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Review

Potential Effects of Prebiotics on Gastrointestinal and Immunological Modulation in the Feeding of Healthy Dogs: A Review

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Abstract: One of the most studied functional foods in dog feed today is the prebiotic. Prebiotics are known for their modulating effects on the intestinal microbiota, fecal characteristics, and the immune system, which promotes beneficial effects to the host. However, with the diversity of prebiotics in the pet market, there are discussions around which prebiotics to use to stimulate these positive effects. In this case, the objective of this review was to demonstrate the main effects of different prebiotics on the feeding of healthy dogs. Platforms such as Embase, PubMed, and Mendeley were accessed to plot all scientific articles in vivo that reported prebiotics to feed adult or senior dogs. After excluding duplicate articles and without the evaluated criteria, we obtained a total of 36 articles. Our results demonstrated the diversity and concentrations of prebiotics in the feeding of healthy adult and senior dogs. The effects of prebiotics differ according to source, concentration, and length of the supplementation period. Several beneficial effects of different prebiotics have been observed in dogs, such as increased fecal *Lactobacilli* and *Bifidobacteria* concentrations and decreased fecal *Clostridium perfringens* and *Escherichia coli* concentrations, increased short chain fatty acids concentrations, decreased colonic ammonia absorption, and immunomodulatory effects, such as improved humoral immune response and increased phagocytic index. Galactooligosaccharides, fructooligosaccharides, mannanoligosaccharides, yeast cell wall, inulin, and beta-glucans were the most studied prebiotics, which showed potentially promising effects. This is a review that brings the importance and the modulating effects of prebiotics in the feeding of healthy dogs; the effects help the gastrointestinal tract and the immune system.

Keywords: canine; fermentative products; fibers; functional foods; intestinal promoters; intestinal health

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1. Introduction

The function of food is to provide enough nutrients to meet all the metabolic requirements of each species. In that regard, studies have brought a new concept of food, considered functional foods, which represent food ingredients that affect the body physiological functions in an oriented way in order to promote beneficial effects that justify health claims. Among functional foods, the most studied and discussed category today is the prebiotic. The current definition of the term "prebiotic", according to the International Scientific Association for Probiotics and Prebiotics consensus panel, is "a substrate that is selectively utilized by the host microorganisms conferring a health benefit" [1]. In the mid-1950s, it was already known that carbohydrates stimulated the growth of beneficial

bacteria in the intestine; however, their use functional food production began to attract interest years later, when many studies on the functionality of the intestinal microbiota in relation to health aspects have demonstrated the real importance of these carbohydrates, better known as prebiotics [2].

The first definition of prebiotics was according to Roberfroid [3], who reported that prebiotics are nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of bacteria in the colon and then improving the health of the host. However, the prebiotic characteristics were attributed to several food components without considering their criteria and conditions to be considered a prebiotic. Most oligosaccharides and polysaccharides were considered like prebiotics, but not all dietary carbohydrates are prebiotics. Therefore, in order to classify a food ingredient as prebiotic it must adhere to the established criteria. Such criteria are to be resistant to the digestion, absorption, and adsorption processes of the host, to be fermented by the microbiota that colonizes the gastrointestinal system, and to selectively and beneficially stimulate the growth and/or activity of one or more bacteria within the gastrointestinal tract [4]. With the development of omics sciences, knowledge regarding the complexity of the intestinal microbiota and its interaction with the host and diet has improved, and the concept of prebiotics has been revised once again [2].

The factors and mechanisms responsible for modulations in the intestinal microbiota due to ingestion of prebiotics have not yet been elucidated, but studies have shown that cross-feeding occurs so that intestinal fermentative products, such as acetate and lactate, which are produced by *Bifidobacteria* and *Lactobacilli*, can be transformed into butyrate by other species of bacteria beneficial to health [5].

This dynamic indicates that the impact of prebiotics on the modulation of the intestinal microbiota is much more complex than we thought. As a consequence, a new definition for prebiotics was recently expressed, which considers not only the functional and ecological characteristics of the microbiota but also the host physiology, as well as the ecosystems diversity, microorganisms consortia, and production of short chain fatty acids [2].

In addition, it is important to filter that the beneficial bacteria responsible for metabolizing the oligosaccharides are strains of *Lactobacilli* and *Bifidobacteria*. The most known prebiotics and used by the petfood industry are lactulose, fructooligosaccharides (FOS), inulin, yeast cell wall, and mannanoligosaccharides (MOS) [6]; moreover, there has been a recent increase in the number of studies that have investigated the potential effects of beta-glucans [7]. However, in addition, there is a range of potential prebiotics that are being studied.

Currently, FOSs are one of the most studied prebiotics due to their beneficial effects to the host and stability characteristics, which means they are stable at low pH and temperature up to 140 °C. This ingredient is a fructose oligomer linked to glucose and fructose molecules which contains up to ten sugar molecules [8]. Because of this bond, their sweetening profile and water retention properties are similar to those of sucrose and sorbitol [9]. In addition, they have a solubility of 80.0% in water at room temperature [10] and are low-calorie carbohydrates, about 1.0–1.7 kcal/g [11]. In humans, due to the beta configuration of the C2 anomeric atom in the fructose residues of the glycosidic bonds of the molecules, FOSs are not hydrolyzed by digestive enzymes; however, the *Lactobacillus* and *Bifidobacterium* bacteria hydrolyze FOS because it contains the enzymes fructan-beta-fructosidase (exo-inulinase) [8]. In dogs, this hydrolysis also occurs by these bacteria and the FOS is not hydrolyzed in the stomach. Given this process, the FOS-based products are short-chain fatty acids (SCFA—acetic, propionic, and butyric acid), carbon dioxide, hydrogen, methane, amino acid, and vitamin. These SCFAs are absorbed immediately in the small intestine and metabolized by the host to produce energy [12].

Although FOS is not digested by enzymes in the small intestine, it contributes to the supply of energy through the intestinal microbiota. According to Oku and Nakamura [13], the energy available through FOS (2 kcal/g, 8368 kJ/g) corresponds to half the energy of sucrose. One of the products of the FOS fermentation, hydrogen—which is found as

ions in the large intestine and thus is directly combined with C to form CH4 and SCFA—is excreted by expiration and can be evaluated to check the available energy in resistant carbohydrates as a possible fermentation indicator by the intestinal microbiota. Considering this, studies have shown that hydrogen has fundamental functions such as antioxidant, anti-inflammatory, and other protective effects. The amount of hydrogen excreted will vary according to the amount that reaches the large intestine and their fermentability.

Repeated ingestion of FOS contributes to increase the amount of SCFA produced by intestinal bacteria and this increase causes changes in the gastrointestinal environment, which leads to a reduction in pH (pH < 7). Due to acidic conditions, pathogenic bacteria proliferation is suppressed, which helps to reduce its putrefactive fermentative products as volatile organic compounds [12]. Considering all these FOS functions and products, it should be noted that these properties depend on the molecular structure, source, and concentration of the prebiotic in the food.

Mannanoligosaccharides is derived from the external cell wall of *Saccharomyces cerevisiae*, one of the substrates also most used in studies associated with dog health [14]. One of its prebiotic properties is due to its resistance to pathogenic bacteria present in the intestinal microbiota. MOS acts as a ligand for type 1 fimbriae, which results in a reduction in pathogenic bacterial proliferation. Studies have shown that MOS was actually able to reduce the concentrations of *Clostridium perfringens* and *Escherichia coli* in the feces of dogs. These bacteria are considered pathogenic because their fermentation products are associated with fecal putrefactive compounds and also because of their association with diarrhea [15,16]. In addition to promoting this intestinal pathogenic reduction, MOS, like FOS, modulates the proliferation of *Lactobacilli* and *Bifidobacteria* [17].

Given the main characteristics of prebiotics, galactooligosaccharides are considered bifidogenic and lactogenic prebiotics due to their selective stimulation of *Bifidobacteria* and *Lactobacilli*. These bacteria take advantage of GOS as the only carbon source, which increasingly encourages the use of this prebiotic. This prebiotic substrate is composed of galactose polymers containing a galactose or glucose monomer and is produced by enzymatic transgalactosylation of glucose, galactose, or lactose. In fact, the interest in GOS is due to their resistance to digestion in the gastrointestinal tract (GI tract), with more than 90.0% of the GOS being available for bacterial fermentation in the distal region in the GI tract. Studies also do not exclude the possibility of cross-feeding within the microbial population [18].

Beta-glucans and mannanoligosaccharides are found in high concentrations in the yeast cell wall. Beta-glucans are one of the prebiotics recently used in studies with dogs in order to investigate their gastrointestinal and immunological effects [19]. According to El Khoury et al. [20], the source of beta-glucans will influence the type of bond, structural branching, solubility, and molecular weight, which can affect their functional properties. These, derived from yeast, contain a linear backbone of beta-1,3-glycosidic bonds replaced by a limited amount of beta-1,6-linked side chains [21]. Rychlik et al. [22] demonstrated that beta-glucans derived from *S. cerevisiae* promoted a reduction in the activity of inflammatory bowel disease, decreasing levels of the pro-inflammatory cytokine IL-6, and increased concentrations of anti-inflammatory IL-10. Additionally, another study reported that 10 μg/mL of beta-glucans increased the production of pro-inflammatory compounds in response to bacterial stimulations. Although the study was in vitro, it indicates that canine macrophages are able to undergo immunological training with the inclusion of beta-glucans, and also promote an increase in TNF-α, IL-6 and IL-1. Trained immunity, evidence of a form of immune memory linked to immunocyte epigenetic reprogramming, acts independently on T and B cells and involves macrophages, dendritic cells, and killer natural cells.

Therefore, this study helps to consolidate the trained immunity to the host's defense mechanisms [23]. The mechanisms of action of beta-glucans without canine metabolism are not at all known, as this prebiotic is recently studied in this species. As a result of the above, most prebiotics are bifidogenic and lactogenic, and go through all the criteria to be considered prebiotics. However, do all prebiotics have beneficial effects in isolation

or together (in a mixture with more substrates) in dogs? Are the used doses considered safe or do they really promote all the potential effects of prebiotics? Or does ingesting prebiotics over long periods promote the same effects? Indeed, the aim of the study was to demonstrate the potential effects of different prebiotics on the feeding of healthy dogs.

2. Development

The articles were searched for by the Embase and PubMed and platforms, based on the keywords described in Table 1. After the research, the articles were plotted in the Mendeley® program in order to exclude duplicates and articles that do not involve the ingestion of prebiotics in healthy adult dogs and healthy senior dogs.

The results considered by the authors were of $p < 0.05$, and tendencies were not considered as significant.

Table 1. Search terms, databases, and number of results found about health studies with prebiotics supplementation.

Database/Date Covered/Number of Results	Search Terms
Embase **Data covered: not specified, all years were searched** **Number of results: 1418**	((‘dog’/exp OR ‘canis canis’ OR ‘canis domesticus’ OR ‘canis familiaris’ OR ‘canis lupus familiaris’ OR ‘dog’ OR ‘dogs’ OR ‘animal model’/exp OR ‘animal model’ OR ‘model, animal’ OR ‘models, animal’) AND (‘prebiotic’ OR ‘prebiotic dog’ OR ‘prebiotics dog’ OR ‘prebiotics’ OR ‘FOS dog’ OR ‘fructoligosaccharide’ OR ‘fructoligosaccharide dog’ OR ‘MOS’ OR ‘MOS dog’ OR ‘mannanoligosaccharide’ OR ‘mannanoligosaccharide dog’ OR ‘inulin’ OR ‘inulin dog’ OR ‘GOS’ OR ‘GOS dog’ OR ‘galactooligosaccharide’ OR ‘galactooligosaccharide dogs’ OR ‘fiber’ OR ‘fiber dogs’ OR ‘fiber blend’ OR ‘prebiotic blend’ OR ‘prebiotic blend dogs’ OR ‘fiber blend dogs’ OR ‘kestose’ OR ‘kestose dogs’) AND (‘article’/it OR ‘article in press’/it OR ‘conference abstract’/it OR ‘conference paper’/it OR ‘short survey’/it))
Pubmed **Data covered: not specified, all years were searched** **Number of results: 1293**	((dog OR canis canis OR canis domesticus OR canis familiaris OR canis lupus familiaris OR dog OR dogs OR animal model) AND (metabolism FOS OR metabolism prebiotic OR prebiotic OR prebiotics dogs OR FOS dogs OR MOS dogs OR GOS OR GOS dog OR galactooligosaccharide OR mannanoligossacharide OR fructoligossacharide OR inulin OR lactic acid dog OR nitrogen dog OR fiber OR fiber blend OR fiber dogs OR prebiotics blends OR fiber blend dogs OR kestose OR kestose dogs) AND (article/it OR article in press/it OR book/it OR chapter/it OR conference abstract/it OR conference paper/it OR conference review/it OR short survey/it)

3. Prebiotics in Dogs

All the articles regarding prebiotics in healthy dogs were obtained and then exported to Mendeley® citation manager. After duplicates were removed, a total of 1709 remained. The authors decided to consider only those with in vivo utilization of prebiotics in healthy adult and old dogs to conduct a more detailed analysis on the subject. The articles selected and added to this review were searched through May 2023. After removing articles regarding in vitro use, use in puppies, and non-healthy adults, and studies that used medication during prebiotic intake, 36 articles were used for the state-of-the-art analysis (Table 2). The results of the articles were discussed afterward.

Table 2. The state-of-the-art analysis of studies conducted with healthy adult dogs with prebiotic supplementation obtained after systematic research.

1. **Diez et al.** [24]	
Population:	Healthy adult dogs.
Sample size:	Eight adult male Beagles.
Intervention details:	These animals were from 1 to 1.4 years old. Three diets were used: control diet (A), 5.0% of fructooligosaccharides (FOS) and sugar beet fiber blend (B), and 10.0% of the same blend on a dry-matter basis (C). The duration of the study was 210 days; each diet was fed for 6 weeks, and each period of the block was followed by a 4-week washout period.
Study design:	Crossover design, randomized block design.
Outcome studied:	To evaluate the inclusion of a blend of FOS and sugar beet fiber at different incorporation rates on nutrient digestibility and plasma glucose, insulin, alpha-amino nitrogen, urea, cholesterol, and triglycerides concentrations.
Main findings: (relevant to PICO question):	The diets with the blend decreased postprandial glucose (C), urea (B and C), and triglyceride (B and C) concentrations. Pre-prandial measurements were decreased urea (B and C), cholesterol (C), and triglycerides (B and C). Diet C decreased protein digestibility.
Limitations:	The authors could have used a diet without sugar beet fiber to better understand which fiber influenced the results.
Conclusions	Chronic intake of fermentable fiber was associated with a slight reduction in protein digestibility and metabolic effects at both pre- and postprandial times.
2. **Howard et al.** [25]	
Population:	Health adult dogs.
Sample size:	Twenty-eight adults female Beagles.
Intervention details:	Four treatments were used: 15 g beet pulp/kg of dry matter (BP), 60 g short chain FOS/kg of dry matter (FOS), 60 g fiber blend/kg of dry matter (FB) and 60 g cellulose/kg of dry matter (C). The duration of the study was 35 days.
Study design:	Dogs were randomly assigned to 1 of 4 treatments (7 dogs/treatment).
Outcome studied:	To use fiber sources, differing in degree of fermentability and end-product formation, to alter N excretion patterns and intestinal microbiota populations.
Main findings: (relevant to PICO question):	When dry matter intake was expressed as a percent of body weight, intake was reduced for dogs fed FOS diet compared to dogs fed C, while intake of FB and BP diets were intermediate between FOS and C diets. Fecal N and microbial N excretion was greater with the FB diet. Bacterial characterization of intestinal contents found that FOS increased total aerobic bacteria in the distal colon. Fiber Blend decreased counts of *Clostridium* spp.
Limitations:	The study has no limitations.
Conclusions:	The results suggest that fermentable fiber sources have the potential to repartition N from urine to feces, so they have the potential to trap and remove N from the body.
3. **Strickling et al.** [15]	
Population:	Healthy ileal T-cannulated adult dogs.
Sample size:	Seven mixed-breed female dogs.
Intervention details:	Four treatments were used: control, 5 g FOS/kg of dry matter (DM), 5 g/kg DM of mannan oligosaccharide (MOS), and xylooligosaccharide (XOS). The duration of the study was 21 days.
Study design:	Incomplete 4 × 7 Latin square design.
Outcome studied:	To evaluated ileal and total tract effects on nutrient digestion and microbial populations when oligosaccharides are added to dog diets.
Main findings: (relevant to PICO question):	There were no differences in DM digestibility, diet, or fecal nitrogen digestibility, ileal or fecal ammonia, fecal consistency, ileal bacteria colony-forming units, blood glucose, or ileal pH. Fecal *Bifidobacteria* numbers were unaffected by dietary treatment.
Limitations:	Each treatment had seven replications except for bacterial samples, which had six.
Conclusions:	The effects of dietary inclusion of oligosaccharides depend on the dose as well as their specific structure. The inclusion of 5 g/kg oligosaccharides in diets offers little benefit for dogs.
4. **Willard et al.** [26]	
Population:	Healthy elderly dogs.
Sample size:	Six female Beagles.

Table 2. *Cont.*

Intervention details:	These animals were 7 and 8 years old. Group A received a diet with supplementation of 1.0% of FOS/kg of diet; Group B received a control diet. Group A received supplementation for 32 days. Before the supplementation period, group A received a diet without supplementation, and group B received diet supplementation for 87 days.
Study design:	Dogs were randomly assigned to two groups of three dogs each.
Outcome studied:	To evaluate fecal concentrations of selected genera of colonic bacteria in healthy dogs, and to investigate effects of dietary FOS.
Main findings: (relevant to PICO question):	Isolated *Bacteroidetes* spp. demonstrated that the diet and time did not affect fecal bacterial concentrations. Group A had higher fecal concentrations of *Bacteroidetes* spp. FOS supplementation did not have a significant effect on fecal concentrations of *Escherichia coli*.
Limitations:	The days of supplementations were different between groups A and B. The authors observed that *Bifidobacterium* spp. and *Lactobacillus* spp. were inconsistently isolated from fecal samples and this was not considered reliable. The study did not evaluate the genera, phylum, family, and bacteria abundance.
Conclusions	Dietary inclusion of 1% FOS was not sufficient to obtain effects on fecal concentrations of *Bifidobacterium* spp. in healthy elderly dogs.
5. **Beynen et al.** [27]	
Population:	Healthy adult dogs.
Sample size:	Six dogs.
Intervention details:	These animals were 2 to 10 years old; there were three male and one female Beagle, and two male Schnauzers. Three diets were used: a control diet (without lactulose), a diet with 1 g of lactulose/MJ of metabolizable energy (ME), and a diet with 3 g of lactulose. The duration of the study was 2 weeks.
Study design:	A 3 × 3 Latin square design.
Outcome studied:	To evaluate the effect of lactulose on the route of nitrogen excretion.
Main findings: (relevant to PICO question):	Fecal pH and apparent nitrogen digestibility decreased in the lactulose diet.
Limitations:	The study has no limitations.
Conclusions:	Lactulose intake increased nitrogen excretion by feces, which may result in lower colonic ammonia absorption, which is considered beneficial for animals with liver disease. There was a dose-dependent effect on the apparent absorption of calcium and magnesium, but not phosphorus.
6. **Beynen et al.** [28]	
Population:	Healthy adult dogs.
Sample size:	Five dogs.
Intervention details:	These animals were three male Beagles and one female and one male Schnauzer, with age ranging between 3 and 11 years old. Two treatments were used: a control diet and a diet with 1.0% of oligofructose on a DM basis (test diet). The duration of the study was 21 days.
Study design:	Cross-over design, placebo-controlled.
Outcome studied:	To evaluate the effects of oligofructose in fecal microflora, nitrogen excretion, and mineral absorption.
Main findings: (relevant to PICO question):	The characteristics of feces and urine did not differ. The total anaerobic bacteria, total aerobic bacteria *Bifidobacteria*, *Streptococci*, and *Clostridia* increased with the test diet. The calcium and magnesium absorption increased with the test diet.
Limitations:	The study has no limitations.
Conclusions:	Dietary inclusion of 1% oligofructose may provide beneficial effects such as fecal microbiota modulation, including increased *Bifidobacteria* concentrations, and significantly increased magnesium and calcium absorption in dogs.
7. **Grieshop et al.** [29]	
Population:	Healthy ileal-cannulated dogs.
Sample size:	Seven purpose-bred female dogs.
Intervention details:	Four treatments were used: control diet, 0.55 g/d, and 1.65 g/d of arabinogalactan (AG) provided via gelatin capsules. Three forms of AG were provided by Larex, raw AG (AG100), Laraceutical (AG1000) and Fiberaid (AG3000). The duration of the study was 10 days.
Study design:	A 7 × 7 Latin square design.
Outcome studied:	To determine the effects of AG on digestive events and immune status of adult dogs.

Table 2. *Cont.*

Main findings: (relevant to PICO question):	Fecal scores increased in dogs supplemented with the low dose of AG1000. Dogs supplemented with the low dose of AG1000 and the high dose of AG3000 had higher concentrations of fecal *Lactobacilli*. AG treatments did not affect serum immunoglobulin (Ig) G, M, or A concentrations.
Limitations:	The study has no limitations.
Conclusions:	Specific forms and doses of AG increased serum lymphocyte concentrations and affected the fecal microbial concentrations.
8. **Swanson et al.** [14]	
Population:	Healthy ileal-cannulated adult dogs.
Sample size:	Four purpose-bred female dogs.
Intervention details:	These dogs were 2.5 years old. Four treatments were used: control diet, 1 g FOS/day, 1 g mannooligosaccharides (MOS)/day, and 1 g FOS plus 1 g MOS/day via gelatin capsule. The duration of the study was 14 days.
Study design:	A 4 × 4 Latin square design.
Outcome studied:	To investigate whether supplemental FOS and MOS influenced the immune function, the microbial populations, and the concentrations of fermentative end products in the large bowel of dogs.
Main findings: (relevant to PICO question):	*Bifidobacterium*, *E. coli*, and *C. perfringens* concentrations were not different among treatment groups. Surprisingly, the supplementation of FOS did not result in any differences in fecal microbial populations. Did not differ fecal short-chain fatty acids (SCFA) concentrations in treatments. Concentrations of ammonia, branched-chain fatty acids (BCFA), indoles, and phenols did not differ among treatments. When lymphocyte data were analyzed as a percentage of total WBC, dogs supplemented with MOS had a greater proportion of serum lymphocytes compared to that of control dogs. No differences were observed in serum IgG or IgM.
Limitations:	The study had a small sample size.
Conclusions:	Fructooligosaccharide plus mannanoligosaccharide supplementation had a positive effect on the intestinal microbiota of healthy adult dogs, as it increased fecal concentrations of *Bifidobacteria*, as well as fecal and ileal *Lactobacilli* concentrations. It was not possible to determine whether the observed effects were due to FOS, MOS, or both.
9. **Swanson et al.** [30]	
Population:	Healthy ileal-cannulated dogs
Sample size:	Eight purpose-bred adult female dogs.
Intervention details:	These dogs were 3.3 years old. Two diets were used: 1 g sucrose (control) and 2 g FOS plus 1 g MOS orally via gelatin capsule. The duration of the study was 32 days.
Study design:	Placebo-controlled, crossover design.
Outcome studied:	To determine whether supplemental FOS and MOS affected ileal and fecal microbial populations as well as indices of immune function.
Main findings: (relevant to PICO question):	Ileal and fecal pH were not different among treatments. The supplemental diet increased ileal and fecal microbial populations, increased *Lactobacillus*, *Bifidobacterium*, and total aerobe populations. *E. coli* and *C. perfringens* did not differ among treatments. The variables of immune function did not differ among treatments.
Limitations:	The study has no limitations.
Conclusions:	MOS alone and MOS plus FOS supplementation had positive effects on gut health. FOS alone did not result in significant differences compared to the control group.
10. **Swanson et al.** [31]	
Population:	Healthy ileal-cannulated adult dogs.
Sample size:	Four purpose-bred adult female dogs.
Intervention details:	Four treatments were used: control, 1 g FOS, 1 g MOS, 1 g FOS, plus 1 g MOS. The administration of the supplement was via gelatin capsules. The duration of the study was 14 days.
Study design:	A 4 × 4 Latin square design.
Outcome studied:	To evaluate whether supplemental FOS and MOS influenced indices of gut health of dogs.
Main findings: (relevant to PICO question):	MOS diet decreased fecal total aerobes. Lymphocytes (% of total white blood cells) were increased in dogs fed a diet with MOS. Total fecal indole and phenol concentrations were decreased in FOS and FOS plus MOS diets.
Limitations:	The study has no limitations.
Conclusions:	MOS supplementation had positive effects on fecal microbiota, fecal nitrogen products, and immunity, while FOS plus MOS supplementation provided beneficial effects on fecal nitrogen products.

Table 2. *Cont.*

11. **Zentek et al.** [32]	
Population:	Healthy adult dogs.
Sample size:	Four adult female Beagles.
Intervention details:	They used six diets: the basal diet I, basal diet plus 1 g/kg BW/d of MOS, basal diet plus 1 g/kg BW/d of transgalactooligosaccharides (TGOS), basal diet plus 1 g/kg BW/d of lactose, basal diet plus 1 g/kg BW/d of lactulose, and basal diet II. The basal diets were fed before and after the supplementation of fermentable carbohydrates. The duration of the study was 42 days.
Study design:	A 4 × 4 Latin square design.
Outcome studied:	To investigate the effects of MOS, TGOS, lactose, and lactulose supplementation on the fecal quality, nutrient, and mineral digestibility, and on some products of intestinal microbial metabolism.
Main findings: (relevant to PICO question):	The apparent digestibility of dry matter, crude protein, and nitrogen-free extracts, unbound water, and fecal pH decreased with MOS supplementation. Apparent absorption rates of minerals were not influenced by the type of diet. The ammonia concentrations were decreased after the addition of MOS than that in basal diet I and the diet with lactulose. The fecal volatile fatty acids (VFA) concentrations did not differ between diets.
Limitations:	They analyzed ammonia and VFA concentrations in in vitro incubation and the results were not considered in the review.
Conclusions:	Supplementation of lactose, lactulose, or transgalactooligosaccharides was not sufficient to change measures of microbial metabolism, compared to those of the control periods. Mannanoligosaccharides resulted in a lower fecal pH, ammonia excretion, and apparent digestibilities of crude protein, nitrogen-free extracts, and dry matter.
12. **Flickinger et al.** [33]	
Population:	Healthy adult dogs.
Sample size:	Exp. 1: sixteen male Beagles, and Exp. 2: four cannulated purpose-bred females.
Intervention details:	Exp. 1: these dogs were 3 years old. Four treatments were used: control diet, and diets with 0.3% of oligofructose (OF-hydrolyzed inulin), 0.6% of OF, and 0.9% OF. The duration was 22 days. The four control-fed animals all remained in the control group, whereas the 12 OF-fed dogs were grouped. After removing five dogs due to outlying values in feed and OF consumption, the OF-supplemented group consumed a diet equivalent to 0.6% dietary OF on a DM basis. Exp. 2: these dogs were 3 ± 1 years old. Four treatments were used: control diet, 1 g/d of short-chain FOS (scFOS), 2 g/d of scFOS, and 3 g/d of scFOS. The experiment lasted 56 days.
Study design:	Exp. 1: completely randomized design. Exp. 2: 4 × 4 Latin square design.
Outcome studied:	To evaluate the effects of selected dietary concentrations of scFOS and OF on nutrient digestibility, gastrointestinal tract microbial populations, and fecal and urinary protein fermentation end-product components of dogs.
Main findings: (relevant to PICO question):	Exp. 1: Total-tract digestibility of DM, original matter (OM), and lipid were decreased in OF treatments; no effects of OF supplementation were observed on fecal DM, pH, and score. Propionate concentrations were increased in OF diet. Other variables did not differ. Exp. 2: Only total aerobe concentrations were linearly increased by scFOS and other variables did not differ.
Limitations:	The study did not use the same days in both experiments and this time could cause different effects. The authors removed five dogs because they had outlying values, and this resulted in different dogs in the control diet (n = 4) and supplemental diet (n = 7).
Conclusions:	Oligofructose supplementation was not sufficient to promote effects on the fecal variables measured, except for nutrient digestibility, in which there was a decrease. Short-chain fructooligosaccharides supplementation was not sufficient to alter fecal concentrations of branched-chain fatty acids, short-chain fatty acids, ammonia, phenols, and indoles; however, it was effective in increasing and decreasing fecal concentrations of total aerobes and *Clostridium perfringens*, respectively.
13. **Hesta et al.** [34]	
Population:	Healthy adult dogs.
Sample size:	Eight Beagles.
Intervention details:	Four dogs received 3.0% of FOS (group I) and four dogs received 3.0% of isomalt-oligosaccharide (IMO) (group II). Both groups received the supplementation for 14 days.
Study design:	Completely randomized design.
Outcome studied:	To evaluate the effect of adding prebiotics to diets enriched with animal-derived protein sources on apparent digestibility and fecal ammonia concentration.

Table 2. *Cont.*

Main findings: (relevant to PICO question):	The supplementation decreased the DM and ether extract apparent digestibility and the bacterial nitrogen content in the feces was highest in the oligosaccharide groups. The fecal production increased by FOS and IMO supplementation with meat and bone meal. The poultry meal with oligosaccharide supplementation decreased ammonia concentration per % DM. Oligosaccharides did not reduce the fecal ammonia concentrations
Limitations:	It was not verified whether the prebiotic added with different protein sources offer a benefit. It does not state how many animals were fed the diet with meat and bone meal protein, crackling, and chicken meal. It does not state how many animals from each protein base received FOS or IMO. Variables could have been analyzed as repeated measures over time.
Conclusions:	Oligosaccharide supplementation decreased nutrient digestibility and was not effective in decreasing fecal ammonia concentrations.
14. **Propst et al.** [35]	
Population:	Healthy ileal-cannulated adult dogs.
Sample size:	Seven purpose-bred female dogs.
Intervention details:	Seven treatments were used: control diet, 0.3% OF, 0.6% OF, 0.9% OF, 0.3% inulin (I), 0.6% I and 0.9% I, all in as-fed basis. The experiment lasted 14 days.
Study design:	A 7 × 7 Latin square design.
Outcome studied:	To investigate the effects of selected oligofructose and inulin concentrations on nutrient intake, nutrient digestibility, stool quality, and fecal protein catabolites in healthy adult dogs fed a meat-based kibble diet.
Main findings: (relevant to PICO question):	Intakes and ileal digestibility of nutrients did not differ. Total tract-digestibility of DM, OM, and crude protein (CP) decreased with OF and inulin supplementation. OF and inulin increased fecal ammonia concentrations, fecal SCFA, and isovalerate. Linear increases were observed in total amines in dogs fed OF. Animals fed inulin had decreased total phenol.
Limitations:	The study has no limitations.
Conclusions:	Dietary inulin and oligofructose supplementation promoted positive effects on intestinal health variables in dogs, without seriously compromising nutrient digestibility or stool quality. The best results were observed in the group that consumed a diet with the inclusion of 0.9% oligofructose.
15. **Grieshop et al.** [17]	
Population:	Healthy geriatric dogs.
Sample size:	Thirty-four Beagles (n = 16) and Pointers (n = 18).
Intervention details:	Beagles were 9 to 11 years old (five females and eleven males) and Pointers were 8 to 11 years old (nine females and nine males). Four treatments were used: control diet (CON), 1.0% dietary chicory (CH), 1.0% dietary MOS, and 1.0% chicory plus 1.0% MOS (CM). The duration of the study was 56 days (4 weeks baseline period and 4 weeks treatment period).
Study design:	Randomly divided into two blocks of 18 animals.
Outcome studied:	To determine the effects of MOS and inulin supplementation on colonic microbial populations and immune characteristics in senior dogs.
Main findings: (relevant to PICO question):	Wet fecal output was increased in dogs supplemented with MOS and CM. CM treatment increased fecal scores, but it was within the acceptable range. CH and MOS supplementation increased concentrations of fecal *Bifidobacteria* compared to CON. MOS supplementation decreased fecal *E. coli* concentrations compared to those of CON. Peripheral lymphocyte concentrations were decreased in MOS and CM supplementation compared to CON.
Limitations:	The author found it difficult to determine exactly when an animal reaches a geriatric or senior age.
Conclusions:	Chicory and mannan-oligosaccharides supplementation promoted beneficial effects on the fecal microbiota of healthy geriatric dogs. In addition, only effect trends were observed in the other variables evaluated.
16. **Middelbos et al.** [16]	
Population:	Healthy ileal-cannulated adult dogs
Sample size:	Six purpose-bred adult female dogs
Intervention details:	These dogs were 4.5 years old. Six treatments were used: control diet, 2.5% cellulose, 2.5% beet pulp, 1.0% cellulose plus 1.5% scFOS (CF), 1.0% cellulose plus 1.2% scFOS and 0.3% yeast cell wall (YCW) (CFY1), 1.0% cellulose plus 0.9% scFOS and 0.6% YCW (CFY2). The duration of the study was 14 days.
Study design:	A 6 × 6 Latin square design.
Outcome studied:	To investigate the effects of fermentable carbohydrates on intestinal health, intestinal microbiota, and immune status.

Table 2. *Cont.*

Main findings: (relevant to PICO question):	Total tract DM and OM digestibility were lowest for the cellulose treatment. The blends decreased crude protein digestibility, increased fecal *Bifidobacteria*, and increased fecal butyrate. The beet pulp diet increased total fecal SCFA when compared with the control and cellulose treatments. Immune indices were not affected by treatment.
Limitations:	The authors did not use a diet with only YCW or FOS.
Conclusions:	The dietary addition of fermentable and non-fermentable fiber blends promoted results similar to the dietary addition of beet pulp on gut health variables of healthy adult dogs.
17. **Middelbos et al.** [36]	
Population:	Healthy ileal-cannulated adult dogs.
Sample size:	Five purpose-bred female dogs.
Intervention details:	These dogs were 4 years old. Five treatments were used: control diet (without YCW), 0.05% DM of YCW, 0.25% DM of YCW, 0.45% DM of YCW, and 0.65% DM of YCW. The YCW was administrated via oral gelatin capsules. The duration of the study was 14 days.
Study design:	A 5 × 5 Latin square design.
Outcome studied:	To evaluate dose-response effects of the YCW on nutrient digestibility, immunological indices, and fecal microbiota in adult dogs.
Main findings: (relevant to PICO question):	Total tract DM digestibility responded cubically to YCW supplementation, as did total tract OM, CP, acid hydrolysis fat (AHF), gas energy (GE), and insoluble dietary fiber (IDF) digestibility. Fecal pH responded cubically to YCW supplementation. Fecal output per gram of feed intake responded quadratically to YCW supplementation, with the lowest fecal production at the 0.45% supplementation level. Monocyte concentrations decreased linearly with YCW supplementation. *Bifidobacteria*, *Lactobacilli*, total anaerobe, and total aerobe populations were not affected by YCW, but *E. coli* populations decreased linearly with YCW supplementation. Analysis of total fecal microbial DNA based on denaturing gradient gel electrophoresis (DGGE) results indicated an increasing quadratic effect of YCW supplementation. Compared with the control treatment, the 0.25% supplementation level had the greatest coefficient, indicating that total fecal microbial DNA.
Limitations:	The study did not analyze SCFA, BCFA, and ammonia.
Conclusions:	Yeast cell wall supplementation in dogs at less than 1% affected ileal and total tract nutrient digestibility, and it decreased fecal *Escherichia coli* concentrations.
18. **Barry et al.** [37]	
Population:	Healthy adult dogs.
Sample size:	Five ileal-cannulated female dogs.
Intervention details:	These dogs were 5.5 years old. Five diets were used: control diet, 0.2% inulin, 0.4% inulin, 0.2% scFOS and 0.4% scFOS. The study lasted 18 days.
Study design:	A 5 × 5 Latin square design.
Outcome studied:	To determine the effects of 0.2 and 0.4% inulin and scFOS on ileal and total tract nutrient digestibility, ileal IgA concentrations, stool protein catabolite concentrations, and microbiota in feces of healthy adult dogs.
Main findings: (relevant to PICO question):	No differences were observed in ileal pH, IgA, ammonia, or fecal concentrations of indole and valerate. Ileal DM, OM, and CP digestibility coefficients; total tract DM and OM digestibility coefficients; and fecal concentrations of phenylethylamine increased linearly, and fecal concentrations of phenol decreased linearly with inulin supplementation. Fecal concentrations of acetate, propionate, and total SCFA decreased quadratically with inulin supplementation. Ileal DM, OM, and CP digestibility coefficients increased linearly, and fecal phenol concentration decreased linearly with scFOS supplementation. Total tract DM and OM digestibility coefficients, as well as fecal butyrate and isobutyrate concentrations, increased quadratically with scFOS supplementation. No significant differences or trends were noted among treatments in fecal microbiota concentrations.
Limitations:	The study has no limitations.
Conclusions:	Low levels of inulin or short-chain fructooligosaccharides were effective in improving nutrient digestibility and in variably modifying the fecal metabolites concentrations, but the levels of prebiotics used were not sufficient to affect the fecal microbiota.
19. **Stuyven et al.** [38]	
Population:	Healthy adult dogs.
Sample size:	Twenty-nine female Beagles.
Intervention details:	These dogs were 3 to 4 years old. Experiment 1: ten and nine dogs fed glucans (Macrogard®, 150 mg beta-1,3/1,6-glucan) and control diet, respectively. Experiment 2: five dogs fed glucans (Macrogard®, 150 mg beta-1,3/1,6-glucan) and five dogs fed the control diet, and all dogs were challenged with vaccination. The duration of the study was 5 weeks.

Table 2. *Cont.*

Study design:	Placebo-controlled.
Outcome studied:	To evaluate the effects of oral supplementation with MacroGard on the isotype-specific serum antibody response in healthy dogs against a subcutaneous vaccine on the total IgA, IgM, and IgG concentrations.
Main findings: (relevant to PICO question):	Exp. 1: At the end of four weeks, the total serum IgA level decreased in the group treated with glucans compared to the control group. The total serum IgM level increased in the group with the supplementation, but no effect was observed on the IgG level. Exp. 2: Similar changes were seen in *Bordetella*-specific IgA and IgM titers following vaccination during the supplementation period. The IgA concentration decreased in the saliva and tears of the glucan-supplemented group. The effects disappeared one week after the cessation of the supplementation.
Limitations:	The study has no limitations.
Conclusions:	Oral administration of Macrogard beta-1,3/1,6-glucan influenced the humoral immune response. Oral administration of beta-glucans had an effect on systemically induced IgA and IgM responses, but not on the IgG response.
20. **Beloshapka et al.** [39]	
Population:	Healthy adult dogs.
Sample size:	Eight adult female hound-mix dogs.
Intervention details:	These dogs were 3.5 years old. Four treatments were used: 0 (control diet), 0.5% DM of polydextrose, 1.0% of polydextrose, and 1.5% of polydextrose. The polydextrose was incorporated into diets before extrusion. The duration of the study was 14 days.
Study design:	A 4 × 4 Latin square design.
Outcome studied:	To determine the effects of polydextrose on fecal characteristics, microbial populations, and fermentative end products.
Main findings: (relevant to PICO question):	Total dietary fiber decreased with increasing polydextrose concentrations. Polydextrose increased fecal scores (loser stools), but no diarrhea was observed. Fecal pH decreased linearly with increasing polydextrose. Fecal acetate, propionate, and total SCFA concentrations increased linearly with increasing dietary polydextrose. Polydextrose had a quadratic effect on fecal isobutyrate concentrations in which concentrations increased by 0.5% and 1.0%, but decreased by 1.5% in comparison with the control diet. Fecal *C. perfringens* decreased linearly with increasing dietary polydextrose concentrations, but *E. coli*, *Lactobacillus* spp. and *Bifidobacterium* spp. were not affected by the inclusion of polydextrose in the diet.
Limitations:	The study has no limitations.
Conclusions:	The polydextrose supplementation provided positive effects on variables that may reflect gut health, such as short-chain fatty acids and fecal pH, and it was efficient in decreasing fecal *Clostridium perfringens* concentrations.
21. **Faber et al.** [40]	
Population:	Healthy adult dogs.
Sample size:	Six female dogs.
Intervention details:	These dogs were 3.4 years old. Six treatments were used: 0 (control diet), 0.5% DM of galactoglucomannan oligosaccharide (GGMO), 1.0% of GGMO, 2.0% of GGMO, 4.0% of GGMO and 8.0% of GGMO. The duration of the study was 14 days.
Study design:	A 6 × 6 Latin square design.
Outcome studied:	To evaluate the nutritional effects and prebiotic potential of a spray-dried GGMO substrate.
Main findings: (relevant to PICO question):	Uncorrected total dietary fiber intake values decreased linearly with increased supplementation of the GGMO substrate. Fecal DM output decreased linearly as the GGMO substrate concentration increased from 0 to 8%. DM and OM digestibility was increased for the 4 and 8% supplemental GGMO treatments. CP digestibility decreased quadratically as dietary GGMO concentration increased. Fecal concentrations of acetate, propionate, and total SCFA increased linearly as GGMO concentrations increased, whereas butyrate concentration decreased linearly. Fecal isobutyrate, isovalerate, and total BCFA concentrations were not different among treatments. Fecal pH decreased linearly as dietary GGMO concentration increased, whereas fecal score increased quadratically. Fecal phenol and indole concentrations decreased linearly as GGMO concentration increased. Phenylethylamine was decreased linearly as GGMO concentration increased. Fecal microbial concentrations of *E. coli*, *Lactobacillus* spp., and *C. perfringens* were not different among treatments. A quadratic increase was noted for *Bifidobacterium* spp. as GGMO concentration increased.
Limitations:	The study has no limitations.
Conclusions:	Dietary inclusion of galactoglucomannan oligosaccharide promoted positive effects on fecal fermentation end-product production and fecal pH, although it had little effect on fecal microbiota.

Table 2. *Cont.*

22. **Beloshapka et al.** [41]	
Population:	Healthy adult dogs.
Sample size:	Six female Beagles.
Intervention details:	These dogs were 5.5 ± 0.5 years old. Six treatments were used: (1) beef control; (2) beef plus 1.4% inulin; (3) beef plus 1.4% YCW; (4) chicken control; (5) chicken plus 1.4% inulin; and (6) chicken plus 1.4% YCW. The duration of the study was 21 days.
Study design:	A 6 × 6 Latin square design.
Outcome studied:	To evaluate the fecal microbial communities of the dogs fed inulin or YCW.
Main findings: (relevant to PICO question):	Diet 1 increased *Escherichia*, but decreased *Anaerobiospirillum* vs. diet 4. Inulin (2) decreased *Enterobacteriaceae* and increased *Megamonas* vs. control diet 1. Inulin (2) also decreased *Escherichia* vs. YCW. YCW increased *Bifidobacterium* vs. inulin and control and inulin increased *Lactobacillus* vs. YCW. A significant increase in fecal SCFA concentrations was evident when dogs were fed beef-based diets with inulin and YCW extract. Fecal spermine concentrations were higher in diets containing inulin and YCW than in control diets.
Limitations:	No limitations worth mentioning were found by the authors of this review.
Conclusions:	The inclusion of inulin or yeast cell-wall in raw meat–based diets for dogs promoted an increase in fecal short-chain fatty acid concentrations, which is considered beneficial for gut health.
23. **Beloshapka et al.** [42]	
Population:	Healthy adult dogs.
Sample size:	Six adult female Beagles.
Intervention details:	These dogs were 5.5 ± 0.5 years old. Six diets were used: beef control, beef and 1.4% DM inulin, beef and 1.4% YCW extract, chicken control, chicken and 1.4% inulin, and chicken and 1.4% YCW. The duration of the study was 21 days.
Study design:	A 3 × 2 factorial Latin square design.
Outcome studied:	To evaluate the effects of raw meat-based diets with and without inulin or yeast cell-wall extract on macronutrient digestibility, blood cell counts, serum metabolite concentrations, and fecal fermentative end-product concentrations in healthy adult dogs.
Main findings: (relevant to PICO question):	The beef with inulin and YCW diets increased fecal SCFA concentrations. The beef and inulin diet increased DM, OM, CP, and energy digestibility, but the beef and YCW diet decreased them. Fecal scores decreased with beef control and beef and inulin diets. The chicken with inulin and YCW diets decreased fecal pH. With the beef-based diets, fecal total SCFA and acetate concentrations increased with the inclusion of inulin or YCW. When dogs were fed the chicken-based diets, fecal indole concentration decreased when inulin or YCW were included, whereas fecal total indole and phenol concentrations decreased only with the inclusion of inulin. Fecal spermine concentration increased with the inclusion of inulin or YCW when dogs were fed either protein source. The beef diet with inulin decreased skin condition score in the tail region.
Limitations:	The authors of this review did not find limitations worth mentioning.
Conclusions:	The dietary inclusion of inulin or yeast cell wall extract promoted some changes in fecal microbiota of healthy adult dogs, but it was not considered a strong prebiotic effect by the authors.
24. **Felssner et al.** [43]	
Population:	Healthy adult dogs.
Sample size:	Eighteen male and female Beagles.
Intervention details:	These dogs were 4 to 7 years old. Three treatments were used: control diet without prebiotics, control diet with blend prebiotics before extrusion process, control diet with blend prebiotics after extrusion process. The blend of prebiotics was composed by 1.0%/kg of dry matter of MOS and FOS (1:1). The duration of the study was 10 days.
Study design:	Random design.
Outcome studied:	To investigate the potential beneficial effects of prebiotics in dogs, we analyzed the effects of the association between FOS and MOS, added before or after extrusion, on fermentative intestinal parameters, nutrient digestibility, and nitrogen metabolism.
Main findings: (relevant to PICO question):	There was no difference in digestibility, metabolizable energy, fecal ammonia content, and short-chain fatty acids (acetic, propionic, and butyric) between the control diet and the one with the addition of prebiotics. The dogs supplemented with prebiotics before and after extrusion had a lower fecal pH compared to those in the control diet and a reduction in the post prandial blood urea concentration was also observed in the animals receiving prebiotics.
Limitations:	No limitations worth mentioning were found by the authors of this review.
Conclusions:	The combination of mannan-oligosaccharide (MOS) and fructoligosaccharide in the diet of healthy adult dogs reduced fecal pH as well as postprandial blood urea concentrations, probably by decreasing intestinal pH.

Table 2. *Cont.*

25. **Garcia-Mazcorro et al.** [44]	
Population:	Healthy young, adult, and elder dogs.
Sample size:	These dogs were 9 months to 10 years old (3 Labrador mix, 1 Boston Terrier, 1 mixed, and 1 Australian kelpie). Experiment 1: 12 adult dogs of different breeds (1 Doberman, 1 Mix Rottweiler, 1 Boston Terrier, 3 Mix, 1 Weimaraner, 1 Pembroke Welsh Corgi, 1 Mix hound/Great Dane, 1 Australian Kelpie). Experiment 2: 10 adult dogs of different breeds were used (1 Boston Terrier, 1 Weimaraner, 2 Mix, 1 Doberman, 1 Dutch Shepherd, 1 Welsh Pembroke Corgi, 1 Australian Shepherd, 1 Pit Bull mix).
Intervention details:	Experiment 1: The dogs were fed 225 mg FOS and inulin once a day for 16 days. Experiment 2: The dogs were fed 99,2 mg FOS and inulin once a day for 16 days. Experiment 1 and 2: The owners collected fecal samples at two time points before prebiotic administration, 8 days and 1 day before initiation of prebiotic administration, and again at two time points after prebiotic supplementation (days 8 and 16). The analysis performed was fecal microbiota. A questionnaire was used for the data collection on acceptance of the prebiotic, attitude, appetite, drinking behavior, fecal consistency, flatulence, and color of feces.
Study design:	The study design was not informed.
Outcome studied:	To evaluate the effect of a commercially available product containing prebiotics on the fecal bacterial composition of healthy cats and dogs.
Main findings: (relevant to PICO question):	Experiment 1: both experiments demonstrated no negative effects from consuming supplementation. Two dogs were excluded because of serum cobalamin and folate concentrations that were below the reference range. Two dogs showed high increases in the order Lactobacillales during supplementation. One dog had high abundances decreased to near 0% at day 16 of prebiotic administration. The highest fecal microbiota abundance was Firmicutes (93.2%). Experiment 2: one dog had flatulence. The fecal microbiota was again dominated by Firmicutes, but with lower proportions when compared to Experiment 1 (78.5%). One dog showed near 0% *Bifidobacterium* at both time points before prebiotic administration, showed an increase to 8.4% on day 8 after prebiotic supplementation, and a further increase to 25.9% on day 16. This same dog also had an increase in *Lactobaillaceae* from <1% before and on day 8 after supplementation to 35.2% on day 16, and *Turicibacteraceae* from 0% before supplementation to 49% and 15% on days 8 and 16 after supplementation, respectively. The prebiotic supplementation showed a lower abundance of *Dorea* (family *Clostridiaceae*) and also higher abundances of *Megamonas* and other members of *Veillonellaceae*.
Limitations:	The authors did not control the intake of food per day for each dog and did not have control animals. The dog food was not standardized and the difference between phylum, genus, and family of the bacteria was not evaluated.
Conclusions:	It was observed variability and discrepancies in microbial abundance between FISH and 454-pyrosequencing.
26. **Ferreira et al.** [45]	
Population:	Healthy adult dogs.
Sample size:	Fourteen adult Beagles.
Intervention details:	These dogs were 6.14 ± 3.13 years old. The dogs were fed a control diet (without prebiotic) and a supplemented diet with 10 g of beta-glucan/kg of food. The supplementation was offered orally after dissolution in 10 mL of water. The duration of the study was 71 days.
Study design:	Randomized complete block design.
Outcome studied:	To evaluate the effects of dietary supplementation with oat beta-glucan extract on physiological, metabolic, immunological, and nutritional parameters.
Main findings: (relevant to PICO question):	The supplemented diet decreased serum concentrations of total cholesterol and density lipoproteins, decreased coefficients of total tract apparent digestibility (CTTAD) of DM, OM, ash, and crude fat (CF), increased fecal output, and decreased fecal consistency. The supplemented diet decreased the number of red blood cells, hematocrit percentage, and hemoglobin concentration on 21 days post-vaccination as well as lower serum concentration of interleukin-4 7 days post-vaccination.
Limitations:	No limitations worth mentioning were found by the authors of this review.
Conclusions:	Dietary inclusion of 1% oat beta-glucan extract was effective in modulating blood lipid variables and decreasing nutrient digestibility in healthy adult dogs; this shows a potential application in obese or hyperlipidemic dogs. In addition, it was shown that the oat beta-glucan extract has the ability to modulate the vaccine response in dogs.
27. **Pinna et al.** [46]	
Population:	Healthy adult dogs.
Sample size:	Twelve dogs of different breeds.

Table 2. *Cont.*

Intervention details:	These dogs were 3.6 ± 1.6 years old. Four treatments were used: low protein diet (LP, 229 g/kg DM), high protein diet (HP, 304 g/kg DM), LP plus 15 g/kg FOS, and HP plus 15 g/kg FOS. The duration of the study was 28 days, with 12-day washout periods in between. On days, 0, 21, and 28 of each feeding period, a fresh fecal sample was collected for fecal microbiota, apparent total tract digestibility (ATTD), fecal pH, VFA, ammonia, and biogenic amines.
Study design:	The design was a 4 × 4 Latin square experimental design.
Outcome studied:	To evaluate the effects of dietary supplementation with FOS on fecal bacterial populations, fecal fermentative end-product concentrations, and ATTD in dogs fed diets with different protein content.
Main findings: (relevant to PICO question):	The results confirmed that the protein concentration and FOS influenced fecal pH, propionic acid, acetic to propionic acid, and acetic plus n-butyric to propionic acid ratios, *Bifidobacteria*, and ATTD of CD and manganese. Supplementation with FOS resulted in lower fecal pH in the HP diet. Fecal concentrations of ammonia were affected by both dietary protein concentrations and FOS. Fecal moisture and concentrations of biogenic amines and total VFA were not affected by treatments. The diets containing FOS resulted in greater ATTD of DM, calcium, magnesium, sodium, zinc, and iron.
Limitations:	No limitations worth mentioning were found by the authors of this review.
Conclusions:	The fructooligosaccharides inclusion in high-protein diets for healthy adult dogs provided positive effects, such as an increase in fecal *Bifidobacterium* spp. concentrations and improvement of some fecal variables, such as pH and propionic acid concentrations. Overall, dietary fructooligosaccharides inclusion increased the apparent digestibility of several minerals.
28. **Ferreira et al.** [47]	
Population:	Healthy adult dogs.
Sample size:	Eighteen female and male dogs.
Intervention details:	These dogs were 2 to 10 years old (English Cocker Spaniel, English Springer Spaniel, Border Collie, Podenco, Boston Terrier, and Labrador Retriever). Two diets were used: control diet and control diet plus 0.5 mL/kg BW of an oral solution of lactulose (3.5 g/5 mL of lactulose solution). The duration of the study was 63 days, and it was divided into three periods: weeks 1–5, usual diet; weeks 6–7, oral lactulose; and weeks 8–9, standardized diet.
Study design:	Completely randomized design.
Outcome studied:	To examine how the fecal microbiota composition changed before, during, and after lactulose treatment in a large animal model.
Main findings: (relevant to PICO question):	Lactulose supplementation decreased microbiota richness/diversity, based on observed operational taxonomic units. At the phylum level, lactulose supplementation was associated with an increase in Firmicutes and Actinobacteria, and a decrease in Bacteroidetes and Fusobacteria, when compared to weeks 5 and 9 without supplementation.
Limitations:	The study has no limitations.
Conclusions:	Lactulose induced a reversible qualitative and quantitative change of the fecal microbiota in healthy adult dogs.
29. **Nogueira et al.** [48]	
Population:	Healthy adult dogs.
Sample size:	Eight intact adult female Beagles.
Intervention details:	These dogs were 4.2 ± 1.14 years old. Four diets containing either 5% cellulose (CT), 5% dietary fiber and prebiotic blend (FP), 0.02% saccharin and eugenol (SE), and 5% fiber blend plus 0.02% saccharin and eugenol (FSE). The dogs were randomly assigned to one of the four dietary treatments. The experimental period consisted of 14 days (10 days of diet adaptation and 4 days of data collection).
Study design:	Replicated 4 × 4 Latin square design.
Outcome studied:	To evaluate the effects of dietary supplementation of a fiber and prebiotic blend alone or in combination with a food additive containing saccharin and eugenol as potential gut health promoters.
Main findings: (relevant to PICO question):	Fecal output and scores did not differ ($p > 0.05$) among dietary treatments, but they were within the ideal range (2.5–2.9) in a 5-point scale. All diets were highly digestible and had similar ($p > 0.05$) ATTD of dry matter, organic matter, and crude protein. Total dietary fiber (TDF) digestibility was greater for dogs fed the FSE diet ($p < 0.05$) in contrast with dogs fed the CT and SE diets. Fecal acetate and propionate concentrations were greater ($p < 0.05$) for dogs fed FP and FSE diets. Fecal concentrations of isobutyrate and isovalerate were greater for dogs fed CT ($p < 0.05$) compared with dogs fed the other three treatments.
Limitations:	The study has no limitations.
Conclusions:	Fiber and prebiotic blend supplementation did not impair nutrient digestibility and stool quality, and it was effective in promoting modulation of fecal metabolites related to gut health in healthy adult dogs.

Table 2. *Cont.*

30. **Theodoro et al.** [49]	
Population:	Healthy adult dogs.
Sample size:	Twenty-four adult male and female Beagles.
Intervention details:	These dogs were 3.5 ± 0.91 years old. Each treatment had eight dogs assigned to it. The study used two different YCW: one conventional and another with higher solubility of the MOS fraction. The three treatments were control, without YCW (CON), control diet with the addition of 0.3% of a conventional YCW, and control diet with 0.3% of a YCW with high MOS solubility (YCWs). The duration of the study was 32 days.
Study design:	Randomized block design with two blocks of 12 dogs each and 4 dogs per diet in each block.
Outcome studied:	To evaluate the effects of the incorporation in extruded diets of two preparations of *Saccharomyces cerevisiae* cell wall, differing in solubility in water of MOS, on nutrient digestibility, microbial fermentation products in feces, and certain immunological parameters of adult dogs.
Main findings: (relevant to PICO question):	YCW reduced fat digestibility, increased the concentration of butyrate and putrescine, and reduced lactate in feces. Lower interleukin 6 (IL-6) on serum was verified for dogs fed the YCWs. A higher phagocytic index was verified for peripheral monocytes after the intake of YCW.
Limitations:	The study has no limitations.
Conclusions:	The solubilization of the mannooligosaccharide fraction interfered with its effects on fecal fermentation products and immunoinflammatory variables in healthy adult dogs.
31. **Ide et al.** [50]	
Population:	Healthy adult dogs.
Sample size:	Six adult Beagles; three neutered females (6 years old), and three intact males (2 years old).
Intervention details:	The dogs were fed the same regular maintenance diet and a tablet form of kestose (2 g/day). The total duration of the study was 12 weeks, but the supplementation was offered for 8 weeks and 4 weeks of follow-up period without kestose supplementation.
Study design:	The study did not provide the design.
Outcome studied:	To elucidate the prebiotic potential of kestose in terms of changes in the intestinal microbiota population and fecal SCFA concentration.
Main findings: (relevant to PICO question):	The results showed an increased genus *Bifidobacterium* after kestose supplementation while genera *Bacteroides* and *Sutterella* decreased. *Clostridium perfringens* decreased in the first 4 weeks of supplementation. Fecal butyrate was increased at week 8 and returned to base level after 4 weeks of the washing period. The phylum Actinobacteria increased after kestose supplementation, at weeks 4 and 8 compared to week 0. The phylum Bacteroidetes, genus *Prevotella*, genus *Megamonas*, genus *Bacteroides*, genus *Butyricicoccus* were decreased in occupancies between week 8 and 12. The phylum Bacteroidetes, genus *Bacteroides,* and genus *Sutterella* demonstrated no change up to 8 weeks during supplementation, but their numbers were significantly decreased at week 12.
Limitations:	The study did not analyze fecal score, fecal pH, BCFA, nutrient digestibility, lactic acid, and ammonia. They did not control food intake.
Conclusions:	Kestose supplementation was effective in decreasing fecal *Clostridium perfringens* concentrations and in increasing fecal butyrate concentrations in healthy adult dogs.
32. **Lin et al.** [51]	
Population:	Healthy dogs.
Sample size:	Twelve adult female Beagles.
Intervention details:	These dogs were 5.16 ± 0.87 years old. Three diets were used: baseline diet, commercial canned diet (CD), and a high-fiber diet (HFD-composed of 22.5 g/d of soluble corn fiber). The experimental diets were supplemented with gelatin capsules of cellulose or YCW. Between days 1 and 14, the dogs were fed the baseline diet and were supplemented with the placebo (22.4 g/d of cellulose) or YCW (365 mg/d), and between days 15 and 28 (transition period). The dogs remained on the placebo or YCW treatments but were fed their new diet: (1) Yeast CD, (2) Yeast HFD, (3) Placebo CD, and (4) Placebo HFD. The duration of the study was 28 days.
Study design:	Replicated 4 × 4 Latin square design, placebo-controlled.
Outcome studied:	To determine the effects of YCW fraction on measures of gut integrity and fecal characteristics of adult dogs undergoing an abrupt diet transition.
Main findings: (relevant to PICO question):	Post-transition diets affected nutrient intake. Intakes of protein, fat, and energy were increased. Fecal characteristics, including pH, score, and DM, were not altered by the YCW treatment before diet transition. HFD resulted in increased fecal scores. Fecal *E. coli*, IgA, and calprotectin were not affected by YCM treatment before diet transition.
Limitations:	The animals received an abrupt diet transition, and this may have influenced the results.

Table 2. *Cont.*

Conclusions:	Supplementation of yeast cell wall fraction was not effective in improving gut health in adult dogs undergoing an abrupt diet transition, because only trending effects were observed.
33. **Perini et al.** [52]	
Population:	Healthy adult dogs.
Sample size:	Twenty-four female and male dogs.
Intervention details:	The breeds of dogs were Irish Setter, Beagle, Labrador, Whippet, French Bulldog, and Cocker Spaniel. Four treatments were used: control diet (CO), 1.0% of GOS (containing a minimum of 0.38% GOS), 0.5% of a prebiotic blend (B1), and 1.0% of the same prebiotic blend (B2). The duration of the study was 60 days, and it was divided into two periods: T30 (30 days) and T60 (60 days). The commercial blend was composed of 120 g/kg FOS, 60 g/kg MOS, 72 g/kg GOS, organic zinc, and 150 g/kg 1,3 beta-glucans.
Study design:	A six-block design.
Outcome studied:	To evaluate the effects of the time of ingestion of prebiotics on fecal fermentation products and immunological features.
Main findings: (relevant to PICO question):	B2: at T60, propionic acid was decreased. At T60, concentrations of IgA, lactic acid, and pH in the feces increased in all treatments regardless of prebiotic inclusion. GOS: increased fecal score and lactic acid concentrations.
Limitations:	The study has no limitations.
Conclusions:	A 60-day intake period of a prebiotic blend was not sufficient to modulate fecal and immune variables in healthy adult dogs.
34. **Rentas et al.** [53]	
Population:	Healthy adult dogs
Sample size:	Twenty-four female and male dogs
Intervention details:	The breeds of dogs were Irish Setter, Beagle, Labrador, Whippet, French Bulldog and Cocker Spaniel. Four treatments were used: control diet (CO), 1.0% of GOS (containing a minimum of 0.38% GOS), 0.5% of a prebiotic blend (B1), and 1.0% of the same prebiotic blend (B2). The duration of the study was 30 days. The commercial blend was composed of 120 g/kg FOS, 60 g/kg MOS, 72 g/kg GOS, organic zinc, and 150 g/kg 1,3 beta-glucans.
Study design:	A 6-block design
Outcome studied:	To evaluate the effects of two prebiotics in different concentrations on nutrient digestibility, fermentative products, and immunological variables in adult dogs.
Main findings: (relevant to PICO question):	Prebiotic supplementation did not affect apparent digestibility coefficients, total stool production, fecal score, fecal pH, ammonia, lactic acid, SCFA, and BCFA. GOS decreased the isovaleric acid. GOS and B2 increased the total number of polymorphonuclear cells and oxidative burst. B2 increased the rate of *S. aureus* phagocytes in relation to CO and GOS, and B2 increased *E. coli* phagocytosis.
Limitations:	The study has no limitations.
Conclusions:	Both prebiotics galactooligosaccharide and blend Yes-Golf at 1.0% inclusion improved the immunity of healthy adult dogs.
35. **Fries-Craft et al.** [54]	
Population:	Healthy adult dogs.
Sample size:	Twenty-four Labrador Retrievers.
Intervention details:	Three dietary treatments were used and consisting of a basal diet (control) supplemented with 0.012% or 0.023% (0.5 or 1×, respectively) yeast 1,3/1,6 beta-glucan.
Study design:	The study did not provide the design.
Outcome studied:	To evaluate systemic immune responses in dogs fed kibble diets with two yeast 1,3/1,6 beta-glucans doses before and after vaccine challenge.
Main findings: (relevant to PICO question):	Prior to vaccination, beta-glucan diets did not affect serum cytokines, antibody titer, or immune cell populations. In the first 7 d post-vaccination (dpv), PBMC CD21 low B cells increased 36.5% to 58.1% in all groups but the magnitude of change was lesser in the 0.5× beta-glucan diet resulting in 25.6% lower CD21 low populations compared to control-fed dogs. By 21 dpv, B-cell populations recovered to baseline levels in dogs fed 1× beta-glucan, but CD21 high cells remained elevated 50.5% in dogs fed 0.5× beta-glucan diets compared with baseline. While no differences in serum titer or cytokines were observed, feeding both beta-glucan diets maintained stable blood monocytes, whereas a 53.0% decrease between baseline and 14 dpv was observed in control-fed dogs.
Limitations:	The study has no limitations.
Conclusions:	Supplementation with 0.023% of yeast 1,3/1,6 beta-glucan was effective in altering monocytes associated with trained immunity and contributing to B-cell population resolution by 21 days post-vaccination in healthy adult dogs.

Table 2. *Cont.*

36. **Wilson et al.** [55]	
Population:	Healthy adult dogs.
Sample size:	Sixteen adult female and male Pointers.
Intervention details:	These dogs were 6.7 ± 2.1 years old. Dogs were fed a control diet for 4 weeks, then randomly assigned to a control or *Saccharomyces cerevisiae* fermentation product (SCFP) supplemented diet (0.13% of the active SCFP ingredient) and fed to maintain BW for 11 weeks. A 6-week washout preceded the second 11-week experimental period with dogs receiving opposite treatments.
Study design:	Randomized crossover design.
Outcome studied:	To measure the effects of SCFP on fecal characteristics, serum oxidative stress biomarkers, and whole blood gene expression of dogs undergoing transport stress.
Main findings: (relevant to PICO question):	Change in serum malondialdehyde concentrations increased and serum 8-isoprostane concentrations tended to increase ($p < 0.10$) in dogs fed SCFP but decreased in control dogs after transport. Fecal dry matter percentage tended to be affected ($p < 0.10$) by diet during transport stress, being reduced in control dogs, but stable in dogs fed SCFP. Blood cyclooxygenase-2 and malondialdehyde mRNA expression was increased in control dogs, but stable or decreased in dogs fed SCFP.
Limitations:	The study has no limitations.
Conclusions:	The addition of Saccharomyces cerevisiae fermentation product-supplemented in the diet of adult dogs under transport stress resulted in stabilization or decreasing in expression of genes associated with activation of innate immunity.

Main Effects of Prebiotics in Healthy Dog Food

Studies that used prebiotics and did not demonstrate their effects, treatments with concomitant probiotics or symbiotics, and studies that used young animals were not considered. According to Table 2, most of the studies included prebiotics in the diet of healthy dogs, aiming to demonstrate its gastrointestinal effects as well as the modulation of the immune system. The main difference between studies is in type and concentration of prebiotic used. Most articles used FOS and YCW supplementation in adult or senior healthy dogs. Other supplements included XOS, IMO, GOS, TGOS, OF, polydextrose, and others.

FOS and YCW were some of the prebiotics studied and are more commonly used. YCW is derived from *Saccharomyces cerevisiae* and is a substrate of moderate fermentation, which contains carbohydrates and proteins and is rich in mannans that can prevent the adherence of bacteria to the intestinal wall [56]. FOS, on the other hand, has been more studied [57] and is considered an inulin-type oligosaccharide, which helps prevent intestinal colonization by pathogenic microorganisms due by stimulating the proliferation of beneficial bacteria [58].

The majority of studies that evaluated FOS at concentrations of 1.0 or 2.0% in DM did not observe effects on important gastrointestinal variables such as SCFA, BCFA, fecal ammonia, fecal pH, fecal score, and fecal microbial population [14,15,26]. Only two studies observed fecal indole and phenol decrease [31] and Bacteroidetes increase [26], which is a bacteria phylum present in low concentrations in dogs [44,59] and has been associated with obesity development [60]. Indole and phenol are products responsible for the fecal odor in dogs and cats, and its decrease can be perceived as beneficial especially by pet owners [61]. According to Swanson et al. [31], FOS supplementation can influence the catabolism or excretion of aromatic amino acids in the colon and all amino acids in the large bowel. It is known that FOS is one of the most studied prebiotics and used both in human and dog food and it is hydrolyzed by the enzyme exo-beta-fructofuranosidase [62]. Furthermore, the metabolism of FOS is still discussed in studies; one study demonstrated that FOS was fermented by 12 strains of *Lactobacillus* and 7 strains of *Bifidobacterium*, in addition to 8 strains of *Escherichia coli*, *Salmonella* spp. [63].

A higher inclusion of FOS (3.0% in DM) in diets with two protein sources (chicken and beef) demonstrated a decrease in the apparent digestibility of dry matter and fat, an increase in feces volume in the beef-based diet, and a decrease in the concentration of ammonia in the chicken-based diet [30]. According to Beloshapka et al. [41], the type of protein can interfere in prebiotic potential effects, which corroborates with findings of the previously

cited study in which the authors demonstrated that beef-based diets increased fecal SCFA with the inclusion of inulin or FOS. Protein content can also influence the prebiotic effect. Pinna et al. [46] observed that a diet with 30.4% protein on a DM basis reduced fecal or pH and increased ammonia and propionic acid concentrations, acetic to propionic acid ratio, and the acetic plus n-butyric to propionic acid ratio. Despite the increase in ammonia being considered a negative effect, the authors suggested that this effect might be associated with a change in nitrogen excretion from urine to feces, which has been previously reported [25]. Furthermore, fecal fermentation products derived from proteins, such as indole, phenol, and BCFA can indicate higher putrefaction of proteins in dogs fed this nutrient from the animal origin when compared to plant-based proteins [64].

A study that included the same concentration of different prebiotics (5 g/kg of FOS, MOS, or XOS) observed that this equivalence of concentrations may have influenced the effects [15]. Swanson et al. [14] observed that the inclusion of a prebiotic blend containing FOS and MOS promoted effects on the serum lymphocytes' proportion. Studies with prebiotic blends containing FOS, GOS, YCW, beta-glucan, or MOS demonstrated better effects in relation to the inclusion of just one prebiotic, such as the increase in *Bifidobacteria*, in the total number of polymorphonuclear cells and oxidative burst and decrease in butyrate, propionic acid, cholesterol, urea, triglycerides, phenol, and indole [16,24,31,52,53]. These blends can promote better effects when combined due to different prebiotic functions.

Studies that included YCW or a prebiotic blend containing YCW observed effects in dogs such as the increase in *Bifidobacteria*, SCFA, and fecal spermine [41,42], decrease in fat digestibility, lactate, and IL-6, and increase in concentrations of butyrate, putrescine, and phagocytic index [49], and an increase in the fecal score [51]. One of the interesting effects observed in some studies is the increase in butyrate and monocyte concentrations. Butyrate, one of the SCFA, is the main source of energy for colonocytes and its increase is related to colonocyte proliferation and intestinal health [16]. Furthermore, butyrate's immunomodulation effect is more powerful than that of acetate or propionate due to its interference in the activity of diacetyl, which is responsible for the decrease in IL-2 and IL-6 secretion [65]. Monocytes, on the other hand, are responsible for the organism's defense against pathogenic microorganisms, and its increase can help in cases of infection or immune system changes [66]. These defense cells perform fundamental roles in cell processes such as regulation of transcription and translation, control of ion channel activity, kinase modulation, protection against oxidative damage, and contribution to structure and stability of nucleic acid [67].

Beta-glucans are one of the most studied prebiotics in humans [68], and there is growing evidence of their use for dogs. They are currently studied in dogs with inflammatory bowel disease [17,69], hyperglycaemic [70], osteoarthritis [71], chronic kidney disease [72], and after vaccination [73–76], due to its immunostimulant function [53]. One study that evaluated the effects of beta-glucans in healthy adult dogs observed that a concentration of 10 g/kg food of dry matter was able to decrease concentrations of total cholesterol and serum LDL and VLDL, as well as decrease nutrient CTTAD. Furthermore, beta-glucans can positively modulate the response to vaccination [45]. Another study that evaluated parameters of innate immunity in the response of rabies vaccination, using 2.5 mg/animal on day 0 and 28, observed an increase in B lymphocytes, which are a predominant factor in the protection against rabies [23].

Other prebiotics are less used in canine nutrition such as polydextrose, lactulose, arabinogalactan (AG), transgalactooligosaccharides (TGOS), oligofructose (OF), isomaltoligosaccharide (IMO), galactoglucomannan oligosaccharide (GGMO), kestose, and galactooligosaccharides (GOS). Among these, OF is the most studied and can be indirectly included in diets because its sources are ingredients such as fruits, vegetables, and grains [76]. Chicory, for example, is rich in inulin, which is used in hydrolysis to produce OF [75]. Beynen et al. [28] observed that the inclusion of 1.0% of OF dry matter increased the populations of *Bifidobacterium*, *Streptococci*, and *Clostridia*, and increased the absorption of calcium and magnesium. This increase in beneficial bacteria population can indicate higher

resistance to pathogenic bacteria and higher competition for nutrients and space [77], as well as a stimulus of the immune system [78]. The increase in calcium and magnesium absorption can be caused by a possible stimulus of these minerals in the canine colon, similar to what has been reported in rats [79] and in dogs supplemented with lactulose [27]. Other studies regarding OF supplementation demonstrated a decrease in the digestibility of DM, OM, and CF, as well as an increase in propionate concentrations [33,35]. These effects on digestibility could indicate an increase in the synthesis of microbial proteins in the colon, which increases fermentable substrates from fructans [35] or a decrease in the average gastrointestinal transit time [33].

Galactooligosaccharides is another prebiotic, with only three articles published that mention it [32,52,53]. Zentek et al. [32] did not observe effects with the inclusion 1 g/kg BW/d of TGOS, one of GOS's structural forms. However, Perini et al. [52] and Rentas et al. [53] observed different effects with the inclusion of 1.0% of GOS dry matter: increase in fecal score and lactic acid, decrease in isovaleric acid, and increase in total polymorphonuclear cells and oxidative burst. The increase in lactic acid is influenced by GOS due to the stimulation of lactic bacteria and *Bifidobacterium* spp. proliferation, which are responsible for the lactic acid production. This does not mean, however, that the SCFA concentrations will increase once SCFAs are absorbed in the colon [80]. The SCFA can bind to receptors of immune compounds and affect innate immunity and inflammatory cell components [81].

Approximately half of the articles (n = 17/36) included in the present study used the prebiotics for a short period (10 to 25 days), which could have influenced the results obtained. According to Perini et al. [52], the period of prebiotic intake increased the concentrations of propionic acid in dogs fed a prebiotic blend. This was the first study that evaluated this time-related response and leads to the conclusion that the period of prebiotic intake is a crucial factor and should be considered in the design of prebiotic research. Another important factor that should be taken into consideration in prebiotic research is abrupt diet modification. This study observed that the daily ingestion of proteins, fat and energy increased while the total fiber dietary and nitrogen-free extract decreased after the diet transition with the supplementation of 0.2% of yeast cell wall per kilo of food in the dry matter. Moreover, they observed a reduction in fecal pH, body weight, and the population of *C. perfringens*, and an increase in the fecal score in dogs that feds abruptly. The study suggests that the yeast cell wall prebiotic assists in gastrointestinal variables in dogs undergoing food transition [51]. The recommendation is to gradually change diets to avoid intake decrease and diet rejection.

From all the published data so far, it can be concluded that prebiotics are important to canine health. However, there is little knowledge of the duration of the organism's response after the removal of prebiotics from the diet. A study that evaluated the effects four months after ceasing the intake of prebiotics observed that fecal butyrate went back to basal levels and populations of the Bacteroidetes phylum, *Bacteroides* genus, and *Sutterella* decreased after the supplementation of 2.0 g of kestose/day at eight weeks [50]. Another study observed that after two weeks of ceasing the supplementation with 3.5 g/5 mL of lactulose solution, the populations of Firmicutes, Actinobacteria, Bacteroidetes, and Fusobacteria increased [47]. Garcia-Mazcorro et al. [44] reported that one of twelve dogs presented a low concentration of *Bifidobacterium* before supplementation of FOS and inulin and an increase in these bacteria at the 8th (8.4%) and 16th (25.9%) days after prebiotic supplementation. Another study observed that the supplementation effects of beta-glucans disappeared after one week without the supplement [38]. Due to differences in effect between types of prebiotics and few studies that evaluated how long the effects last, more studies should be performed to investigate the period of supplementation efficacy after ceasing the prebiotic intake.

In most cases, prebiotics used in foods for dogs influenced gastrointestinal and immunological parameters. The gastrointestinal benefits include higher SCFA concentrations, better fecal score, and fecal pH. Some of the fermentation products that are increased by prebiotic consumption, like butyrate, propionate, and acetate, are an important source of

energy for the colonocytes [14]. SCFAs, however, are volatile and there may be a loss in samples during processing for analysis, because it depends on the sample collection method as well as the way the samples were stored and the time spent until the analysis [52], which can influence the results of SCFA measurement. Prebiotics are currently studied due to their modulating effect on intestinal microbiota, as they can increase the proliferation of beneficial bacteria in the host's gut [78,82]. Canine microbiota is abundant in Firmicutes, Bacteroidetes, Proteobacteria, Fusobacteria, and Actinobacteria [6]. FOS and MOS promote proliferation of *Bifidobacteria* and *Lactobacilli* [16], GOS and inulin favors proliferation of only *Bifidobacteria* [17,81], and AG can stimulate *Lactobacilli* [29]. These bacteria are considered beneficial for the gastrointestinal tract and their proliferation is associated with a decrease in the concentration of putrefactive compounds and population of pathogenic bacteria such as *Clostridium perfringens* [17]. However, the intestinal microbiota is not only affected by daily food intake, but also by general health condition [6,83,84], body composition [85–87] and the surrounding environment [88]. Without considering these factors, the prebiotics effects will be committed, and the microbiota modulation will be influenced first by these factors. In healthy conditions, the relation between the host and the microorganisms develops a homeostatic balance of bacteria, called eubiosis, which is responsible for intestinal health, beneficial bacteria growth and prevent excess of potentially pathogenic bacteria. When this condition is stopped, the animal is afflicted with dysbiosis, which is responsible for unbalance in the composition and bacterial activity present in the microbiota, which generates a harmful and unstable microbiome-host interaction. Dysbiosis has three specific characteristics: bacterial diversity reduction, pathogens excessive growth, and beneficial bacteria reduction [89].

Why should we study these intestinal and immunological variables in the effects of prebiotics? The final products in the fermentation process affect the acids concentrations in the feces and, consequently, the fecal pH. Compounds that change the pH in the intestinal lumen are mostly starches, fermentable fibers, amino acids, and, to a lesser extent, fatty acids. In addition, with variation in the food's composition and in the nutrient's digestibility, the prebiotics inclusion is essential in order to maintain the intestinal pH in an interval which will favor the beneficial bacteria proliferation. The ideal interval to favor this proliferation is still discussed in most animal species [43]. In a study with humans, it was observed that amino acids that reach the large intestine promoted an increase in intestinal pH and this resulted in an increase in the production of undesirable compounds such as biogenic amines, phenol, and ammonia. These compounds in high concentrations can harm the intestinal epithelial cells, which leads to a predisposition to the development of metabolic changes [90]. According to Felssner et al. [43], most of the ammonia produced is absorbed by the intestinal mucosa cells; their production and absorption are directly affected by the intestinal pH, in addition to their fecal quantification not being an adequate indicator.

Ammoniacal nitrogen is one of the variables studied in the prebiotics effects and is present in the form of proteins, amino acids, nucleic acids, purines, pyrimidines, vitamins, hormones, antibodies, enzymes, urea, ammonia, and other compounds, being excreted mainly as undigested protein and microbial protein in feces, and as urea in urine, which is produced in the liver by the catabolism of amino acids [43]. Another important variable to be analyzed is ammonia, as it presents toxicity in high concentrations. Most of the ammonia is reabsorbed and metabolized in the liver by the carbamoyl phosphate synthase enzyme and is then converted to urea again and excreted in the urine [91]. On the other hand, the formation and absorption of intestinal ammonia from urea depends on the activity of the urease enzyme and under favorable pH conditions. Approximately 99.0% of the ammonia produced in the large intestine is absorbed by colonocytes through the non-ionic diffusion mechanism. However, when the intestinal pH decreases, the ability of urea to diffuse from the intestine into the bloodstream also decreases, and as a result, more ammonia is excreted in the feces [92].

Short-chain fatty acids, better known for providing energy to colonocytes, demonstrated satiety functionality by stimulating gastrointestinal satiety hormones, such as

peptide 1 (GLP-1) and peptide YY. The GLP-1 secretion from the enteroendocrine L cells, present in abundance in the distal region of the gastrointestinal tract, was increased with the supplementation of dietary fibers [19], which contributes to satiety.

In sum, the propionate is correlated with the increase in the phylum Bacteroidetes and also for the two families *Porphyromonadaceae* and *Prevotellaceae*. Lactate is considered an intestinal health promoter since it creates effects against pathogenic bacteria and is converted into butyrate through interactions in the bacteria cross-feeding [93].

4. Conclusions

After carefully reviewing all data included in the present study, it can be concluded that prebiotics are beneficial components to healthy adult and senior dogs. As the effects of prebiotics differ according to source, concentration, and length of the supplementation period, more research is necessary to evaluate their short and long-term effects on gut microbiota and health in general, as they have great beneficial potential and are widely used in pet food. Moreover, the prebiotics revised in this review were considered potential prebiotics for dogs because their effects improving intestinal and immunological health. Based on the data observed in the literature, animals with diseases that can cause dysbiosis, such as exocrine pancreatic insufficiency, chronic enteropathy, and obesity, could be the animals that would most benefit from prebiotic supplementation, as well as animals with immunosuppression. Therefore, it is important to consider future studies that investigate the potential effects of prebiotics in sick animals.

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