacy

Rigorous clinical trials and biomarker identification: while the potential health benefits of functional foods for dogs are widely recognized, robust evidence from well-designed clinical trials is essential to substantiate their efficacy and safety. Challenges arise in identifying appropriate biomarkers of gut health, immune function, and overall well-being in dogs, as well as in standardizing methodologies for assessing functional food interventions. Addressing these challenges necessitates the validation of objective biomarkers through longitudinal studies and the integration of omics technologies to elucidate molecular mechanisms underlying functional food effects.

### 18.3. Standardization of functional food formulations

Ensuring consistency and quality: variability in the composition, quality, and dosage of functional food products presents challenges for both researchers and consumers in evaluating product efficacy and safety. Standardizing formulations and manufacturing processes, as well as implementing rigorous quality control measures, are imperative to ensure consistency and reproducibility across different batches and brands of functional pet foods. Collaboration between industry stakeholders, regulatory agencies, and academic institutions is essential to establish standardized protocols and guidelines for the production

and labeling of functional pet foods.

### 18.4. Advancing understanding of canine gut microbiome dynamics

The canine gut microbiome plays a pivotal role in modulating host metabolism, immune function, and overall health. However, challenges persist in characterizing the complex dynamics of microbial communities in response to dietary interventions, particularly regarding species-specific variations and temporal fluctuations. Addressing these challenges requires longitudinal studies employing high-throughput sequencing technologies and multi-omics approaches to unravel the intricate interactions between diet, microbiota, and host physiology in dogs.

### 18.5. Navigating regulatory frameworks for functional pet foods

Harmonizing standards and ensuring transparency: regulatory oversight of functional pet foods varies widely across jurisdictions, posing challenges for manufacturers, retailers, and consumers in navigating disparate standards and requirements. Harmonizing regulatory frameworks, establishing evidence-based criteria for health claims, and enhancing post-market surveillance mechanisms are critical to ensure consumer safety and promote transparency in the functional pet food industry. Collaborative efforts between regulatory agencies, industry stakeholders, and scientific experts are essential to foster trust and accountability in the marketplace.

### 18.6. Educating stakeholders and promoting informed decision-making

Enhancing public awareness and education about the importance of canine nutrition and the potential benefits of functional foods is essential to empower pet owners to make informed decisions about their pets' diets. Challenges include addressing misconceptions, disseminating evidence-based information, and fostering collaboration between veterinarians, nutritionists, and pet owners. Developing accessible educational resources, professional training programs, and community outreach initiatives can facilitate knowledge transfer and promote responsible pet ownership practices.

### 18.7. Sustainability and ethical considerations

Balancing health, environmental, and social impact: as the demand for functional pet foods grows, ensuring sustainability and ethical sourcing of ingredients becomes increasingly imperative. Challenges include reconciling nutritional quality with environmental impact, promoting ethical sourcing practices, and minimizing packaging waste. Adopting holistic approaches to sustainable pet food production, such as utilizing novel protein sources, implementing eco-friendly packaging solutions, and supporting local supply chains, can mitigate environmental

degradation while safeguarding animal welfare and public health.

## 19. PROSPECTS

### 19.1. *Advancements in omics technologies*

The integration of omics technologies, including genomics, metagenomics, metabolomics, and proteomics, holds promise for elucidating the intricate interactions between diet, gut microbiota, and canine health. Harnessing big data analytics and machine learning algorithms will enable researchers to identify biomarkers of nutritional status, gut microbial composition, and host immune response, facilitating personalized nutrition interventions for dogs.

### 19.2. *Targeted therapeutic nutrition*

As our understanding of canine physiology and pathophysiology advances, there is growing interest in developing targeted therapeutic nutrition strategies for managing specific health conditions in dogs. Tailored diets enriched with bioactive compounds, nutraceuticals, and therapeutic agents can complement traditional veterinary therapies, offering adjunctive support for conditions such as gastrointestinal disorders, joint diseases, and immune-mediated conditions.

### 19.3. *Microbiota-based therapies*

Manipulating the gut microbiota through dietary interventions represents a promising avenue for promoting canine health and preventing disease. Future research may explore microbiota-based therapies, such as fecal microbiota transplantation (FMT), microbial consortia supplementation, and microbial metabolite modulation, to restore eubiosis and mitigate dysbiosis-associated disorders in dogs.

### 19.4. *Nutrigenomics and nutrigenetics*

Nutrigenomics and nutrigenetics research in dogs can elucidate how individual genetic variations influence nutrient metabolism, dietary requirements, and susceptibility to diet-related diseases. Integrating genomic information with dietary recommendations can enable precision nutrition approaches tailored to each dog's unique genetic makeup, optimizing nutrient utilization and health outcomes.

### 19.5. *Functional ingredients discovery*

Continued exploration of novel functional ingredients derived from natural sources, biotechnology, and food processing by-products holds promise for expanding the repertoire of functional foods for dogs. Screening bioactive compounds for their efficacy in modulating canine physiology, gut health, and immune function will drive innovation in pet food formulation and product development.

### 19.6. *Validation of health claims*

Rigorous scientific validation of health claims associated with functional pet foods is essential to ensure transparency, credibility, and consumer trust. Future research should focus on conducting well-designed clinical trials, intervention studies, and mechanistic investigations to substantiate the health benefits attributed to specific functional ingredients and formulations in dogs.

### 19.7. *Environmental sustainability*

The environmental sustainability of pet food production is an emerging concern, prompting the exploration of eco-friendly ingredients, sustainable sourcing practices, and novel production technologies. Research efforts aimed at reducing the ecological footprint of pet food manufacturing while maintaining nutritional quality and palatability will align with growing consumer demand for environmentally responsible pet products.

## 20. CONCLUSION

Canine nutrition is evolving towards tailored interventions with balanced diets and functional foods fortified with prebiotics and probiotics. Prebiotics selectively promote beneficial gut bacteria, aiding gastrointestinal resilience and immune function in dogs. Challenges like optimal dosages and interindividual variability require further investigation. Evidence-driven research is vital to harness prebiotics' potential in canine nutrition, potentially revolutionizing veterinary practice for improved health and longevity.

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