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Review

Influence of Probiotic Supplementation on Health Status of the Dogs: A Review

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Featured Application: The current review summarizes the effects of probiotics on canine microbiota and the protective effect of probiotic interventions against several diseases and gastrointestinal discomforts. This review is a contribution that aims to assist in the development of new dietary or pharmaceutical products supplemented with probiotics for dogs.

Abstract: Most commonly, pet dogs suffer from gastrointestinal (GI) diseases due to careless eating behaviors, such as eating food other than dog food; excess or insufficient nutrient intake of food leading to malnutrition, which could be harmful to dogs; a lack of digestive enzymes; food intolerance or allergies; infections; and/or breed-related hypersensitivities. Probiotics are live microorganisms that deliver health benefits to the host when administrated in an adequate amount. The possible mechanism behind probiotics' beneficial effects could be their positive regulation of the host's intestinal microbiota. Probiotics are reported to have therapeutic properties against canine GI and other diseases. The most suitable dosages and applications of probiotics have not been evaluated extensively. The present review summarizes current knowledge regarding the benefits of probiotics and the changes in canine microbiota during probiotic interventions. This literature review provides clinical evidence for probiotics' beneficial effects in preventing or treating canine ill-health conditions. Based on current knowledge, subsequent researchers could develop or improve probiotics-based canine pharmacological products.

Keywords: canine; probiotics; microbiota; *Lactobacillus*

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1. Introduction

Companion dogs need nutritional care to maintain good health [1]. Dogs are fed with foods supplemented with appropriate nutrients such as choline, vitamins (E, B5, B3, B2, B1, K, B6, A, B9, B12, D), and minerals (calcium, potassium, phosphorus, magnesium, etc.) in addition to the dietary components, such as proteins, carbohydrates, fats, and fibers [1]. Malnutrition can occur due to an excess or a deficiency in nutrient intake, which could be harmful to dogs [2]. The nutritional requirements of each dog can differ based on the dog's breed, size, developmental stage, and level of activity [1]. Even if companion animals are fed with nutritionally balanced diets to maintain good health, any differences in their normal microbiota can facilitate illness upon exposure to harmful environmental influences and pathogens [3,4]. The supplementation of probiotics positively regulates the microbiota of the host and improves their health [5–7]. The joint Food and Agriculture Organization (FAO) of the United Nations and World Health Organization (WHO) defines the term "probiotics" as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" [8]. The term "probiotics" refers to live microbes (isolated from different sources, such as gut commensals, fermented foods, and any other source) that have been characterized and evidenced in adequate controlled studies to have a health beneficial effect, and their safety has been verified. Even though microbial components,

dead microbes, and microbial products exhibit health beneficial effects, they should not be considered as probiotics [9]. The ideal characteristics of probiotics include being non-pathogenic to the host, the ability to tolerate low pH levels and high concentrations of bile acids in the gastrointestinal environment, and the ability to proliferate in the gut by adhering to the intestinal epithelium. For economically viable production, the probiotics should also preferably have the ability to grow well on inexpensive media and tolerate manufacturing, transportation, and storage processes [10,11]. Probiotic supplementation aids in maintaining gut health by preventing or controlling pathogens in the gastrointestinal tract and promoting the population of favorable gastrointestinal tract microflora. The modes of action of probiotics might be strain-specific [11]. Since the supplementation of probiotics is not universally safe and effective in humans [12], probiotics should also be used with caution in companion animals until the limitations of probiotics usage in companion animals are found. Probiotics have been reported to have several health benefits in humans, such as facilitating dental health [13], aiding in cancer management [14], exerting cholesterol-lowering abilities [15], controlling diabetes mellitus [16], and exhibiting immune-stimulating properties [17]. Generally, probiotics that are beneficial to humans are considered to be safe, but canine-derived probiotics could be used safely for canine animals [18].

The intervention of a probiotic-supplemented diet can protect dogs from various health effects. The supplementation of probiotics has been used for the alleviation or prevention of atopic dermatitis [19,20] and treatment of gastrointestinal diseases, such as diarrheal diseases [21–25] in companion animals.

An updated literature review on the role of probiotic supplementation in the health status of domestic dogs is necessary to contribute to the development of new probiotics-based nutraceuticals. This review summarizes the impact of probiotics interventions on domestic dogs and the changes that occur in the microbiota of dogs upon probiotic intervention.

Scientific evidence was collected from Scopus, Web of Science, PubMed, and Google Scholar using the keywords “Probiotics” and “Dogs”. Blinded controlled clinical trials (published from 1998 to February 2021) that were published with details such as a probiotic name, probiotic dose (CFU), and the details of animals used in the study were selected manually and used to prepare the manuscript.

2. Characterization of the Microbiota in Dogs

The microbiomes of humans and pet animals have similarities [26], and the microbiota play a critical role in health and diseases among pet animals [27,28]. Previous bacterial-culture-based studies showed that healthy dogs contain a 10^2 to 10^{11} CFU/g bacterial load in their gastrointestinal (GI) tracts. Recently, high-throughput DNA sequencing techniques were implemented to determine the microbiomes of canines [29]. Current molecular methods revealed that the microbial loads of healthy dogs can range from 10^{12} to 10^{14} [30–32]. Garcia–Mazcorro et al. [33] reported that Firmicutes was the predominant phylum, followed by Actinobacteria, Proteobacteria, and Bacteroidetes, which were identified in the fecal microbiome of healthy dogs. Fusobacteria and Acidobacteria were the least abundant phylum compared to the other identified phyla in the fecal microbiome of healthy dogs [33]. Jha et al. [34] reported the same set of phyla as the predominant one found in the fecal samples of dogs [34].

The microbial load of each segment in the GI tract varies based on that section’s physiological functions [35,36]. For instance, the small intestine is rich in aerobic and facultative anaerobic bacteria, while the colon harbors anaerobic bacteria [35]. Individual variations in the microbiota of different intestinal compartments (such as higher diversity indices for the colon, rectum than those of duodenum, jejunum, and ileum within individual dogs) may also be present, and these variations must be taken into account to explain the microbiota of healthy dogs [36]. The results can fluctuate based on the sequencing method and depth of the study. Some studies showed that Firmicutes (abundant in duodenum, jejunum, ileum,

and colon), Fusobacteria (abundant in ileum, and colon), Bacteroidetes (abundant in ileum, and colon) [37], Proteobacteria (abundant in duodenum [37,38], jejunum, ileum [38], and rectum [37]), and Actinobacteria [37] are the primary phyla identified in the GI tract of the dog microbiota [37,38].

Clostridia is the most abundant bacterial class. Clostridium clusters XI (more abundant in the small intestine) and XIVa (more abundant in the colon) are the dominant clusters of Clostridia [37–39]. In addition to Clostridia, *Bacilli* (genera *Streptococcus* and *Lactobacillus*) and *Erysipelotrichi* (genera *Turicibacter*, *Catenibacterium*, and *Coprobacillus*) are the dominant classes belonging to Firmicutes [39]. *Prevotella*, *Bacteroides* [39,40], *Sutterella* [40], SMB53, Enterococcus, Fusobacterium [34], and, *Megamonas* [34,39] are the most abundant genera among the gut microbial populations in dogs.

3. Health Benefits of Probiotic Supplementation in Healthy Dogs

3.1. Microbiota Changes and Other Benefits in Healthy Dogs upon Probiotic Supplementation

The supplementation of *Enterococcus faecium* NCIB 10415 (derived from healthy adult canine feces) (9.2×10^9 CFU/dog/day) for 18 days reduced the fecal count of *Clostridium* spp. in healthy dogs. *E. faecium* NCIB 10415 lowered the fecal count of *Campylobacter* spp. and *Salmonella* spp. in a few healthy dogs and insignificantly increased the fecal count of *Campylobacter* spp. and *Salmonella* spp. in the majority of the healthy dogs, indicating that *E. faecium* NCIB 10415 was not effective in inhibiting the growth of *Campylobacter* spp. and *Salmonella* spp. in the majority of healthy dogs. Although the study results suggested the beneficial effects of *E. faecium* NCIB 10415 in inhibiting the pathogenic *Clostridium* spp., the effects of probiotics should be evaluated with caution [41] (Table 1).

Biagi et al. [42] studied the effects of *Lactobacillus animalis* LA4 (derived from healthy adult canine feces) on the composition and metabolism of dog intestinal microbiota under both in vitro and in vivo conditions. The in vitro evaluation showed that upon exposure of *L. animalis* LA4 to adult dog fecal cultures, *L. animalis* LA4 increased the count of *Lactobacillus* and reduced the *Clostridium perfringens* count. Moreover, LA4 increased the level of lactic acid in fecal cultures. LA4 reduced the ammonia concentration under in vitro conditions, while fecal concentration of ammonia was not influenced by LA4 under in vivo conditions. When healthy dogs (n = 9 adult) were fed with *L. animalis* LA4 (10^9 CFU/g; 0.5 g per dog per day) for 10 days, the count of *Lactobacillus* was increased, and the enterococci count was reduced. The in vivo evaluations of *Lactobacillus* count also supported the findings of the in vitro experiments. The in vivo results showed that the strain LA4 could withstand the gastrointestinal environment and proliferate in the intestines of the studied adult dogs [42].

The supplementation of *Bacillus amyloliquefaciens* CECT 5940 and *E. faecium* CECT 4515 (0.2 g of a probiotic blend = 1×10^8 CFU of each probiotic strain per dog per day; each strain = 5×10^8 CFU per gram food) in the food of healthy dogs for 39 days did not affect the nutrient digestibility coefficients, fecal scores, or microbiota, whereas reduction was observed in the pathogenic clostridia count in the feces of healthy dogs administered probiotics. This study revealed that the supplementation of CECT 5940 and CECT 4515 reduced the pathogenic clostridia count without influencing food digestibility in healthy dogs [43].

A probiotic preparation (daily dose: 1 g of probiotic supplement/10 pounds of body weight) containing *Lactobacillus* (>64×10^9 CFU/g), *Bifidobacterium* (30×10^9 CFU/g), and *Bacillus* species (24×10^9 CFU/g) was orally administered to healthy female dogs for 14 or 28 days. The study results showed that probiotic supplementation orally for 14 or 28 days did not increase the vaginal lactic acid bacteria (LAB) in the healthy dogs [44].

Park et al. [45] investigated the effect of the supplementation of Queso Blanco cheese containing *Bifidobacterium longum* KACC 91563 (5×10^8 CFU/10 g cheese/kg of body weight/day) on intestinal microbiota and short chain fatty acids (SCFA) in healthy companion dogs. The fecal samples were collected at different stages such as before intake of cheese (week −2 and 0), during cheese intake (week 4 and 8), and after intake of cheese

(week 10). The microbiota analysis revealed that the cheese containing KACC 91563 supplementation effectively reduced the Enterobacteriaceae and *Clostridium* count and increased *Bifidobacterium* in the feces of the companion dogs. The results showed that KACC 91563 intervention for eight weeks reduced the harmful bacterial load and increased the beneficial microbes in the dog intestine. Moreover, the SCFA, such as acetic and propionic acid levels, were raised during probiotic supplementation [45]. Similarly, Queso Blanco cheese containing *B. longum* KACC 91563 (5×10^8 CFU/10 g cheese/day) altered the fecal microbiota by effectively increasing the Bifidobacterium and decreasing *C. perfringens*, Enterobacteriaceae, *Collinesella*, *Blautia*, and *Fusobacterium* in healthy beagle dogs. Overall, these results demonstrated that the supplementation of probiotic Queso Blanco cheese could improve the health status of companion dogs [45,46].

A non-medicated milk replacement containing *Bacillus amyloliquefaciens* CECT 5940 (1×10^9 CFU/g dry matter of probiotic product/dog/day) was administered to healthy dogs for 60 days, and the changes in microbiota were assessed. The results revealed that the bacillus count increased, and the coliform count decreased, in the probiotic-supplemented group compared to the control. The stool consistency was not affected by the probiotic supplementation. This study showed that CECT 5940 could survive the gastrointestinal passage and colonize in the dog intestine, further positively altering the intestinal microbial composition [47].

A probiotic food additive comprising *Lactobacillus casei* Zhang, *Lactobacillus plantarum* P-8, and *Bifidobacterium animalis* subsp. *lactis* V9 (equal proportion of 3 probiotic strains in a final concentration of 2×10^9 CFU/g; Dosage: 2 g/day for each young dog; 4 g/day for each training dog; 10 g/day for each elderly dog) was administered to healthy dogs of different age groups (young, training, and an elderly group) for two months. Probiotic supplementation increased the load of *Lactobacillus* spp. and *Faecalibacterium prausnitzii* but decreased the abundance of *Sutterella stercoricanisin* and *Escherichia coli* in the feces of the experimental elderly dogs. Moreover, the induction of cytokines and antibodies was positively regulated in the probiotic-treated group. Two months of probiotic treatment also shifted the gut microbiota of elderly dogs towards the composition of microbiota found in younger dogs at the end of the experimental study. Changes in some microbial species showed a correlation with some immune factors (such as fecal secretory IgA, serum IgG, TNF-α), indicating that probiotic intervention governs the immune system by changing the gut's microbial composition in dogs [48].

Kefir, a probiotic dairy product (200 mL of kefir/day; Kefir contains LAB (9.32 ± 0.23 log CFU per mL), and yeast (7.12 ± 0.36 log CFU per mL)), was administered orally to healthy dogs (n = 6 adult; 2 females, 4 males) for two weeks, and the changes in microbiota were assessed. The load of *Fusobacteria*, *Clostridiaceae*, *Fusobacteriaceae*, *Ruminococcaceae*, and the *Firmicutes*: *Bacteroidetes* ratio decreased, whereas quantities of LAB, *Sutterellaceae*, *Prevotellaceae*, and *Selenomonadaceae* and the LAB: *Enterobacteriaceae* ratio increased in the group that consumed kefir. This study revealed that kefir supplementation effectively improved the dogs' intestinal microbiota, and that kefir could be used as a functional food to improve dogs' health [49].

Lactobacillus kefiri (10^7 cells per day) was administered to healthy dogs (n = 10) for 30 days. The changes in gut microbiota composition were assessed in the fecal samples of the experimental dogs. The results suggested that the fecal microbiota of the experimental dogs were not influenced upon administration of *L. kefiri* [50].

Table 1. The effects of probiotic supplementation on the health status of healthy dogs.

Ref.	Model	Probiotic Used	Dose per Animal	Duration	Effect of Probiotic Supplementation
[41]	Healthy dogs (Probiotic group, n = 12)	*Enterococcus faecium* NCIB 10415	9.2×10^9 CFU/day	18 days	Reduced the fecal count of *Clostridium* spp. Not effective in inhibiting the growth of *Campylobacter* spp. and *Salmonella* spp.
[42]	Healthy dogs (Probiotic group, n = 9)	*Lactobacillus animalis* LA4	10^9 CFU/g; 0.5 g per day	10 days	LA4 could withstand the gastrointestinal environment and proliferate in the dog intestines
[43]	Healthy dogs (Probiotic group, n = 8; Control group, n = 8)	*Bacillus amyloliquefaciens* CECT 5940 and *E. faecium* CECT 4515	0.2 g of a probiotic blend = 1×10^8 CFU of each probiotic strain per day	39 days (probiotic supplementation period); 6 days adaptation period before the supplementation period	Reduced fecal clostridia count
[44]	Healthy dogs (Probiotic group 1, n = 23; Probiotic group 2, n = 12)	*Lactobacillus*, *Bifidobacterium*, and *Bacillus* species	Daily dose: 1 g of probiotic supplement/10 pounds of body weight; *Lactobacillus* (>64 $\times 10^9$ CFU/g), *Bifidobacterium* (30×10^9 CFU/g), and *Bacillus* species (24×10^9 CFU/g)	14 days (probiotic group 1); 28 days (probiotic group 2)	No increase in the prevalence of vaginal lactic acid bacteria
[45]	Healthy dogs (Probiotic group, n = 5; Placebo group, n = 5; Control group, n = 5)	Queso Blanco cheese containing *Bifidobacterium longum* KACC 91563	5×10^8 CFU/10 g cheese/kg of body weight/day	8 weeks	Reduced the fecal Enterobacteriaceae and *Clostridium* count Increased the fecal *Bifidobacterium* count Increased the short-chain fatty acids such as acetic and propionic acid levels
[46]	Healthy dogs (Probiotic group, n = 4; Placebo group, n = 4; Control group, n = 4)	Queso Blanco cheese containing *Bifidobacterium longum* KACC 91563	5×10^8 CFU/10 g cheese/day	8 weeks	Increased the fecal Bifidobacterium Decreased the fecal *C. perfringens*, Enterobacteriaceae, *Collinesella*, *Blautia*, and *Fusobacterium*
[47]	Healthy dogs (Probiotic group, n = 4; Control group, n = 4)	*Bacillus amyloliquefaciens* CECT 5940	1×10^9 CFU/g dry matter of probiotic product/day	30 days (probiotic supplementation period); 6 days adaptation period before the supplementation period	Increased the fecal bacillus count Decreased the fecal coliform count
[48]	Healthy dogs (Elderly probiotic group, n = 15; Elderly control group, n = 15; Young probiotic group, n = 12; Young control group, n = 12; Training probiotic group, n = 18; Training control group, n = 18)	*Lactobacillus casei* Zhang, *Lactobacillus plantarum* P-8, and *Bifidobacterium animalis* subsp. *lactis* V9	Equal proportions of 3 probiotic strains in a final concentration of 2×10^9 CFU/g; Dosage: 10 g/day (Elderly group); 2 g/day (Young group); 4 g/day (Training group)	60 days	Increased the fecal load of *Lactobacillus* spp. and *Faecalibacterium prausnitzii* and decreased the abundance of *Sutterella stercoricanisin* and *Escherichia coli* in the elderly dogs.
[51]	Healthy dogs (Probiotic group, n = 11)	*E. faecium* EE3	10^9 CFU/mL	1 week	Reduced the fecal staphylococci and *Pseudomonas* load Reduced total lipid levels in most of the dogs
[52]	Healthy dogs (Probiotic group, n = 5; Placebo group, n = 5; Control group, n = 5)	*Lactobacillus johnsonii* CPN23 or *L. acidophilus* NCDC15	10^8 CFU per mL; 0.1 mL per kg of body weight	13 weeks (9 weeks probiotic supplementation period) (next 4 weeks to study the withdrawal effects)	Reduced the plasma glucose levels in the dogs of both probiotic groups Superoxide dismutase and glutathione peroxidase activity were higher in *L. johnsonii* CPN23-treated dogs
[53]	Healthy dogs (Probiotic group, n = 18; Control group, n = 18)	*E. faecium* SF68	5×10^8 CFU/g/day	14 days	An effective cobalamin reduction was observed during the 28th day (after a 14-day follow-up)
[54]	Healthy dogs (Probiotic group, n = 18; Control group, n = 18)	*E. faecium* SF68	5×10^8 CFU/g/day	14 days	No changes in serum alanine transferase and alkaline phosphatase activity

Table 1. *Cont.*

Ref.	Model	Probiotic Used	Dose per Animal	Duration	Effect of Probiotic Supplementation
[55]	Healthy dogs (Probiotic group supplemented with two different concentrations of probiotic, n = each 5; Control group, n = 5)	*Weissella cibaria* JW15	50 g per day; 3×10^8 CFU/g or 3×10^9 CFU/g	14 days	Decreased the serum TG levels and fecal ammonia emissions in the probiotic group. Improvement of fecal lactobacilli load and serum HDL-C levels
[56]	Healthy dogs (Probiotic group, n = 8; Control group, n = 8)	*Bacillus subtilis* and *Bacillus licheniformis*	3.66×10^7 CFU of each bacterial strain/kg of the diet	20 days	Improved the fecal consistency and reduced the occurrence of fetid feces Reduced the fecal biogenic amine content, thereby reducing the fecal odor
[57]	Healthy dogs (Probiotic group, n = 14; Control group, n = 16)	*L. acidophilus* D2/CSL	5×10^9 CFU/g of feed additive; Pre-mixture contains 50 g of feed additive + 9950 g of maltodextrin; 20 g of pre-mixture per day	35 days (probiotic supplementation period); 7 days adaptation period before the supplementation period	Maintained a perfect body condition score throughout the study Improved the fecal moisture content
[58]	Healthy dogs (n = 15)	*Lactobacillus fermentum* AD1	10^9 CFU	7 days	Increased the fecal count of lactobacilli and enterococci and, also increased serum level of total lipid and total protein Reduced the serum glucose level
[59]	Healthy dogs (Probiotic group 1, n = 12; Probiotic group 2, n = 11)	*Lactobacillus fermentum* AD1-CCM7421	Probiotic group 1 (2 g per day; 10^8 CFU/g of milk powder); Probiotic group 2 (1 g per day; 10^7 CFU/g of milk powder)	14 days (Probiotic group 1); 7 days (Probiotic group 2)	Reduced the fecal count of clostridia and increased the total fecal concentration of SCFA such as butyric, formic, succinic, and valeric acid in probiotic group 1 Reduced the fecal count of *Aeromonas* sp., *E. coli* and *Pseudomonas* sp. in probiotic group 2

The administration of *E. faecium* EE3 (10^9 CFU per mL) to healthy dogs (n = 11) for one week reduced the fecal staphylococci and *Pseudomonas* load. Most of the dogs showed a reduction in total lipid levels. Cholesterol levels were also impacted in dogs upon probiotic intervention (i.e., probiotic supplementation increased the cholesterol levels to reach physiological level in the dogs those showed low cholesterol values in their blood samples and also decreased the cholesterol levels to reach physiological level in those with high levels). Moreover, the strain EE3 was found in the experimental dogs' feces after three months of the study. The results showed that EE3 could improve the health condition of healthy dogs [51].

The healthy dogs were administered *Lactobacillus johnsonii* CPN23 or *Lactobacillus acidophilus* NCDC15 (10^8 CFU per mL; 0.1 mL per kg of body weight) for nine weeks, and the biochemical profile and antioxidant capacity were assessed. Probiotic supplementation reduced the plasma glucose levels in the dogs of both probiotic groups (each group n = 5) compared to that of the control group (n = 5). There was no change in catalase, reduced glutathione, or glutathione S-transferase levels in the probiotic-treated dogs, while superoxide dismutase and glutathione peroxidase activity were higher in the *L. johnsonii* CPN23-treated dogs [52].

The administration of *E. faecium* SF68 (5×10^8 CFU/g/day) for 14 days to healthy dogs (non-hypocobalaminaemic breeds; Age = 2–5 years old) (probiotic group, n = 18, 10 females, 8 males) reduced the mean serum cobalamin when compared with the baseline and at day 14. In this study, cobalamin concentration is considered clinically relevant when the reduction is 25% to 30% from the mean baseline concentration of cobalamin (301.7 pg/mL). An effective cobalamin reduction was observed during the 28th day (after a 14-day follow-up). There was a non-significant increase in the serum folate level at day 14; however, this level was reduced at day 28. The canine IBD activity index score was nil for both probiotic

and control groups during the study. All dogs of both group showed no alterations on the general attitude, appetite, weight, consistency or frequency of feces and there were no adverse effects. The results showed that short-term *E. faecium* SF68 supplementation could reduce the serum cobalamin in healthy dogs [53]. The supplementation of *E. faecium* SF68 (5×10^8 CFU/g/day) for 14 days was unable to change the liver function (serum alanine transferase and alkaline phosphatase activity) in healthy dogs [54].

Adult beagles were supplemented with *Weissella cibaria* JW15 (probiotic strain isolated from kimchi) in two different concentrations (50 g per day; 3.0×10^8 CFU/g or 3.0×10^9 CFU/g) along with regular food for 14 days. The serum TG levels and fecal ammonia emissions were decreased in the probiotic-treated group. The fecal lactobacilli load and serum HDL-C levels were also improved by the probiotic intervention. The results suggested that JW15 supplementation could improve dogs' health [55].

Bacillus subtilis (3.66×10^7 CFU/kg of the diet) and *Bacillus licheniformis* (3.66×10^7 CFU/kg of the diet) administration to healthy dogs (n = 8, 4 females, 4 males) for 20 days did not affect nutrient digestibility but improved fecal consistency and reduced the occurrence of fetid feces. The fecal biogenic amine content was decreased, thereby reducing the fecal odor in dogs of the probiotic group [56].

L. acidophilus D2/CSL (5×10^9 CFU/g of feed additive; pre-mixture contains 50 g of feed additive + 9950 g of maltodextrin; 20 g of pre-mixture per day) supplementation maintained a perfect body condition score for the whole duration of the study and improved the fecal moisture content in healthy dogs (n = 16) compared to that of the control group (n = 14). The study demonstrated that including *L. acidophilus* D2/CSL in a dog's food could maintain weight and improve GI tract health in healthy dogs [57].

Supplementation of *Lactobacillus fermentum* AD1 (10^9 CFU) for 7 days showed that *L. fermentum* AD1 can survive in the canine GI tract, and increased fecal count of lactobacilli and enterococci and, also increased serum level of total lipid and total protein, and reduced the serum glucose level in healthy dogs [58]. Strompfová et al. [59,60] demonstrated that *L. fermentum* CCM 7421 (AD1 is denoted as CCM 7421; 10^7–10^8 CFU per dog per day) can survive in the canine GI tract and alter the microbiota (increased fecal count of LAB and reduced fecal count of clostridia, *Aeromonas* sp., *E. coli* and *Pseudomonas* sp.) and metabolites (increased the total fecal concentration of SCFA such as butyric, formic, succinic, and valeric acid) [59,60] in healthy dogs.

3.2. Immune Immunomodulatory Properties

The serum levels of IL-6 and TNF-α were increased in the *B. longum* KACC-91563-treated group compared to the control, indicating the immune–modulatory effects of the probiotics [46].

The serum levels of IgG, IFN-α, and fecal secretory IgA were increased and serum levels of TNF-α was reduced in the probiotic (*L. casei* Zhang, *L. plantarum* P-8, and *B. animalis* subsp. *lactis* V9)-treated group [48].

IgA levels was not influenced upon administration of *L. kefiri* in healthy dogs [50].

The administration of 5×10^8 CFU/day of *E. faecium* SF68 to healthy young dogs at 8 weeks of age up to one year of age increased the fecal IgA level, canine-distemper-virus vaccine-specific IgG and IgA levels, and the proportion of the $CD21^+$/major histocompatibility complex class II^+ compared to the placebo controls [61] (Table 2). Similarly, the administration of *Lactobacillus murinus* LbP2 (5×10^9 CFU/day) on alternating days for two weeks increased the total fecal IgA concentration in healthy dogs, indicating the immune immunomodulatory properties of LbP2 [62]. These findings will aid in using probiotics in canine nutrition for immune enhancement.

Table 2. The immune-modulatory effect of probiotic supplementation on the health status of dogs.

Ref.	Model	Probiotic Used	Dose per Animal	Duration	Effect of Probiotic Supplementation
[46]	Healthy dogs (Probiotic group, n = 4; Placebo group, n = 4; Control group, n = 4)	Queso Blanco cheese containing *Bifidobacterium longum* KACC 91563	5×10^8 CFU/10 g cheese/day	8 weeks	Increased the serum levels of IL-6 and TNF-α
[48]	Healthy dogs (Elderly probiotic group, n = 15; Elderly control group, n = 15; Young probiotic group, n = 12; Young control group, n = 12; Training probiotic group, n = 18; Training control group, n = 18)	*Lactobacillus casei* Zhang, *Lactobacillus plantarum* P-8, and *Bifidobacterium animalis* subsp. *lactis* V9	Equal proportions of 3 probiotic strains in a final concentration of 2×10^9 CFU/g; Dosage: 10 g/day (Elderly group); 2 g/day (Young group); 4 g/day (Training group)	60 days	Increased serum IgG, and IFNα levels, fecal SIgA levels in the probiotic-treated group
[61]	Healthy young dogs (Probiotic group, n = 7; Placebo group, n = 7)	*Enterococcusfaecium*	5×10^8 CFU/day	1 year	Increased the fecal IgA, and canine distemper virus vaccine-specific circulating IgG and IgA. Increased the proportion of mature B cells.
[62]	Healthy dogs (Probiotic group, n = 7; Placebo group, n = 6)	*Lactobacillus murinus* LbP2	5×10^9 CFU/day; dosage on alternative days	2 weeks	Increased the total fecal IgA concentration
[63]	Healthy dogs (Probiotic group, n = 5; Placebo group, n = 5)	*Lactobacillus johnsonii* CPN23	2–3 $\times 10^8$ CFU/day	9 weeks	Improved the phytohaemagglutinin-P reaction and the concentration of acetate and butyrate. Reduced fecal ammonia. No change in antibody reaction to sheep erythrocytes.

IL-6: Interleukin-6; IFN-α: TNF-α: tumoral necrosis factor-alpha; IgG: Immunoglobulin G; Interferon-alpha; IgA: Immunoglobulin A; sIgA: secretory IgA.

L. johnsonii CPN23 (canine origin) administration to healthy adult Labrador dogs improved the fecal acetate and butyrate levels. *L. johnsonii* CPN23 also reduced fecal ammonia concentrations. Cell-mediated immune response was also evaluated as type-IV delayed-type hypersensitivity reaction to intradermally injected phytohaemagglutinin-P. CPN23 administration improved the cell-mediated immune response (as delayed-type hypersensitivity response in the form of increase in skin induration) in healthy dogs. There was no change in the antibody response to sheep erythrocytes. The study concluded that *L. johnsonii* CPN23 could be used to achieve improvements in canine health [63].

4. Health Benefits of Probiotic Supplementation in Diseased Dogs

4.1. Chronic Kidney Disease

VSL#3 supplementation effectively increased the glomerular filtration rate (GFR) in dogs with chronic kidney disease. In more detail, the 60-day administration of VSL#3 (a mixture of probiotics containing *L. casei*, *L. plantarum*, *L. acidophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *B. longum*, *Bifidobacterium breve*, *Bifidobacterium infantis*, and *Streptococcus salivarius* subsp. *thermophilus*; 112 to 225 $\times 10^9$ CFU/10 kg body weight; every 24 h) to dogs (n = 30) with chronic kidney disease increased the GFR compared to that of the control group (n = 30) and baseline values [64].

4.2. Atopic Dermatitis

Marsella [19] reported that the prenatal and postnatal supplementation of *Lactobacillus rhamnosus* GG did not affect the clinical scores of atopic dermatitis (AD) in puppies. In detail, the mother dog was orally administered with *L. rhamnosus* GG (20×10^9 CFU per capsule; 10 capsules per day) starting at the third week of gestation during the dog's second pregnancy and continuing throughout lactation. Puppies (n = 9; 3 females, 6 males) of

the second litter (probiotic group) were administered *L. rhamnosus* GG (five capsules per day) starting at 3 weeks of age until 6 months of age. The puppies (at 3 weeks of age) of the first and second litters from same parents (two adult beagles with severe AD) were epicutaneously sensitized with *Dermatophagoides farinae*. The blood samples were then collected every 6 weeks (from 6 weeks of age until 6 months of age) to assess the serum-allergen-specific IgE. Severity of the clinical signs of AD were evaluated using Canine Atopic Dermatitis Extent and Severity Index (CADESI) scoring system before and after (initially after 6 h and then every 24 h for 5 days) allergen exposure. The puppies (n = 7; 5 females, 2 males) of the first litter (control group) developed clinical signs of AD and were strongly seropositive for IgE. The puppies of the second litter presented a lower serum titer against IgE and no significant change in AD clinical score. This study suggested that the supplementation of *L. rhamnosus* GG reduced the immunologic indicators of AD [19] (Table 3). As a follow-up [20] to the previous study [19], all the dogs (at the age of 2) in both litters (both the control and probiotic groups) were epicutaneously sensitized (biweekly application for 4 months) with ragweed, timothy, and *D. farinae* [20]. The clinical signs of the experimental dogs in both the control and probiotic groups were then evaluated. Allergen-specific IgE, TGF-β, and IL-10 levels were also measured. During the study, the dogs in the probiotic group were 3 years old, and the dogs in the control group were 4 years old. Normal dogs (n = 10 non-atopic Beagles; 4 females, 6 males; 4 years old) were used as the control group. IL-10 was higher in the control group, whereas IgE and TGF-β levels did not differ among the dogs of the control and probiotic groups. The CADESI scores of clinical signs were improved in the probiotic group. This study demonstrated that early administration of *L. rhamnosus* GG has long-term immunological and clinical effects in dogs [20].

Table 3. The effects of probiotic supplementation on the health status of dogs with atopic dermatitis.

Ref.	Model	Probiotic Used	Dose per Animal	Duration	Effect of Probiotic Supplementation
[19]	Dog puppies (Probiotic group, n = 9; Control group, n = 7)	*Lactobacillus rhamnosus* strain GG	20×10^9 CFU per capsule; 10 capsules per day (mother); 5 capsules per day (puppy)	Prenatal and postnatal (mother dog at third week of gestation and continuing throughout lactation); postnatal (Starting at the age of 3 weeks until 6 months old)	Reduced the immunologic indicators of AD. No significant reduction in clinical signs of AD.
[65]	Dogs with AD (Probiotic group, n = 32; Placebo group, n = 10)	*Lactobacillus sakei* probio-65	2×10^9 CFU/day	2 months	Reduced the CADESI score
[66]	Dogs with AD (Probiotic group, n = 7; Placebo group, n = 4)	*Bifidobacteriumlongum*	5×10^{10} CFU/day	12 weeks	Reduced the CADESI score

AD: Atopic dermatitis; CADESI: Canine atopic dermatitis extent and severity index.

The oral administration of probiotic strain *Lactobacillus sakei* probio-65 (2×10^9 CFU per gram per day for two months) to dogs (n = 32) diagnosed with canine AD effectively reduced the disease severity index measured by CADESI scoring system. The double-blind placebo-controlled study demonstrated that *L. sakei* probio-65 could be considered an adjuvant therapeutic agent to manage canine AD [65].

B. longum (5×10^{10} CFU/day) was orally supplemented in dogs with AD for 12 weeks, and the severity of the skin lesions was assessed using the CADESI score. Pruritus severity was also assessed using the pruritus visual analog scale (PVAS). The transepidermal water loss (TEWL) and the medication score was also recorded. The data were collected every four weeks of the study. The results revealed that the administration of *B. longum* progressively reduced the CADESI score compared to the baseline, whereas probiotic supplementation was not effective in reducing the TEWL, PVAS, and medication scores. The study determined that *B. longum* could improve skin lesions in dogs [66].

4.3. Gastrointestinal Diseases and Abnormalities

4.3.1. Chronic Enteropathy

The dogs with chronic enteropathies were administered *Saccharomyces boulardii* (1×10^9 CFU/kg/12 h) for 60 days in addition to the standard treatment. The Canine Chronic Enteropathy Clinical Activity Index (CCECAI), stool consistency and frequency, and body condition score were improved after the probiotic treatment. This study suggested that *S. boulardii* could be used as a probiotic for canines to treat enteropathies [67] (Table 4).

Table 4. The effects of probiotic supplementation on the health status of dogs with gastrointestinal diseases and abnormalities.

Ref.	Model	Pro/Prebiotic Used	Dose per Animal	Duration	Effect of Probiotic Supplementation
[21]	Dogs with FRD (Probiotic group, n = 11; Placebo group, n = 10)	*Lactobacillus acidophilus* NCC2628, *L. acidophilus* NCC2766, *Lactobacillus johnsonii* NCC2767	10^{10} CFU of each probiotic strain/day	4 weeks	Reduced Enterobacteriaceae count, Increased *Lactobacillus* spp. count, Improved clinical signs
[22]	Dogs with diarrhea (Probiotic group, n = 25; Placebo group, n = 19)	*Lactobacillus fermentum* VET 9A, *Lactobacillus rhamnosus* VET 16A, and *Lactobacillus plantarum* VET 14A	Daily dose: 2×10^9 CFU/mL of 3 bacterial strains	7 days	Improved the stool consistency, normalized the appetite, decreased *Clostridium perfringens* and *Enterococcus faecium* count
[23]	Dogs with acute diarrhea (Probiotic group, n = 57; Placebo group, n = 61)	*E. faecium* 4b1707	2×10^8 CFU/gram paste; dosage according to body weight; 3 times per day (every 8 h)	10 days	Reduced the duration of diarrhea and improved acute diarrhea
[24]	Dogs with ACD (Probiotic group, n = 19; Placebo group, n = 19; Metronidazole group, n = 19)	*Bifidobacterium bifidum* VPBB-6, *Bifidobacterium longum* VPBL-5, *Bifidobacterium animalis* VPBA-4, *Bifidobacterium infantis* VPBI-6, *L. acidophilus* VPLA-4, *L. plantarum* VPLP-5, *Lactobacillus casei* VPLC-1, *Lactobacillus brevis* VPLB-5, *Lactobacillus reuteri* VPLR-1, and *Lactobacillus bulgaricus* VPLB-7.	30×10^9 CFU/425 mg/capsule *; 125 mg/4–10 kg body weight; 250 mg/10.1–20 kg body weight; 400 mg/20.1–45 kg body weight; dose twice a day	10 days	Improved stool consistency
[25]	Dogs with diarrhea (Probiotic group, n = 16; Placebo group, n = 16)	Metronidazole and *E. faecium* SF68	5×10^8 CFU/day	7 days	Eliminated *Giardia* cysts and cured diarrhea
[67]	Dogs with chronic enteropathies (Probiotic group, n = 6; Placebo group, n = 7)	*Saccharomyces boulardii*	1×10^9 CFU/kg/12 h	60 days	CCECAI, fecal consistency and frequency, and body condition score were improved.
[68]	Dogs with chronic enteropathy (n = 12; Diseased dogs during 1st visit (Control) and 2nd visit (Treatment) after 6 weeks of treatment	*E. faecium* and FOS	1×10^9 CFU/day	6 weeks	No change in the expression of the inflammasome or its components
[69]	Dogs with IBD (Probiotic group, n = 10; Control group, n = 10)	VSL#3 (*L. casei*, *L. plantarum*. *L. acidophilus*, and *Lactobacillus delbrueckii* subsp. *bulgaricus*, *B. longum*, *Bifidobacterium breve*, *B. infantis*, and *Streptococcus salivarius* subsp. *thermophilus*)	$112–225 \times 10^9$ CFU/10 kg/day	60 days	Decreased the clinical and histological scores. Normalized dysbiosis. Enhanced regulatory T-cell markers.
[70]	Dogs with diarrhea (Probiotic group, n = 20; Placebo group, n = 20)	*L. casei* Zhang, *L. plantarum* P-8, and *Bifidobacterium animalis* subsp. *lactis* V9	Probiotic mixture (equal proportion of 3 probiotic strain) containing 3×10^9 CFU/g (each *Lactobacillus* strain), 4×10^9 CFU/g (*Bifidobacterium* strain); Dosage: 10 g/day (elderly dogs); 4 g/day (young dogs); 2 g/day (adult dogs)	60 days	Increased the abundance of *L. johnsonii*, *L. reuteri*, *L. acidophilus*, *Butyricicoccus pullicaecorum* Reduced the count of *C. perfringens* and *Stenotrophomonas maltophilia*
[71]	Dogs with DAD (Probiotic group, n = 13; Placebo group, n = 7)	*Lactobacillus murinus* LbP2	5×10^9 CFU/day	5 days	Improved the clinical score

FRD: Food responsive diarrhea; DAD: Distemper-associated diarrhea; AHDS: Acute hemorrhagic diarrhea syndrome; ACD: Acute canine diarrhea; IBD: Inflammatory bowel disease; FOS: Fructo-oligosaccharides; CCECAI: Canine Chronic Enteropathy Clinical Activity Index. * 30×10^9 CFU/capsule as per the label of the probiotic product, 70×10^9 CFU/capsule as per the assessment at the initiation of the study [23].

The supplementation of *E. faecium* (1×10^9 CFU/day) and FOS for six weeks did not change the expression of the inflammasome and its associated components in dogs with

chronic enteropathy. The study observed that the inflammasome and its components could be partially involved in chronic enteropathy's inflammatory process [68].

VSL#3 containing *L. casei*, *L. plantarum*, *L. acidophilus*, *L. delbrueckii* subsp. *bulgaricus*, *B. longum*, *B. breve*, *B. infantis*, and *S. salivarius* subsp. *thermophilus* (112 to 225 $\times$ 10^9 CFU/10 kg/day) was administered to dogs suffering from idiopathic inflammatory bowel disease (IBD, which is a chronic enteropathy) for 60 days. The probiotic supplementation improved the clinical and histological scores and reduced CD3+ T-cell infiltration. The probiotic treatment enhanced regulatory T-cell markers, such as FoxP3+ and TGF-β+. IBD-associated dysbiosis was normalized in the probiotic treatment group. Clinical and histological improvements were not observed in the control (prednisone- and metronidazole-treated) group. This study noted that future, more extended, studies are needed to confirm the efficiency of VSL#3 [69].

The supplementation of a probiotic mixture (*L. acidophilus* NCC2628, *L. acidophilus* NCC2766, and *L. johnsonii* NCC2767; 10^{10} CFU of each probiotic strain/day) effectively reduced the expression of IL-10 and increased the expression of IFN-γ in dogs suffering from food responsive diarrhea (which is a chronic enteropathy) treated with an elimination diet; this mixture also reduced and increased the abundance of *Enterobacteriaceae* and *Lactobacillus*, respectively. Overall, the clinical signs of diarrhea were improved after the intervention of the probiotic mixture [21].

4.3.2. Acute Diarrhea

Daily intervention of fermented sour milk products containing 2 $\times$ 10^9 CFU/mL of *L. fermentum* VET 9A, *L. rhamnosus* VET 16A, and *L. plantarum* VET 14A for seven days normalized the stool consistency, and improved appetite in the dogs with acute diarrhea. The abundance of pathogenic strains such as *C. perfringens* and *E. faecium* were also decreased during the probiotic treatment [22]. *E. faecium* 4b1707 (2 $\times$ 10^8 CFU/gram paste; dosage according to body weight; three times per day (every 8 h)) intervention for 10 days reduced the duration of diarrhea and improved acute diarrhea in dogs [23].

Intervention of probiotic mixture containing *B. bifidum* VPBB-6, *B. longum* VPBL-5, *B. animalis* VPBA-4, *B. infantis* VPBI-6, *L. acidophilus* VPLA-4, *L. plantarum* VPLP-5, *L. casei* VPLC-1, *Lactobacillus brevis* VPLB-5, *Lactobacillus reuteri* VPLR-1, and *Lactobacillus bulgaricus* VPLB-7 (30 $\times$ 10^9 CFU/425 mg/capsule as per the label of the probiotic product, 70 $\times$ 10^9 CFU/capsule as per the assessment at the initiation of the study; 125 mg/4–10 kg body weight; 250 mg/10.1–20 kg body weight; 400 mg/20.1–45 kg body weight; dose twice a day) for 10 days improved the stool consistency in dogs with acute canine diarrhea [24].

4.3.3. Nonspecific Diarrhea

In addition to metronidazole, *E. faecium* SF68 (5 $\times$ 10^8 CFU/day) was administered for seven days to dogs with diarrhea. Normalization of the stool and the elimination of *Giardia* spp. cysts were observed in dogs treated with dual therapy compared to the group treated with the drug alone. Completely cured diarrhea was observed among dogs in the dual-therapy group [25]. But due to the small number of positive samples in the group supplemented with *E. faecium* SF68, they have not statistically compared the prevalence of *Giardia* and clinical response rates between groups, which is the limitation of this study [25], so further studies with larger sample sizes and statistical comparison between groups are required to conclude the efficacy of SF68 on Giardia control.

The intervention of a probiotic cocktail containing equal proportion of *L. casei* Zhang, *L. plantarum* P-8, and *B. animalis* subsp. *lactis* V9 (probiotic mixture containing 3 $\times$ 10^9 CFU/g (each *Lactobacillus* strain), 4 $\times$ 10^9 CFU/g (*Bifidobacterium* strain); Dosage: 10 g/day for elderly dogs; 4 g/day for young dogs; 2 g/day for adult dogs) for 60 days effectively altered the gut microbiome of dogs with diarrhea. The abundance of *L. johnsonii*, *L. reuteri*, *L. acidophilus*, and *Butyricicoccus pullicaecorum* increased, and the count of *C. perfringens* and *Stenotrophomonas maltophilia* decreased. Moreover, the pathways involved in the metabolism of amino acids and the biosynthesis of secondary metabolites were activated,

and the virulence-associated pathways were downregulated. This study suggested that a probiotic intervention could improve dog health through positive regulation of the gut microbiome [70].

4.3.4. Other Diseases Associated with Diarrhea or Prevention of Gastrointestinal Infections

The effect of the oral supplementation of *Lactobacillus murinus* LbP2 to dogs suffering from canine distemper virus-associated diarrhea was also studied. In detail, an intervention of LbP2 (5×10^9 CFU/day) administered to dogs for five days improved the clinical score of the disease, stool consistency, stool output, appetite, and mental health. The results suggested that LbP2 could be used to manage canine distemper virus-associated diarrhea [71].

German Shepherd (n = 36, 18 females, 18 males) and Yorkshire puppies (n = 36, 18 females, 18 males) were vaccinated for distemper and parvovirus at 6 weeks of age, and were vaccinated for distemper, hepatitis, leptospirosis, parainfluenza, parvovirus at 8 weeks and 12 weeks of age. These puppies were randomly grouped to fed with diet supplemented with either of the probiotic, *L. rhamnosus* MP01 (~9 $\log_{10}$ CFU/day) or *L. plantarum* MP02 (~9 $\log_{10}$ CFU/day) (two probiotic groups) or diet without probiotics (control group) for 8 weeks (started at 5 weeks of age). Each group (two probiotic groups and one control group) had German Shepherd (n = 12, 6 females, 6 males) and Yorkshire puppies (n = 12, 6 females, 6 males). GI infection (symptoms such as diarrhea in the presence or absence of fever or vomiting) during the duration (2 months) of the study. *L. rhamnosus* MP01 and *L. plantarum* MP02 interventions effectively prevented gastrointestinal infections in German Shepherd and Yorkshire puppies compared to that of the control group, and also increased the SCFAs concentration in the feces of dogs of probiotic group [72].

Since only single study are available for distemper virus-associated diarrhea [71], and for the prevention of gastrointestinal infections in puppies [72], more studies are needed to suggest the health promoting probiotic effect in those cases.

5. Discussion

Microbiota shape the health status of pets. Subsequently, dog owners play a role in determining the microbiota of dogs through their food. Pet foods are formulated to contain the carbohydrates, proteins, fats, minerals, and vitamins, etc. Presently, some pet foods include probiotics in their formulations. Several probiotic supplements have been reported to have health-promoting properties for both healthy and diseased dogs. Probiotics isolated from fresh fecal swabs of healthy dogs could also be used as a potent probiotic candidate as a dietary supplement for dogs [73,74].

Positive regulation of the microbial ecology in the GI tract has systemic effects. Studies have confirmed that the intervention of probiotics could change the microbiota of dogs. The supplementation of probiotic preparations was shown to increase the abundance of beneficial microbes, such as *Lactobacillus* spp. and *Bifidobacterium* spp., and suppress the growth of harmful microbes, such as *Enterobacteriaceae* and *C. perfringens* [70].

The additional functional foods that are nutritive or nonnutritive and required dietary components to promote the host health are referred as functional nutrition. Functional nutrients and functional foods with immune-regulatory properties are termed as immunonutrition. Vitamins (D and E), minerals (Zinc), omega-3 polyunsaturated fatty acids, phytochemicals such as epigallocatechin-3-gallate, microbial metabolites such as SCFA, and probiotics have immunomodulating potentials. The vitamins, zinc, and SCFA are involved in intestinal homeostasis and maintain intestinal integrity [75–77]. Supplementation of vitamin E and selenium enhanced the immune response in vaccinated dogs, and especially increased the IgG concentration compared to the control, and offered the highest protection [78]. The supplementation of eicosapentaenoic acid (EPA) and docosahexaenoic acid showed significant health improvement in diseased dogs [79]. The intestinal dysbiosis affects the availability of beneficial microbial metabolites, such as SCFA. The use of antibiotics might deplete the anaerobic bacterial load, and drain the SCFA content, particularly

butyrate concentrations. Besides, over-immunomodulation has to be taken into account so that the naïve functions, such as eliminating microbial infections, of the immune system can work accordingly. Probiotic supplementation resumes the healthy intestinal microbiota and promotes the ideal immune regulation. Thus, factors such as selection of the probiotic strain, dose, and duration have to be optimized properly before practical applications [77].

Dogs cannot synthesize vitamins, but vitamins can be obtained from their diet and the gut microbiota. The gut microbiota has been considered as a rich source of vitamins [80]. The deficiency of folate and cobalamin is considerably related to and maintains gastrointestinal homeostasis. The deficiency of folate might lead to the reduction of regulatory T (Treg) cells (subpopulation of T cells involved in the immune homeostasis), which cause impaired gut homeostasis, and are prone to intestinal inflammation [81]. Cobalamin is a marker of gastrointestinal diseases in companion animals due to the availability and detectability of cobalamin in serum, even in the serum of animals fed with a low cobalamin diet. Hypothetically, reduced serum cobalamin is due to the utilization of available cobalamin by the increased number of intestinal bacteria [82]. The dogs with diarrhea were reported for the low serum cobalamin concentration [83]. Allenspach et al. [84] reported that a decrease in serum cobalamin concentration was observed in dogs with chronic enteropathies [84], and the oral supplementation of cobalamin rectified the deficiency [85]. Sauter et al. [21] reported that the probiotic mixture intervention did not significantly affect the cobalamin concentration, while the serum folate concentration was increased [21]. Hypocobalaminemia is associated with negative outcomes in dogs with chronic gastrointestinal disease [86]. The probiotics were used to treat GI disorders [58], and their potential effect hinges on the bacterial strain [3]. Though probiotic bacteria have been reported to produce vitamins [80,87], the administration of *E. faecium* SF68 resulted in moderate hypocobolaminaemia in healthy dogs [53]. Thus, the intake of *E. faecium* SF68 longer than 14 days might lead to an intense decrease in the levels of cobalamin in healthy dogs, which should be noted, and further studies are required to know whether SF68 is safe or not. The selection of probiotics, principally to treat diseased dogs, is more considerable and prompt the need for caution as the mechanism of action of probiotics might be affected by many factors and some clinical aspects are not perfectly known, especially in diseased dogs.

The production of SCFA in dogs is not fully dependent on the dietary carbohydrate content [88]. The altered SCFA level affects gastrointestinal health. SCFAs, particularly butyrate, might be used as a preferred energy source for the colonocytes [89]. It has been reported that the SCFA has a regulatory effect on gastrointestinal motility [90]. The gastrointestinal disease developments may be related to the changes in intestinal microbial composition and its function, especially the reduced production of SCFA and other metabolites [91]. For example, dogs with acute and chronic diarrhea who had dysbiosis were found to have reduced SCFA-producing bacteria [92,93], and significant reduction of propionate concentration has been reported in dogs with acute diarrhea [94]. Probiotic supplementation amended the microbial population in dogs and augmented the production of SCFA. *B. longum* KACC 91563 [45] and *L. fermentum* CCM 7421 [59] intervention, which significantly reduced the harmful bacterial count and increased the SCFA levels in healthy companion dogs [45,59]. Nevertheless, not all the probiotic interventions increased the SCFA level in experimental dogs. Gagné et al. [95] reported that the intervention of synbiotic preparation (5 g of synbiotic per dog per day) containing *E. faecium* SF68 (56.7 mg/g; 5.67×10^8 CFU/g), *Bacillus coagulans* (2.5 mg/g; 3.75×10^7 CFU/g), *L. acidophilus* (14.4 mg/g; 7.2×10^8 CFU/g), fructooligosaccharides (400 mg/g), mannanoligosaccharides (80 mg/g), Vitamin B1 (2.5 mg/g), Vitamin B2 (0.8 mg/g), Vitamin B3 (19.2 mg/g), Vitamin B6 (0.8 mg/g) for 6 weeks showed no significant changes in acetate, propionate, and butyrate concentrations in healthy training sled dogs of synbiotic group (n = 9) compared to that of the placebo group (n = 8) [95].

A recent systemic review of 12 studies that reported the clinical effect of probiotics in dogs with GI disease stated that probiotic supplementation showed a very limited and unimportant effect on the treatment or prevention of acute GI disease in dogs, and

non-significant improvement in chronic GI disease [96]. A very recent review stated that probiotics are beneficial in some acute or infectious GI conditions in dogs and are slightly beneficial in food-responsive or antibiotic-responsive chronic enteropathies in dogs [97].

The evidence suggests that nutrition influences the composition of the microbiota and is thus strongly associated with gastrointestinal health [98]. The development of probiotic-based foods for dogs is required to promote the health benefits for both healthy and diseased companion pets. However, further research is needed to develop effective probiotic preparations to improve the well-being of domestic dogs.

6. Conclusions

The literature survey provided in this paper revealed that probiotics might promote the health and well-being of healthy dogs, that they could be considered as an adjuvant therapeutic agent to manage AD [19,20,65], and could help dogs by improving their clinical symptoms of diarrhea [21–25]. The possible mechanisms of action of probiotics include persistent colonization in the host and restoring the normal microbiota by modulating the commensal microbiota, thereby improving the availability of beneficial microbial metabolites, such as SCFA, and reducing the pathogenic microbial load in the host system, and/or improving the host immune response by exhibiting an immunomodulatory effect on maintaining or improving the health status of dogs (Figure 1).

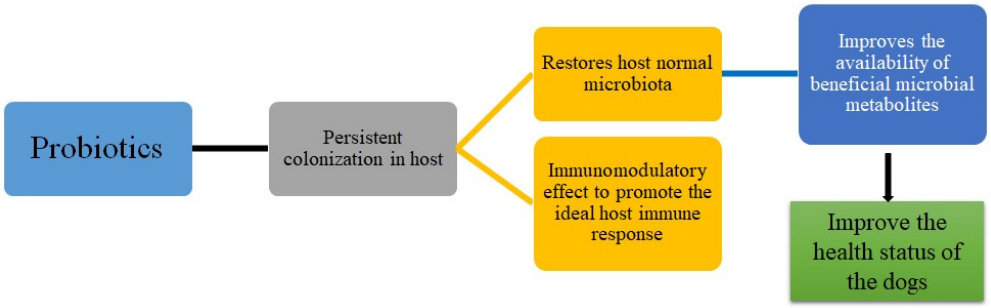

Figure 1. Possible mechanism of probiotic action on improving the health status of dogs.

However, a few studies [41,44,50] showed no positive effects on the health status of dogs upon intervention of *E. faecium* NCIB 10415 (isolated from healthy dog feces) [41], *Lactobacillus*, *Bifidobacterium*, and *Bacillus* species [44], and *Lactobacillus kefiri* [50]. Thus, more studies are required to discover the negative effects and positive effects of probiotics in healthy and diseased dogs for the evidence-based use of probiotics in dogs. The influence of sources of probiotic bacteria could be concluded by comparing the health beneficial effects of same probiotic LAB species/strains derived from different origins. Thus, an abundance of studies based on the same probiotic species/strains of different origin is required to compare the health beneficial effect between them and conclude the influence of origin on the health status of dogs. The protective effects of probiotic supplements depend on the strain, dose, duration of the intervention, and combinations with other strains. Therefore, more controlled trials are required to find effective and safe probiotics by characterizing the safe probiotic preparations in terms of the optimum dose, either for specific dog breeds or for all dog breeds, to discover their efficacy when used as a single species/strain or multi-species/strain, and for the treatment of different diseases in compliance with ethical standards. In conclusion, further, much larger studies are needed to explore the beneficial implications of probiotics at a broader level, paving the way for the development and/or improving the pharmacological activity of nutraceutics, which include probiotics for dogs.

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