The influence of 'biotics' on the gut microbiome of dogs and cats

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ABSTRACT

A global rise in pet ownership and an increasing tendency towards the humanisation of pets have resulted in a greater focus on improving animal health and longevity. These developments coincide with the increased recognition of the role of the gut microbiome in animal health. The gut microbiome has been shown to play a prominent role in gastrointestinal health, and it is becoming increasingly clear that these health benefits extend beyond the gut and into different physiological systems, such as the immune system. Dietary supplementation with products known as 'biotics', which include probiotics, prebiotics, synbiotics and postbiotics, is a strategy used to modify the gut microbiome and promote host health. Although biotics have been successfully used in companion animals, questions remain regarding appropriate biotic selection, mechanisms of action, optimum inclusion levels and safety. This review aims to summarise the effects of biotics on the gut microbiome of dogs and cats and assess their potential role in supporting gastrointestinal health.

DOGS and cats are being kept by an increasing number of families worldwide, leading to steady growth in the pet food and supply industries. Euromonitor International estimated global pet food sales to be worth 133.9 billion US dollars in 2023, representing a 43.9 per cent increase from 2018.[1] Alongside this increase in market size, there has been a trend towards premiumisation, with the rising tendency of pet owners to humanise their animals meaning that they are willing to spend more in an effort to enhance pet health and longevity.

In addition to nutritional adequacy and ingredient transparency, pet owners are increasingly interested in the presence of functional ingredients in pet food because they are thought to provide health benefits beyond the provision of essential nutrients (ie, water, vitamins, minerals, proteins and fats). For example, 90 per cent of dog owners reported changing their dog's food after a cancer diagnosis.[2] Similarly, 38 per cent of cat owners started administering dietary supplements to their cats after a diagnosis of chronic renal failure.[3] However, the interest in functional ingredients goes beyond their potential benefits to unwell animals, with their inclusion now being common in pet foods and treats for healthy animals.

A wide range of functional ingredients may be present in these products, including antioxidants (eg, vitamin C, vitamin E, selenium), chondroprotective agents (eg, chondroitin sulfate, glucosamine sulfate), mitochondrial cofactors (eg, L-carnitine, coenzyme Q10), plant-derived phytochemicals (eg, anthocyanidins, carotenoids, flavonoids, polyphenols, sterols), intrinsic or isolated dietary fibres with varying degrees of solubility and fermentability, and various preparations of live microorganisms (yeast and bacteria), microbial substrates and their bioactive products.[4-6]

Mounting evidence suggests that the gastrointestinal (GI) microbiome can influence host health, primarily through contributions to nutrient metabolism, production of beneficial metabolites, enhancement of intestinal barrier function and education of the immune system.[7,8] Therefore, modulating the GI microbiome's composition and/or activity with functional ingredients may be a helpful strategy to prevent or manage GI disorders, which are common in cats and dogs presented at veterinary clinics.

Diarrhoea in dogs and cats is often caused by chronic enteropathy (CE),[9,10] but it can also be initiated by common life events, such as acute

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stress[11,12] and adverse food reactions.[13] Although the pathogenesis of CE is not well understood, it is thought to develop from a combination of factors (eg, genetics, intestinal barrier integrity, immune function, gut microbiota) that result in clinical signs such as diarrhoea, vomiting, weight loss, inappetence and abdominal discomfort.[14-16]

Common treatment strategies for dogs and cats with CE include dietary interventions (elimination trials or prescription diets) and medications (antibiotics and immunosuppressive agents) directed towards suppressing the aberrant immune responses that are thought to cause clinical signs.[17] Individual dogs and cats have been shown to respond to these traditional interventions at different rates,[18,19] and there is increasing evidence that these interventions may negatively affect the commensal gut microbiome. Even in healthy pets, the overall composition of the gut microbiome can shift due to environmental factors, such as antibiotic administration, dietary changes and pathogen exposure.[20-27] Large shifts in the GI microbial population can ultimately lead to community imbalance, a state referred to as dysbiosis.[28] Dysbiosis is implicated in the development of GI disorders (eg, inflammatory bowel disease [IBD], ulcerative colitis, Crohn's disease) in people, dogs and cats.[27, 29-36] Because dysbiosis is commonly observed in these conditions, strategies designed to manipulate the GI microbiome, including the use of nutraceuticals known as 'biotics', have been suggested to promote the health of companion animals (Fig 1).

The GI microbiome is recognised as playing a substantial role in animal health. Indeed, it has recently become clear that these health benefits extend beyond the gut into different physiological systems, such as the gut-brain axis[37,38] and the immune system.[39,40] Commensal bacteria in the gut are not only essential in maintaining intestinal homeostasis via pathogen resistance but are also important in the education and maturation of the host immune system and for maintaining immune tolerance. The composition of the gut microbiome is influenced by many factors, including host species, age, dietary history, environmental conditions and disease status.[41] Additionally, both functional redundancy[42] and complex microbial interactions[43] exist within the gut microbiome, highlighting the importance of supporting stability, diversity and functionality within the overall microbial ecosystem to maintain health.

It should be noted that, as in people, faecal sampling is the primary method used to analyse changes in the gut microbiome composition and activity of companion animals. Time of day, stool frequency and other factors are known to influence faecal metabolite concentrations and bacterial populations.[44] Although faecal samples may provide some insight into the colonic microbiome, they provide an incomplete picture of the metabolites produced in the colon, especially those that are rapidly absorbed by the intestine before defecation (eg, short-chain fatty acids [SCFAs]). For example, most butyrate produced in the colon is rapidly absorbed by colonocytes, serving as a key energy source.[45,46] While advances continue to be made in this area, GI sample type must always be considered when testing and interpreting treatment response.

Research on microbiome-modulating dietary interventions and their effects on host health has primarily focused on supplementation with biotics, including probiotics,[47] prebiotics,[48] synbiotics[49] and postbiotics.[50] Although biotics have been demonstrated to support the health of companion animals (Table 1), questions remain regarding appropriate biotic selection, mechanisms of action, optimal inclusion levels and safety. This review aims to summarise the influence that biotic substances have on the gut microbiome of dogs and cats and assess their role in maintaining GI health. It is not meant to offer exhaustive coverage of all published work in the field. Instead, it provides a discussion of relevant articles and key insights into biotic research in pets.

Probiotics

Probiotics are defined as 'live microorganisms that, when administered in adequate amounts, confer a health benefit on the host'.[47] Mechanisms by which probiotics are thought to exert their effects within the GI tract and gut-associated lymphoid tissue include displacing pathogens and restricting their growth,[51,52] improving intestinal barrier function and reducing inflammation,[53,54] production of antimicrobial substances,[55-57] and regulation of innate and/or adaptive immune responses.[58,59] Probiotics are commonly incorporated into commercial pet foods, treats and dietary supplements, but the clinical application of probiotics for dogs and cats has produced conflicting reports, especially regarding the management of GI disease.[60-62]

A systematic review by Jensen and Bjørnvad,[63] published in 2019, concluded that there was minimal evidence to support using probiotics to prevent or treat acute or chronic GI disease in dogs. However, only five of the 17 included studies concerned

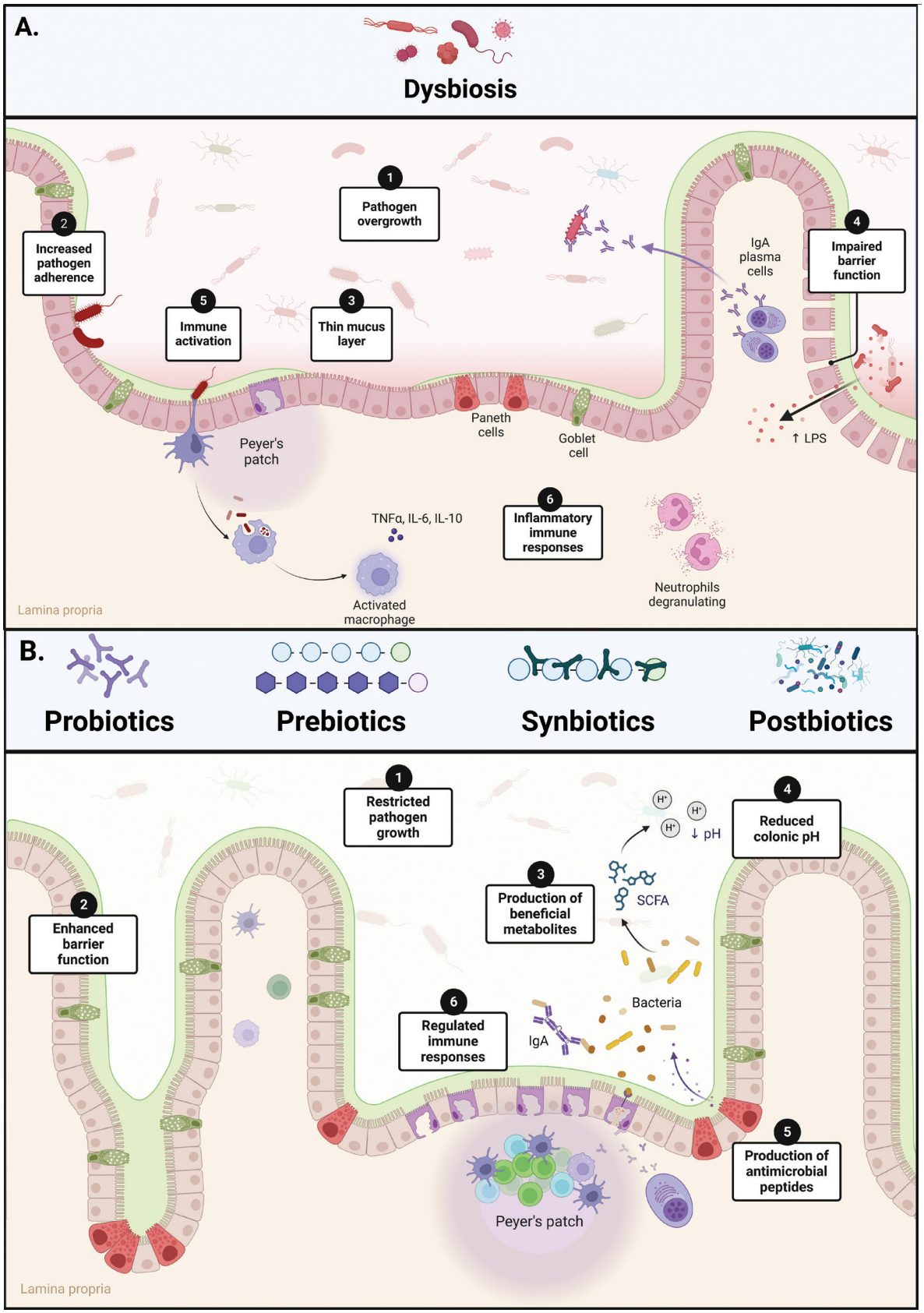

Fig 1: (a) Microbial community imbalance (ie, dysbiosis) may lead to the development of gastrointestinal disorders. Dysbiosis is characterised by (1) pathogen overgrowth, (2) greater bacterial adhesion to the gut epithelium, (3) impaired barrier integrity, (4) greater intestinal permeability, (5) activation of the immune system and (6) proinflammatory immune responses. (b) Biotics are thought to modulate the gut microbiome and promote microbial community balance through (1) displacing pathogens and restricting their growth, (2) enhancing intestinal barrier function, (3) the production of bioactive metabolites (eg, short-chain fatty acids), (4) a reduction of colonic pH, (5) secretion of antimicrobial peptides (eg, α-defensins, lysozymes, C-type lectins, cathelicidins) and (6) regulation of innate and/or adaptive immune responses

Table 1: Examples of the successful use of biotics in dogs and cats

Biotic treatment	Study population	Primary beneficial outcomes	Reference
scFOS or inulin (1 per cent of diet); 14-day intervention	Twelve-week-old hound-cross puppies infected with *Salmonella* Typhimurium DT104	Reduced fever and anorexia Maintained intestinal glucose transport Reduced enterocyte sloughing	Apanavicius et al[88]
scFOS (1 per cent of diet); six-week intervention	Obese adult beagles	Increased insulin sensitivity Altered adipose tissue mRNA expression of genes associated with fatty acid and glucose metabolism	Respondek et al[89]
Bifidobacterium animalis AHC7 (2×10^{10} CFU/day); 14-day intervention	Young adult dogs diagnosed with acute idiopathic diarrhoea	Reduced number of days to resolution of diarrhoea	Kelley et al[128]
Probiotic paste (2.85×10^{9} CFU live strains of *Lactobacillus farciminis*, *Pediococcus acidilactici*, *Bacillus subtilis* and *Bacillus licheniformis*, plus 1.35×10^{9} CFU thermostabilised *Lactobacillus acidophilus* per ml), with dose based on bodyweight (1–10 kg: 1 ml; 10–25 kg: 2 ml; 25–50 kg: 3 ml); given three times daily, starting with a double dose, until normalisation of stools	Hospitalised adult dogs diagnosed with acute gastroenteritis	Reduced number of days to resolution of diarrhoea	Herstad et al[65]
Enterococcus faecium SF68 (2.1×10^{9} CFU/day); four-week intervention	Healthy cats in an animal shelter	Reduced incidence of diarrhoea	Bybee et al[66]
Enterococcus faecium, *Streptococcus thermophilus*, *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Lactobacillus casei*, *Bifidobacterium bifidum* and *Lactobacillus plantarum* (5×10^{9} CFU/day) and two undisclosed prebiotics; 21-day intervention	Adult cats with chronic diarrhoea	Improvement of diarrhoea	Hart et al[106]
Enterococcus faecium NCIMB 10415 4b1707 (2×10^{9} CFU/day) and a combination of FOS and acacia gum (46.4 mg/day)	Dogs with shelter-associated diarrhoea	Reduced incidence of diarrhoea A lower proportion of treated dogs had a diarrhoea duration of more than one day	Rose et al[114]
1 per cent oat β-glucan extract; 71-day intervention	Healthy adult beagles	Reduced blood lipid concentrations (total cholesterol, low-density lipoprotein and very low-density lipoprotein)	Ferreira et al[129]
Streptococcus thermophilus DSM32245, *Lactobacillus acidophilus* DSM32241, *Lactobacillus plantarum* DSM32244, *Lactobacillus casei* DSM32243, *Lactobacillus helveticus* DSM32242, *Lactobacillus brevis* DSM27961, *Bifidobacterium lactis* DSM32246, *Bifidobacterium lactis* DSM32247 (2×10^{11} total CFU per 5 kg bodyweight); 90-day intervention	Cats with chronic constipation or idiopathic megacolon	Reduced feline chronic enteropathy activity index, faecal consistency score and mucosal histology score Increased number of interstitial cells of Cajal	Rossi et al[71]
Enterococcus hirae strain 1002-2 (1×10^{8} CFU/day); administered until kittens reached 900 g or more (14–26 days)	Fostered shelter kittens less than 12 weeks old	Reduced incidence of diarrhoea	Gookin et al[72]

CFU, colony-forming units; scFOS, short-chain fructooligosaccharides

dogs with chronic GI disease, with the remaining 12 studies focused on acute GI signs.[63] Similarly, a more recent systematic review and meta-analysis evaluating the effectiveness of antimicrobials and biotics for treating canine acute diarrhoea reported that biotic preparations did not significantly shorten the duration of clinical signs, based on very low to moderate certainty evidence.[64] However, the studies included in these reviews were conducted with small sample sizes, which may impede the interpretation of their findings.[63,64]

Despite inconsistent reports in the literature, some lactic acid-producing bacteria, such as *Lactobacillus* species, as well as *Bifidobacterium* and *Enterococcus* species and yeast of the genus *Saccharomyces* have been successfully used in single- or multistrain probiotic supplements for dogs and cats.[65-73] *Enterococcus faecium* is among the most studied single-strain probiotics in dogs and cats, with evidence of GI and immunological modulation. Puppies supplemented with *E faecium* SF68 (5×10^{8} colony-forming units [CFU]/day) from weaning to one year of age had greater proportions of mature B cells and higher faecal IgA concentrations compared with unsupplemented controls, demonstrating a potential benefit to adaptive immunity.[74] The same strain of *E faecium* improved clinical abnormalities in healthy cats with antibiotic-induced diarrhoea after 14 days of probiotic supplementation.[69] Supplementation with *E faecium* (5×10^{8} CFU/day) from seven to 27 weeks of age was also shown to increase the proportion of circulating $CD4^{+}$ lymphocytes in healthy kittens.[75] In another study, a large cohort of shelter dogs and cats was supplemented with *E faecium* SF68 for four weeks. Although no differences in the occurrence of diarrhoea were observed between control and treated dogs in this study, the percentage of cats with diarrhoea was lower in the probiotic group than in

the control group.[66] Unfortunately, faecal microbiome populations were not evaluated or reported in any of these studies, so any potential microbial shifts due to treatment are unknown.

The multistrain probiotic VSL#3 (containing *Lactobacillus plantarum*, *Streptococcus thermophilus*, *Bifidobacterium breve*, *Lactobacillus paracasei*, *Lactobacillus delbrueckii* subspecies *bulgaricus*, *Lactobacillus acidophilus*, *Bifidobacterium longum* and *Bifidobacterium infantis*) has been demonstrated to benefit dogs with IBD by enhancing gut barrier integrity[68] and by reducing clinical scores and normalising intestinal dysbiosis.[67,70] Although faecal *Faecalibacterium* and *Turicibacter* abundances were lower in dogs with IBD at the beginning of the trial, Rossi et al[67] observed an increase in *Faecalibacterium* abundance in dogs treated with the probiotic. Similarly, Ziese et al[70] reported a greater faecal abundance of *Faecalibacterium* and a lower faecal abundance of *Clostridium perfringens* compared with the placebo group after one week of probiotic intervention.

Another study found that a different probiotic blend (2×10^{11} total bacteria per 5 kg bodyweight) containing *S thermophilus*, *L acidophilus*, *L plantarum*, *Lactobacillus casei*, *Lactobacillus helveticus*, *Lactobacillus brevis* and two strains of *Bifidobacterium lactis* reduced clinical severity scores and increased the number of interstitial cells of Cajal in cats with idiopathic constipation or megacolon after 90 days. Faecal abundances of *Lactobacillus* species and bacteria of the phylum Bacteroidetes were increased after treatment, but no other changes in faecal microbiome composition were reported.[71]

In healthy cats, a combination probiotic containing *Saccharomyces boulardii* (2.0×10^{10} CFU/g) and *Pediococcus acidilactici* (2.5×10^{10} CFU/g) was shown to increase faecal butyrate and total SCFA concentrations, reduce faecal inflammatory markers (calprotectin and myeloperoxidase) and increase faecal antioxidant capacity (superoxide dismutase and glutathione activity) compared with control cats.[76] No significant changes to the faecal microbiome composition or diversity were noted.

In summary, single and multistrain probiotics offer an alternative strategy to manage and prevent GI disorders, and their application may reduce the use of antibiotics, which are known to cause adverse effects and contribute to antimicrobial resistance. However, when prescribing a commercially available probiotic, it is important to choose strains that have demonstrated clinical efficacy, documented stability and proven safety. Although several studies on probiotic use in dogs and cats report changes in gut microbiome composition and metabolites (eg, faecal SCFA concentrations) or modulation of immunological or haematological parameters, outcome measures are often nonspecific and fail to explain potential mechanisms of action.[60-62]

Probiotic effects are typically strain specific and dose dependent. Therefore, it is recommended to consult the primary literature to verify strain identity and ensure that appropriate amounts are being administered. Moreover, host-derived strains are thought to maximise the clinical potential of probiotics, as the origin influences the adaptability of the microorganism to the native gut environment and resident microflora.[77,78] Clinicians and manufacturers must also consider the stability of probiotics after processing, shipping and storage to maintain viability through to the end of the guaranteed shelf life. Finally, it is recommended that investigators conduct whole genome sequencing and longitudinal research on probiotic candidates to allow strain-level identification and safety assessment for antimicrobial resistance genes and pathogenicity.[79]

Prebiotics

A prebiotic is defined as 'a substrate that is selectively utilised by host microorganisms to confer a health benefit'.[44] To be effective, a prebiotic must be able to resist digestion by the host, be fermentable by intestinal microorganisms and selectively stimulate the growth and/or activity of the intestinal bacteria that confer a health benefit.[48] To date, all recognised prebiotics fall into the category of non-digestible oligosaccharides. Most of these substances contain carbohydrate chains with a low degree of polymerisation (typically three to 10 monosaccharide units), although some definitions include those containing up to 20 monosaccharide units or more.[80,81]

Due to their chemical structure, prebiotics are resistant to digestion by mammalian enzymes. However, microbial enzymes are capable of hydrolysing non-digestible oligosaccharides, leading to the production of SCFAs and other bioactive substances. Through microbial fermentation of prebiotics, SCFAs can alter the intestinal environment by reducing colonic pH and modulating the resident microbiome, which can positively influence barrier integrity and immune function in the intestine.[82,83]

The most extensively studied prebiotics include the fructans inulin and fructooligosaccharides (FOS), as well as galactooligosaccharides (GOS) and lactulose.[84,85] Although published data on these

well-accepted prebiotics were primarily derived from studies conducted in people, they have been shown to reduce insulin resistance, support metabolism, improve immune function and beneficially modulate the gut microbiome when fed to healthy dogs and cats.[86-92] However, there is little evidence to support prebiotic use in dogs and cats with GI conditions. Prebiotic studies in clinical populations are generally limited, possibly due to the potential for fermentable oligosaccharides to exacerbate GI signs (eg, bloating, flatulence, diarrhoea and abdominal discomfort).

The currently available evidence suggests that prebiotics may aid more in the prevention of GI disease than in its treatment because the selective utilisation of prebiotics by SCFA-producing bacteria leads to enhanced barrier function and reduced pathogen adhesion to the intestinal epithelium.[93] In one study, weanling puppies were fed diets containing either no prebiotic (control), 1 per cent short-chain FOS (scFOS) or 1 per cent inulin for 14 days before being challenged with *Salmonella*.[88] Infected puppies consuming fructans had reduced anorexia and enterocyte sloughing compared with controls, with puppies consuming inulin having greater faecal SCFA concentrations and *Lactobacillus* abundances than scFOS-fed puppies and controls.[88] In another study, healthy cats were fed diets containing no prebiotic (control), 0.5 per cent scFOS, 0.5 per cent GOS or 0.5 per cent scFOS and 0.5 per cent GOS for 14 days, and the effects of supplementation on nutrient digestibility, faecal fermentative end-product concentrations and faecal microbiome populations were assessed.[90] Cats fed supplemented diets had a greater faecal *Bifidobacterium* abundance than the control cats. Moreover, faecal pH was lower and faecal SCFA concentrations were higher for cats fed the scFOS and GOS-supplemented diet than for the control cats.[90]

A meta-analysis evaluating the effects of prebiotics on nutrient digestibility, faecal SCFA concentrations, faecal bacterial populations and immune function in dogs reported that although digestibility coefficients and immune indices were unaffected, prebiotic doses of up to 1.4 per cent on a dry matter (DM) basis may increase beneficial bacterial populations and faecal SCFA concentrations.[94] Similarly, a review evaluating the effects of prebiotics in healthy dogs reported that previously established prebiotics (ie, inulin, FOS and GOS) and some prebiotic candidates (ie, mannanoligosaccharides [MOS], yeast cell wall [YCW] and β-glucans) have demonstrated beneficial effects, such as modulation of the gut microbiome, increased SCFA concentrations, decreased colonic ammonia absorption and modulated immune function.[83]

Santos et al[95] evaluated the effects of increasing concentrations of the prebiotic candidate YCW (0 per cent, 0.2 per cent, 0.4 per cent and 0.6 per cent) in diets for healthy adult cats. The inclusion of YCW resulted in a reduction in the abundance of potentially pathogenic bacteria (eg, *C perfringens* and *Escherichia coli*) and increases in the abundance of beneficial bacteria (eg, *Bifidobacterium* and *Lactobacillus* species) in the faeces of treated cats. Interestingly, faecal protein catabolite (ie, valerate, total biogenic amines, putrescine, cadaverine and histamine) and butyrate concentrations increased linearly with YCW concentration.[95]

Prebiotic supplementation has also been shown to affect circulating metabolite concentrations and cell populations. For example, Rentas et al reported that the inclusion of a prebiotic blend (GOS, MOS, FOS and β-glucans; 1 per cent of extruded diet) increased circulating polymorphonuclear cell numbers, oxidative burst and phagocytic activity against *Staphylococcus aureus* and *E coli* in dogs.[96]

When it comes to prebiotics, dose and purity are key factors that influence efficacy. For example, crude preparations of FOS and GOS have been demonstrated to be less easily fermented by probiotic bacteria than high-purity substrates.[97,98] Furthermore, low inclusion rates (ie, less than 1 per cent) of prebiotic blends (ie, GOS, MOS, FOS and β-glucans) or a low inclusion rate of YCW (ie, 0.3 per cent) have been shown to have no effect on faecal characteristics and faecal ammonia and total SCFA concentrations.[96,99] However, it should be noted that the efficacy of prebiotics will often depend on the host population studied and the specific outcomes measured.

Prebiotic type and complexity are also important. While many individual prebiotic substances have been studied, much of the most recent research has focused on prebiotic blends for commercial use in pet foods. Determination of selective use by the host microbiome is not possible with blends, so each component should be studied individually, at least initially. However, prebiotic blends may be beneficial in practice, potentially providing complementary support for a more diverse range of microbes and immune functions.[90, 96, 100-103]

Synbiotics

In 2019, an International Scientific Association for Probiotics and Prebiotics (ISAPP) expert consensus panel reviewed the definition and scope of synbiotics, updating the definition to 'a mixture comprising live

microorganisms and substrate(s) selectively utilised by host microorganisms to confer a health benefit on the host'.[49] The ISAPP panel defined two subsets of synbiotics: complementary and synergistic.

A complementary synbiotic is composed of a probiotic plus a prebiotic designed to target commensal organisms. It is important to note that the existing probiotic and prebiotic criteria must be met for the complementary components to be considered a synbiotic.[47-49] Meanwhile, a synergistic synbiotic is a synbiotic in which the substrate is designed to be selectively utilised by the co-administered microorganism(s).[49] For synergistic synbiotics, evidence of selective utilisation of the substrate must be demonstrated in the same study that initially establishes the health benefit to confirm that the combined effect is better than the estimated effects of each component separately. This is not required for complementary synbiotics, wherein the probiotic(s) are chosen based on the benefits provided to the host, while the prebiotic is designed to promote the growth and function of commensal bacteria and provide a health benefit.[104] Regardless of the approach, it is required that the health benefit of the synbiotic mixture be confirmed in the target host species at doses necessary to confer the stated health benefit throughout the product's shelf life.

Although various studies have investigated combined probiotic and prebiotic preparations in companion animals, sufficient evidence is not always provided to satisfy the defined criteria of a synbiotic. Also, determining the potential mechanisms of action and comparing results between synbiotic studies is often challenging due to the variety of probiotic and prebiotic combinations and doses used.[105-113]

Several studies have investigated the effects of *E faecium* combined with FOS and gum Arabic in healthy dogs[114] and dogs with CE,[115-117] but the outcomes of these trials were mixed. In healthy shelter dogs treated with *E faecium* (2×10^9 CFU/capsule) combined with FOS and gum Arabic, the incidence of diarrhoea was lower than in untreated controls.[114] However, *E faecium* combined with FOS and gum Arabic had little effect on faecal microbial diversity, clinical scores or immune response in dogs with CE.[115-117]

In another study, an extruded kibble diet containing dried beet pulp (3.7 per cent DM), dried chicory (1.4 per cent DM) and FOS (1.3 per cent DM) formulated with or without live *E faecium* (1×10^9 CFU/kg) was tested in healthy dogs, leading to lower diarrhoea incidence, lower serum cholesterol concentrations, greater faecal SCFA (acetate and butyrate) concentrations and greater microbial diversity.[112] This increased faecal acetate was shown to correlate with decreases in the faecal abundance of *Fusobacterium*, *Streptococcus*, *Blautia* and *Clostridium* species. However, the authors attributed most benefits to the fibre and prebiotic component of the diet, as all outcomes except for reduced circulating cholesterol and diarrhoea occurrence were demonstrated regardless of *E faecium* inclusion.[112]

Hart et al[106] reported firmer stools in cats with chronic diarrhoea after supplementation with a commercially available synbiotic formulation comprised of *E faecium*, *S thermophilus*, *L acidophilus*, *L bulgaricus*, *L casei*, *Bifidobacterium bifidum* and *L plantarum* ($5x10^9$ CFU/capsule) combined with a proprietary blend of FOS and arabinogalactan. However, the same mixture of probiotics and prebiotics was unable to prevent or ameliorate dysbiosis in healthy research cats given clindamycin (75 mg orally once daily for 21 days).[109]

In another study, dogs with CE were fed a hydrolysed protein diet with or without a synbiotic supplement for six weeks. The synbiotic paste contained $1x10^9$ CFU/ml of *L acidophilus*, *L casei*, *E faecium* and *Bacillus subtilis* and was combined with proprietary yeast derivatives and IgY derived from chicken egg yolk.[113] Although both treatment groups had lower clinical disease activity and endoscopic scores at the end of the trial, dogs supplemented with the synbiotic had lower faecal inflammatory marker (ie, calprotectin and C-reactive protein) concentrations and a more favourable colonic microbiome population (ie, increased mucosal *Clostridium* and *Bacteroides* species and decreased colonic Enterobacteriaceae) than dogs fed the hydrolysed protein diet alone.[113] The gut microbiome changes were considered favourable based on previous studies demonstrating that dogs with CE had a lower intestinal abundance of *Fusobacterium*, *Clostridium* and *Bacteroides* and a greater abundance of Enterobacteriaceae.[33,35,118-120]

In summary, while some probiotic and prebiotic combinations demonstrate complementary synbiotic potential, the mechanisms of action are often unclear. Although total probiotic counts are typically provided in synbiotic formulations (ie, CFU per capsule), the count of each bacterial taxa in multistrain probiotics is typically unknown. Moreover, the doses, sources and proportions of prebiotics and dietary fibres used in synbiotic formulations are rarely disclosed and may differ from those when the components are tested individually. Unfortunately, modern pet foods and supplements often contain various blends of fibres

and probiotics without a clear understanding of their mechanisms of action, emphasising the need for additional research and transparency in this area.

Postbiotics

Alongside the development of nutritional supplements containing live microorganisms (ie, probiotics), there has been interest in determining the potential influence of non-viable bacterial cells and their components on host health in recent years. An ISAPP expert consensus panel recently defined a postbiotic as 'a preparation of inanimate microorganisms and/or their components that confers a health benefit on the host'.[50] In addition to modulating the GI microbiome, the potential functions of postbiotics on host health include enhancing epithelial barrier function, modulation of local and systemic immune responses and modulation of systemic metabolism. Sources of postbiotics include inactivated bacteria, bacterial lysates and fermentation products. Postbiotic modulators of systemic metabolism also include microbial-derived metabolites, including vitamins and SCFAs such as butyrate, that may increase the production of the antioxidant glutathione and reduce oxidative stress in the colon.[121]

The technological factors that may influence the characterisation and bioactivity of postbiotics include the microorganisms used as the starting material, the growth and/or fermentation conditions, the inactivation procedure and the composition of the final product.[50] By definition, viable cells in postbiotics are inactivated by thermal (eg, pasteurisation, autoclaving) or non-thermal (eg, electric field, ultrasonication, high pressure) inactivation techniques, although some cells may inevitably survive depending on the processing conditions. It is possible that viable cells may interact differently with the host's immune system, metabolism and/or microbiome compared with inactivated cells, cell fragments or metabolites.

Overall, the non-viable microorganisms, microbial cell components and metabolites or end-products comprising a postbiotic will likely affect its biological activity and potential health benefits. Therefore, it is important that postbiotics produced for commercial use are the same as those tested and shown to provide health benefits in the target host.[50]

Given the newness of the term, research investigating the effects of postbiotic supplementation in companion animals is limited, especially in cats. A recent review paper found no studies investigating postbiotic use in dogs with CE.[122] However, in healthy dogs, supplementation with a *Saccharomyces cerevisiae* culture product has been shown to modulate the gut microbiome, enhance innate and adaptive immune responses and modulate markers of inflammation and oxidative stress.[123-125] Lin et al[123] reported that, in dogs fed an *S cerevisiae* culture product (125, 250 or 500 mg/day), the faecal relative abundance of *Bifidobacterium* and *Prevotella* species increased linearly, while the relative abundance of *Fusobacterium* species decreased linearly. Furthermore, immune cells derived from *S cerevisiae* culture product-supplemented dogs produced lower levels of proinflammatory cytokines than cells from control dogs when stimulated with agonists mimicking bacterial and viral challenge.[123] Similarly, in healthy cats, an *S cerevisiae* culture product increased crude fibre and ash digestibility, reduced faecal *C perfringens* abundance and increased faecal lactic acid concentrations compared with unsupplemented controls. However, gross energy digestibility was reduced in cats fed a higher dose of the postbiotic.[126]

In another study, a dried *Lactobacillus* fermentation product (consisting of two proprietary strains of heat-killed *Limosilactobacillus fermentum* and *L delbrueckii*, culture media and bacterial fermentation metabolites) tended to improve faecal consistency and increase faecal bacterial diversity in healthy dogs, with greater faecal abundances of *Faecalibaculum*, *Bifidobacterium* and Butyricicoccaceae compared with unsupplemented controls.[127] The dogs supplemented with the *Lactobacillus* postbiotic may also have had enhanced protection against oxidative damage, as treated dogs had lower circulating oxidative stress markers (eg, serum superoxide dismutase enzyme) after transport stress.[127] However, more research is necessary to fully elucidate the potential benefits of postbiotics in both healthy animals and those with GI disorders.

Conclusions

Biotics are frequently administered to dogs and cats with the goal of beneficially modifying the abundance and/or activity of GI microbial populations and providing health benefits such as promoting or maintaining intestinal health, enhancing immune function or improving metabolism. While there is a large body of foundational evidence supporting the use of biotics in the prevention or treatment of GI disorders, studies in companion animals have not always reported consistent health benefits and/or changes in the GI microbiome.

Some of the inconsistencies may be due to studies having low statistical power, poor designs (eg, lack of control group) or ineffective strategies or doses. The heavy reliance on faecal sampling may also be a factor in the apparent lack of response in some studies. It is also often difficult to compare outcomes across studies due to the variation in biotic preparations, their dose, purity and modes of administration, and the health status of the study populations. The study population is of great importance, as some products (eg, prebiotics, postbiotics) are aimed at preventing GI disorders, whereas others (eg, probiotics, synbiotics) may be better suited to managing or treating disease.

While more research in the area is needed, several effective biotic interventions exist for dogs and cats. Veterinarians and consumers should select products that are designed for the host species and life stage of interest, contain an effective dose and have been backed by published studies.

Conflict of interest statement

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