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Pro-, Pre-, Syn- and Postbiotics

Pro-, Pre-, Szin- és Postbiotikumok

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Abstract

Pro-, pre-, syn- and postbiotics show a vast potential in curing, treating and preventing systemic and local disease and aiding the reduction of antimicrobial use and antimicrobial resistance occurrence. Nowadays, dogs and cats are not only pets, but family members, whose health is of great importance. This has led to growing interest in companion animal health. Pro-, pre-, syn- and postbiotics are powerful treatment tools for health modulation in dogs and cats, which is why this thesis summarises their definitions, health benefits, risks, and technological advances and discusses newest research in this field. These products have systemic health benefits as well as local health benefits and can be used not only in the medical field, but also in food production.

A pro-, pre-, szin- és posztbiotikumok alkalmazásának nagy jelentősége lehet a szisztémás és helyi betegségek gyógyításában, kezelésében és megelőzésében, valamint az antimikrobiális szerek használatának és az antimikrobiális rezisztencia előfordulásának csökkentésében. Napjainkban a kutyák és macskák nemcsak háziállatok, hanem családtagok, akiknek egészsége nagy jelentőséggel bír. Ez növekvő érdeklődéshez vezetett a kedvtelésből tartott állatok egészsége iránt. A pro-, pre-, szin- és posztbiotikumok hatékony kezelési lehetőségek a kutyák és macskák egészségének megőrzésében és befolyásolásában, ezért ez a dolgozat összefoglalja definícióikat, egészségügyi előnyeiket, kockázataikat és technológiai fejlődésüket, és tárgyalja a legújabb kutatásokat ezen a területen. Ezek a termékek az egészségre vonatkozó szisztémás és helyi hatással is bírnak, és nemcsak az orvosi területen, hanem az élelmiszertermelésben is használhatók.

Contents

Abbreviations

AHDS	Acute haemorrhagic diarrhoea syndrome
AHR	Aryl hydrocarbon receptors
BBB	Blood brain barrier
BL	Bifidobacterium longum
BL + B-SA	Bifidobacterium longum in combination with iron single-atom nanoenzyme treated with C18-PEG-B
BL@B-SA_{50}	Artificially-enzyme-modified Bifidobacterium longum
B-SA	Iron single-atom nanoenzyme treated with C18-PEG-B
CAD	Canine atopic dermatitis
CADESI	Canine atopic dermatitis extent and severity index
CD	Chron's disease
CE	Chronic enteropathy
CFU	Colony forming unit
CNS	Central nervous system
CON	Conventionalized offspring mice
DI	Dysbiosis index
EFSA	European Food Safety Authority
FDA	Food and drug administration
FEEDAP	EFSA panel on Additives and Products or Substances used in Animal feed
FMT	Fecal matter transplant
FOS	Fructo-oligosaccharides
GALT	Gut associated lymphoid tissue
GF	Germ free
GI	Gastrointestinal
GIT	Gastrointestinal tract
HPA	Hypothalamic pituitary axis
IBD	Inflammatory bowel disease
ICU	Intensive care unit
IgA	Immunoglobulin A
MAMP	Microbe-associated molecular pattern

MOS	Mannan-oligosaccharides
MUO	Meningoencephalomyelitis
PO	Per os
PRR	Pattern recognition receptor
ROS	Reactive oxygen species
SCFAs	Short chain fatty acids
SCFAs	Short chain fatty acids
SIMBA^{TM} capsules	Small Intestinal MicroBiome Aspiration capsules
SPF	Specific pathogen free
UC	Ulcerative colitis
VFAs	Volatile fatty acids
WHO	World health organisation

1. Introduction

Nowadays, dogs and cats are not just best friends but family members of humans [1]. The health of those animals has become as important to us as our own health. Almost identical nutritional and microbiome associated diseases are recognized in dogs, cats and humans. This is due to the increasing similarities between our pets' lifestyles and our own, such as them living in big cities with humans and eating similar carbohydrate rich feed as humans. The microbiome in animals is as complex as in humans, with dogs and cats having an even higher number of microorganisms living in the gastrointestinal tract (GIT) consisting of bacteria, protozoa, fungi, archaea and viruses [2–4] and is approximated to consist of 10^{13}-10^{14} cells [5]. Bacteria represent with ~98% the largest portion of the microbiome [6]. The presence of the gut microbiome has been known for decades, however, we only started to slowly study its importance and effect on the overall health of an organism in the last 25 years [7]. The definition for the term microbiome has changed over the years. It was originally defined by Whipps et al. in 1988 but a new extended definition has been suggested by Berg et al. in 2020 and reads as follows: *"The microbiome is defined as a characteristic microbial community occupying a reasonable well-defined habitat which has distinct physio-chemical properties. The microbiome not only refers to the microorganisms involved but also encompass their theatre of activity, which results in the formation of specific ecological niches. The microbiome, which forms a dynamic and interactive micro-ecosystem prone to change in time and scale, is integrated in macro-ecosystems including eukaryotic hosts, and here crucial for their functioning and health."*. The "theatre of activity", which is influenced by the surrounding environmental conditions, refers to molecules produced by microbiota, such as metabolites (e.g. signal molecules, toxins) and structural elements (e.g. mobile genetic elements like relic DNA, polysaccharides, lipids). The microbiota includes bacteria, archaea, fungi, protists and algae found in a certain part of the body. The "theatre of activity" is part of the microbiome but not the microbiota [8]. Therefore, simplified, we could say that the microbiota are the living elements of the microbiome, with the microbiome also including the "theatre of activity".

Today we know that the genome of dogs microbiome is 150 times larger than that of humans [5]. The dogs' microbiome is not just important to grand the pet's health, but it also plays a pivotal role in granting the health of all family members. Diseases can be transmitted easily from pets to humans, and a small study has shown that even the composition of gut microbiota in children can be modified by the gut microbiome of their household dogs [9].

The microbiome influences the GIT and overall health in various ways: by production of mucus and protection from colonization of pathogens, by maintaining gut homeostasis, by creating intestinal immunity [10], by educating the immune system, and by supporting the host's metabolism and digestion of complex molecules. Microbiota also take part in digestion by converting feed to metabolically active compounds or modulating gene expression [2, 11]. The gut microbiome of canine and feline healthy adults from the US mostly consists of *Firmicutes* (like *Clostridia* or *Lactobacillales*), *Proteobacteria* and *Bacteroidetes* with great intraspecies and individual differences as well as fungi, of which the most abundant fungi are Saccharomyces [12, 13]. The microbiome can be greatly influenced by nutrition, however, diet-induced changes of the microbiome are usually considered less severe compared to dysbiosis connected to underlying disease, since a simple change in diet can resolve the nutritionally induced dysbiosis [14]. The composition of microbiome greatly depends on species, breed, age and sex, but also extrinsic factors such as nutrition, lifestyle, stress, medication or geographical region [12, 15–17]. Healthy older dogs have a greater abundance of *Fusobacterium* in the GIT microbiome compared to younger animals, whereas overweight animals have more *Actinobacteria* compared to lean dogs, whose microbiome hosts more *Anaerobiospirillum succiniciproducens, Beduini massiliensis, Rouboutsia timonensis*, and *Streptococcus equins* [16].

The exact causes of dysbiosis are not always known, but it is certain that breed and therefore genetic predisposition, like breed related anatomical malformations (at which we can see bacterial overgrowth), can influence the microbiome in addition to metabolic disease influencing the gut pH, motility disorders or drugs like antibiotics or NSAIDs [2, 14]. The uptake of milk after birth plays a significant role in the development of the microbiome of young animals due to the contact to the skin as well as the milk itself, which contains microbiota that were translocated from the GI tract to the mammary ducts [18, 19]. Nowadays, we know that the healthy uterus is not sterile, and that the foetus already harbors bacteria. The composition of microbiome of the newborn depends on various things, like the type of parturition. It is suggested that even the nutrition of a bitch and therefore her microbiome and microbial metabolites can influence the development of foetus or newborns and their microbiome [18]. The geographic region where an individual lives can greatly influence the composition of microbiome. Even the microbiomes of ticks living on dogs and cats have different diversity and community structure depending on the geographic region of origin in the US [20]. However, the GIT microbiome is not the only microbiome of our bodies. There are several different microbial communities spread all over one's body, ranging from skin to urinary tract, genitals, conjunctiva, respiratory system and many more. The compositions of all microbial communities differ from each other, which is why, occasionally, interactions between them can cause problems. Depending on the location/type of microbiome and composition as well as the hosts symptoms, not all microbiome related problems can be treated the same way [21, 22].

Today we know that an alteration in the gut microbiome can be linked to several different metabolic and immune associated diseases in dogs and humans, like obesity, atherosclerosis, neurological disease or asthma [2, 23], due to the gut microbiome not only affecting the gut and its digestion directly, but also the brain, immune system [24], urinary tract and metabolic system (like glucose homeostasis in connection to diabetes mellitus) [3]. Dogs can also develop diseases like Chronic enteropathy (CE) or Inflammatory bowel disease (IBD) in connection to the microbiome and its immune functions [25, 26], which, regarding the latter, is diagnosed by the exclusion of parasitic, infectious, extraintestinal and other intestinal diseases. CE is most likely caused by imbalances in the enteric microenvironment (dysbiosis) along with genetics, yet causal genetic defects have not been found (as of 2011) [27]. Nevertheless, one should not mistake the correlation of dysbiosis occurring alongside certain disease as causation, since dysbiosis can be a symptom of other diseases. Dysbiosis being the cause of some of the former mentioned disease is not yet proven in all cases and can be falsely assumed when interpreting newest studies [2]. Yet, causative treatment as well as symptomatic treatment are justified to be used in practice to ensure the patients maximised wellbeing. This is why this thesis will cover possibilities of how to manage gut microbiome related diseases in dogs and cats by using pro-, pre-, syn- and postbiotics.

To decide whether pro-, pre-, syn- or postbiotics have the desired effect on the microbiome or to diagnose a dysbiosis, we assess the composition of microbiome. This can be done by bacterial cultures [28] or doing DNA extractions and gel gradient electrophoresis which shows the quantitative composition of the microbiota. Real-time quantitative PCR (qPCR) and 16s rRNA pyrosequencing makes it possible to show small qualitative changes as well as quantitative changes of the microbial composition [29]. Also, fluorescent in situ hybridisation (FISH), 454 pyrosequencing and many other methods can be used [30]. In experimental studies, samples are not just derived from feces but also from the GIT directly via fistulas [28] or post mortem examinations [31] which is not feasible in clinical settings [32]. In 2023, non-invasive sampling of the small intestinal chyme using Small Intestinal

MicroBiome Aspiration capsules (SIMBA^{TM} capsules) were tested for their applicability in eleven beagle dogs, posing a possible easily applicable sampling method of small intestinal chyme microbiome and metabolites. In this small study the capsules were proven to provide a safe and reliable sampling method, showing similar results in microbial composition as described in previously published papers using endoscopic small intestinal GI biopsy sampling or post-mortem small intestinal chyme sampling. However, the metabolite examination results differed from some previously published studies [33]. Depending on the type of test, type of sample and GI section, the results can differ from each other. In case of suspected dysbiosis, a microbial fecal sample examination can be done to create the Dysbiosis index (see chapter 2 Dysbiosis Index), which makes it possible to compare the severity of dysbiosis between different patients [34].

Pro-, pre-, syn- or postbiotics might be the future for curing various local and systemic diseases in dogs and cats as well as humans and are therefore in focus of researchers driving the medical field into a new promising direction, potentially one day replacing the use of antibiotics and combating antibiotic resistance.

The objective of this review is to summarise the risks, advantages, disadvantages, new findings and possibilities regarding the use of pre-, pro-, syn- and postbiotics in the veterinary field, and therefore to provide a foundation of knowledge on this topic. Inconsistencies, conflicts and gaps in the reviewed literature will be identified and discussed.

2. Dysbiosis Index

The dysbiosis index (DI) is a tool to compare the changes in microbial composition in case of dysbiosis using qPCR [34]. The term gut dysbiosis describes the state of altered gut microbiome which causes functional changes [35]. DI and therefore the degree of dysbiosis will be expressed in one single numerical value, where negative numbers express normobiosis and positive numbers express dysbiosis with a specificity of 95% and a sensitivity of 74%. Eight different bacterial groups are examined for this purpose: total Bacteria, *Blautia*, *Clostridium hiranonis*, *Escherichia coli*, *Faecalibacterium*, *Fusobacterium*, *Streptococcus* and *Turicibacter* [34, 36]. Reference values have been created for dogs to assess the severity of dysbiosis, to make it comparable between patients and to measure the success of a treatment [37].

The DI is a sequencing technique that was originally designed to be able to differentiate the bacterial shift in dogs with acute diarrhoea and CE compared to healthy dogs. Naturally passed fecal samples are examined using qPCR. In general, *Proteobacteria* like *Escherichia coli* increased, whereas *Faecalibacteriae*, *Ruminococci* and *Blautiae* decreased in case of CE. Overlapping DI can be seen between healthy and diseased dogs since CE is a multifactorial disease, however, assessing the DI will help in developing a treatment plan [34]. Indications for DI include comparison of responses to therapy, comparison of studies with each other or comparison of patient to healthy individuals [2]. Cats and dogs with a DI under 0 (negative DI) are considered to be without dysbiosis whereas a dog with DI between 0 to 2 are considered to have a mild to moderate dysbiosis. In case of a DI above 2, the shift in overall diversity of microbiota is considered to be significant [5]. Cats with a DI between 0 and 1 are unlikely to show any clinical signs compared to cats with a DI above 1 [38]. The DI is mostly only used for dogs and rarely for cats due to a lack of cat specific research (as of 2024).

According to latest research it is important to note that this index is used to compare adolescent/adult dogs, since it has been noticed that the microbiome of young healthy puppies differs significantly from that of their adult conspecifics. The DI of suckling dogs is high and decreases slowly after weaning, becoming more comparable to that of adult dogs. It has been noticed that right after weaning of puppies the amount of *Clostridium difficile* decreases significantly, whereas *Clostridium hiranonis* increases [18, 36]. It was suggested in 2017 that the age, body weight and gender of adult individuals does not influence the DI [34], which was confirmed by a study in 2023 testing 106 dogs from different age groups

(0,2 to 15 years old). The author of this study, however, suggested further studies to prove the result [5]. Another study about healthy mixed breed and purebred cats (n = 80) found similar results stating that their DI did not differ significantly based on age (0,5 to 15 years old) [38]. It was suggested that after 6 months of age the abundance of bacteria in cats does not undergo big changes anymore, with the biggest changes between 2 to 6 months of age [39]. It is possible that DI only differs in very young age, causing this discrepancy in results regarding the applicability of DI to different age groups. However, this discrepancy shows the need for further studies. It also raises the question whether different probiotics must be developed for different age groups.

The composition of microbiome changes in individuals depending on species, breed, body condition score and age with similarities between them [16]. As mentioned before, the gut microbiome of canine as well as feline healthy adult animals in the US mostly consists of *Bacillota* (formerly known as *Firmicutes*, like *Clostridia* or *Lactobacillales*), *Proteobacteria* (now called *Pseudomonadota*) and *Bacteroidetes* with great intraspecies and individual differences, as well as fungi, of which the most abundant fungi are Saccharomyces. Cats have a different composition of microbiome, showing a higher bacterial diversity compared to dogs' GIT microbiome and having a higher relative abundance of *Actinobacteria, Adlercreutzua, Alistipes, Bifidobacterium, Carnobacterium, Collinsella, Coprococcus, Desulfovibrio*, *Faecalibacterium*, *Oscillospira*, *Parabacteroides*, *Peptococcus*, *Peptostreptococcus*, *Ruminococcus*, *Slackia*, and *Sutterella*. Dogs relative abundance of *Enterococcus, Fusobacterium, Megamonas* and *SMB53 (Clostridia)* are higher with *Bacteroides* being the most abundant regarding bacterial taxa [12, 13]. The normal microbiome of an adult dog changes in number and composition and microbial number based on the section of the GI tract. A small study (n = 6) suggests the following: the most abundant bacterial order in the duodenum and jejunum is *Clostridales,* whereas the most abundant bacterial order of the ileum and colon were anaerobic *Fusobacteriales* and *Bacteroidales. Enterobacteriales* are more abundant in the small intestine [31]. The small intestine is therefore comprised of a mix of anaerobic and aerobic bacteria, whereas the abundance of anaerobic bacteria is predominant in the large intestine.

The fecal bacterial count of healthy dogs mostly comprises *Bacteroides* (13,66%), *Fusobacterium* (24,32%), and *Prevotella* 9 (15,18%), whereas the feline feces show higher abundance in *Prevotella* 9 (18,92%), *Bacteroides* (8,32%), *Clostridium sensu stricto 3* (5,32%).and *Romboutsia* (5,28%) [40].

Due to the small patient size, more studies defining the GIT microbiome in different sections should be done. The number of bacteria colonizing the stomach ranges from 10^4 to 10^5CFU/ml. In the duodenum and proximal jejunum, it ranges from 10^5 to 10^6 CFU/ml, with 10^6 CFU/ml in the distal jejunum. In the colon, the bacterial count of dogs and cats reaches 10^9 to 10^{11} CFU/ml. The fecal bacterial count ranges from 10^8 to 10^{11} CFU/ml [4, 41–43]. Also, the age changes the occurrence and number of bacteria comprising the microbiome of dogs, with Bacteroidales being the highest in 0-8 month old dogs, Phascolarctobacterium in highest abundance in junior (9-24 month old) dogs, Fusobacterium in adult (25-96 month old) dogs and Roseburia in senior (<97 month old) dogs [17].

Table 1- Abundance of bacteria along the gastrointestinal tract of healthy dogs and cats

	Small Intestine	**Colon**	**Feces**
Number of total Bacteria	10^5 to 10^6 CFU/ml	10^9 to 10^{11} CFU/ml	10^8 to 10^{11} CFU/ml
Most abundant Bacteria – Dogs	*Clostridales, Fusobacteriales, Bacteroidales*	***Bacillota, Proteobacteria, Bacteroidetes,*** *Enterococcus, Fusobacterium, Megamonas, Clostridia*	*Bacteroides*, *Fusobacterium, Prevotella* 9
Most abundant Bacteria – Cats	*No reliable data was found*	***Bacillota, Proteobacteria, Bacteroidetes,*** *Actinobacteria, Adlercreutzua, Alistipes, Bifidobacterium, Carnobacterium, Collinsella, Coprococcus, Desulfovibrio*, *Faecalibacterium*, *Oscillospira*, *Parabacteroides*, *Peptococcus*, *Peptostreptococcus*, *Ruminococcus*, *Slackia*, *Sutterella*	*Prevotella* 9, *Bacteroides, Clostridium sensu stricto 3, Romboutsia*

Antibiotics do not only kill pathogenic bacteria, but also other members of the microbiome, potentially leading to dysbiosis and decreased diversity of the microbiome. Antimicrobials are the number one cause of long-term disturbances in the microbial balance. Commonly administered antimicrobials in dogs with GI disorders, like metronidazole or tylosin, cause long-term microbial imbalances, decrease species richness, diversity and increase dysbiosis index. Their use might lead to a transient reduction in clinical signs but also to negative progress in dysbiosis, driving the disease even further [44]. This is why pro-, pre-, syn and postbiotics must be considered as alternative, potentially superior, treatment options.

3. Probiotics

Probiotics are *"live microorganisms which when administered in adequate amounts confer a health benefit on the host"* [45].

Commonly, the term Probiotics is used incorrectly in practice, not meeting the strict definition of WHO, using theoretical models explaining the theoretical benefit rather than evidence-based studies proving the claimed benefit of the products. Probiotics can be supplements, drugs, found in food/medical food [32] or beverages or even in topical products like toilet paper for humans [46]. Many theoretical mechanisms of actions of probiotics have not been confirmed to be effective in small animals yet. In the USA, the FDA approval is only needed for probiotics marketed as drugs, and therefore only those were proven safe and effective regarding the intended use through clinical trials. Probiotics not labelled as medication do not need FDA approval, and therefore the effectivity was commonly not proven by clinical studies, since those are not intended to treat or prevent disease, and therefore do not need to be safe for a vulnerable patient population [32, 47]. In Europe, the European Medicines Agency (EMA) regulates the approval of drugs on the European drug market, having strict rules for the approval of probiotics as drugs, demanding efficacy, safety, and quality evidence, whereas, like in the USA, the approval for probiotics as food supplement is not as strict, however, the European Food Safety Authority (EFSA) decreed that any health claim for food marketed in Europe has to be evidence based [48]. In 2007, the quality presumption of safety (QPS) concept was introduced by EFSA, assessing the taxonomic identity of microorganisms, body of knowledge and potential safety concerns like antimicrobial resistance genes or toxin production. The latest update of QPS-recommended microorganisms plus scientific opinion was published in 2022, with possible additions every 6 months (latest update December 2023), and can be viewed on efsa.europa.eu homepage under the name "Updated list of QPS-recommended microorganisms for safety risk assessment carried out by EFSA" (https://doi.org/10.5281/zenodo.1146566) [49].

During the research for this paper, studies have been found concerning *Lactobacillus reuteri* (*Limosilactobacillus reuteri*), which highlight an autoimmune disease stimulating effect of the bacterium in mice [50, 51]. This bacterial species is recommended according to "Updated list of QPS-recommended microorganisms for safety risk assessment carried out by EFSA". It has many positive effects, mentioned later in this paper, like stress reduction, and has been used in commercially available stress relieving probiotic products for dogs [52]. This discrepancy in results may be due to the fact that *L. reuteri* has many different strains, and highlights the necessity to study each individual bacterial strain when assessing the risks and benefits of probiotics.

When administering probiotics to canines, one shall consider that there is a host specificity [53]. A lot of research has gone into studying the exact composition and diversity of dogs' microbial communities of different species allowing a deeper understanding of an individual's physiological and pathogenic microbial compositions making it more likely to find suitable treatments [21]. The effect of the treatment also strongly depends on the concentration, duration and type of probiotics used, as well as the underlying disease. Already in 2008, Minelli and Benini stated that more studies have to be done for every individual disease and probiotic to find the ideal probiotic concentration for each case [54]. In 2021, Sivamaruthi et al. summarized the effect of probiotics in healthy and diseased dogs at certain dose and duration. For instance the administration of different probiotics in different stages of life against atopic dermatitis were compared, showing that *Lactobacillus sakei* probio-65 in $2 * 10^9$ CFU/day (colony forming unit per day) given for 2 months, as well as *Bifidobacterium* in $5 * 10^{10}$ CFU/day for 3 months, can reduce the CADESI score (canine atopic dermatitis extent and severity index) effectively, however, the administration of *Lactobacillus rhamnosus* strain GG to mother dogs at the third week of gestation and during lactation, as well as to puppies of 3 weeks to 6 months old, will lead to reduced immunological indicators for atopic dermatitis, but no reduction in clinical signs [55].

Possible mechanism of action for probiotics are complex, including stimulating local immune response by increased TNF-α production or increasing epithelial barrier function, detoxifying toxins or host products, or directly affecting pathogenic and non-pathogenic microorganisms [46, 56, 57]. Probiotics have different multi-action mechanisms of actions, making it possible to not only treat bacterial imbalances, but also viral infections. They can show higher specificity in short time treatment compared to antibiotics, and show other advantageous effects like growth promotion and increased performance. Due to the increasing occurrence of antibiotic resistance, probiotics are increasingly considered to be used instead of antibiotics, despite their potential possession of antibiotic resistance genes [47, 58–61]. Lee et al. (2023) observed that *Ligilactobacilus animalis SWLA-1* and its cell-free supernatant has inhibitory effect on *Salmonella gallinarum* (Gram-negative) and *Salmonella enteritidis* (Gram-negative) on an agar spot essay, which can also be seen in case of other *Lactobacillus* strains. *L. animalis* as well as *L. plantarum ATCC 14917* are able to inhibit *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive) [60].

Probiotic mixtures like Slab51® (SivoMixx®) containing *Streptococcus thermophilus* DSM 32245*, Bifidobacterium lactis* DSM 32246, *Bifidobacterium lactis* DSM 32247, *Lactobacillus acidophilus* DSM 32241, *Lactobacillus helveticus* DSM 32242, *Lactobacillus paracasei* DSM 32243, *Lactobacillus plantarum* DSM 32244, and *Lactobacillus brevis* DSM 27961 were given to healthy dogs in a small study for 60 days in 400 billion cfu and showed to reduce the abundance of *Clostridium perfringens* [62].

If probiotics, which are considered to be safe in the regard to antibiotic resistance genes, are used instead of antibiotics in the treatment or prevention of certain infectious diseases, the spread of antibiotic resistance can be reduced [63]. Probiotics are not only used instead of antibiotics, but also together with antibiotics. The combination of probiotics and antibiotics can reduce or prevent the occurrence of antibiotic-associated diarrhoea [64, 65]. Newer research has shown that the combined administration of probiotics and antibiotics is not justified to maintain the diversity of gut microbiome, however, probiotic supplementation may be considered to prevent antibiotic-associated diarrhoea or *Clostridioides difficile* (former *Clostridium difficile*) overgrowth [66]. The effectivity of probiotics used to prevent antibiotic associated diarrhoea depends on the initial baseline diarrhoea risk as well as dose. Higher effectivity was seen if a higher dose (greater then $10 * 10^9$cfu/day) was administered and no benefit can be seen in low baseline diarrhoea risk patient [67]. For humans commercially available products have already been designed to be taken during the course of antibiotics. OMNi-BiOTiC® advertise that their human probiotic mixture OMNi-BiOTiC® AAD 10 must be taken at least 1h apart from antibiotics but preferably as far apart as possible. This product promises to modulate the imbalance of the microbiota during antibiotic therapy and ensures potential long-term colonization of the gut. © Institut AllergoSan [68].

When producing a probiotic, it is not only important to take the preparation's shelf life, cell viability, feasibility, targeted action, safety and functionality into consideration, but also the preferred route of administration. Encapsulation of probiotics is a better option compared to loose probiotics regarding these factors. If administered per os (PO), encapsulated probiotics provide protection from bile and gastric acid, as well as antibiotics showing a significant higher efficacy in colonization (even in the large intestine) and enhanced mucoadhesive properties [61].

Depending on where in the body a probiotic shall act, the preferred route of administration defers. Probiotics that are meant to modulate the gut microbiota can be administered orally (capsule, microcapsule, hydrogels, microcapsule with modified release, capsule in capsule, nanoencapsulation, non-encapsulated powders or granules, tablets, orally dissolving or disintegrating films), rectally via enema or endoscopically, where rectal administration makes it possible to introduce higher concentrations into the rectum and colon. Other routes of administration influencing mainly other parts of the body are vaginal (tablet, gel, suppository, transdermal (microneedle) or intranasal (nasal spray) [69–75].

Probiotics are most commonly administered PO. Physical, chemical, biological and pathological microenvironmental challenges have to be faced, like low gastric pH, digestive enzymes, bile salts and intestinal juice, altered transit times, turnover epithelial cells, competition with other bacteria, individual specific selective bacterial colonization, inflammation, mucosal integrity, altered intestinal volume, diarrhoea, and more [73, 76–79]. Powders/granules have a low cost in production, dissolve rapidly and can be administered in large doses, but can be damaged during the preparation process (freeze or spray drying/wet or dry granulation), therefore, non-encapsulated probiotics can potentially have a reduced effectivity. However, Lee et al. found in a study (rats) that encapsulated probiotics did not have a greater effect than non-encapsulated probiotics. Granules are slightly superior to powders because they can be consumed easier (no dust formation) and can potentially also be encapsulated increasing the survival rate of bacteria [72, 75].

Capsules in general are easy to dose, cheap, easy to administer, provide good probiotic stability and are widely used. Some capsules are less durable than tablets, needing dry environmental conditions for storage. A Duocapsule (capsule in capsule) makes it possible to combine different active ingredients and to release them at different locations of the small intestine. The combined administration of active ingredients which are normally not compatible will be possible at a preset dosing schedule [80, 81]. Production of probiotics for delivery via tablet form is cheap, however, the bacterial viability during production and storage is low, but better than in case of powders. In case of coated tablets, the bacterial viability and shelf life can be significantly increased, making a storage for 6 months in the refrigerator possible [74, 75]. Microencapsulated and nanoencapsulated probiotics administered PO make a controlled targeted delivery to certain intestinal areas possible (controlled release nano-/microencapsulation), increase stability by resisting harsh microenvironment, therefore reducing cell death during GI passage, and increase shelf life.

Hydrogels are microencapsulating polymers used to carry probiotics [71, 75, 79]. Nanoencapsulated probiotics are superior in all these properties, making an even delivery of bigger fractions of viable probiotics to the lower GI tract possible [73]. In pathological microenvironmental conditions (sick animals), changes in pH, gastrointestinal transit time or microbial compositions can influence the targeted delivery systems negatively and prevent the proper release of bacteria [79]. New methods of micro- or nanoencapsulation have been designed to combat this issue. More can be read about the advances, challenges and possibilities in "Delivery Strategies of Probiotics from Nano- and Microparticles: Trends in the Treatment of Inflammatory Bowel Disease—An Overview" by Lopes et al. (2023) [73]. Rudenko et al. suggested in 2020 that there is not enough practical knowledge about the impact and interactions of nanoparticles and biological objects, yet indicating potential unknown health risks [82].

Rectal administration of probiotics in colitis can potentially have improved effectiveness compared to oral administration [69]. This makes it seem like potentially rectal administration of probiotics might be superior to oral administration in treating inflammatory bowel diseases, however, due to the small study size more studies specifically for dogs and cats must be done.

Via rectal administration, the harsh environment of the other parts of the GI tract can be circumvented, preventing damage, therefore allowing targeted delivery in higher doses and increased anti-inflammatory effect. [75] Colonoscopy can be done for rectal administration of fecal matter, making controlled delivery possible and potentially increasing the retention of fecal matter before elimination, however, this is not proven [83]. Sedation and anesthesia needed for colonoscopy could induce hypotension and decreased GIT perfusion in severely sick patients [84].

Table 2 - Advantages and disadvantages regarding common routes of administration of probiotics in case of gastrointestinal diseases

	Advantages	Disadvantages
PO: Capsule	Easy dosing, easy administration, cost effective, good shelf life	Sensitive to humidity (some types), less durable than tablets (some types)
PO: Microencapsulation (e.g. hydrogels)	Reduced cell death during GI passage, controlled release possible/more targeted delivery to certain intestinal areas, improved shelf life, easy to administer	Potentially unreliable targeted release in pathological microenvironment
PO: Nanoencapsulation	Reduced cell death during GI passage, controlled release possible/more targeted delivery to certain intestinal areas, easy to administer	Unreliable targeted release in pathological microenvironment, potential unknown health risk
PO: Duocapsule (capsule in capsule)	Targeted release of different active ingredients at different locations, easy dosing, easy administration, good probiotic stability	Inflexible dosing schedule
PO: Tablet	Cost effective, easy dosing, easy to administer	Variable bacterial viability, variable shelf life
PO: Non-encapsulated powders	Low cost, rapid dissolution, dose adaptability	Potential decreased effectivity
PO: Non-encapsulated granules	Convenient to consume, rapid dissolution, dose adaptability	Potential decreased effectivity (but if encapsulated the effectivity increases)
Rectally (e.g. enema)	Potential superior effectivity in case of colitis treatment compared to oral administration, targeted delivery to colon	Sedation/anesthesia (not always necessary), time consuming
Endoscopically	Controlled delivery to certain areas	Sedation/anesthesia, more expensive, time consuming

Intranasal administration of probiotics poses a potentially more effective administration route in case of treatment of intranasal pathogen carriage in dogs [85]. In human respiratory diseases, it is suggested that administration of probiotics intranasally might be more effective than orally, but more studies are needed [86]. In broiler chicken we commonly administer probiotics via drinking water, litter, oral gavage or feed. At experimental settings Soumeh et al. suggested better effectivity in improving growth and performance in case of administration of probiotics via water, whereas Olnood et al. found no improvement of performance or significant difference in delivery methods (drinking water, litter, oral gavage and feed) [87, 88]. These differences in result might be due to different probiotic strains, preparates and doses studied, suggesting that for each probiotic in every species and health status we need individual studies to ensure the best possible effectivity.

Probiotics can have an effect not only on the GI tract itself, but also on the immune system, brain, skin and other organ systems [55]. This will be explained in more detail in the next four subchapters.

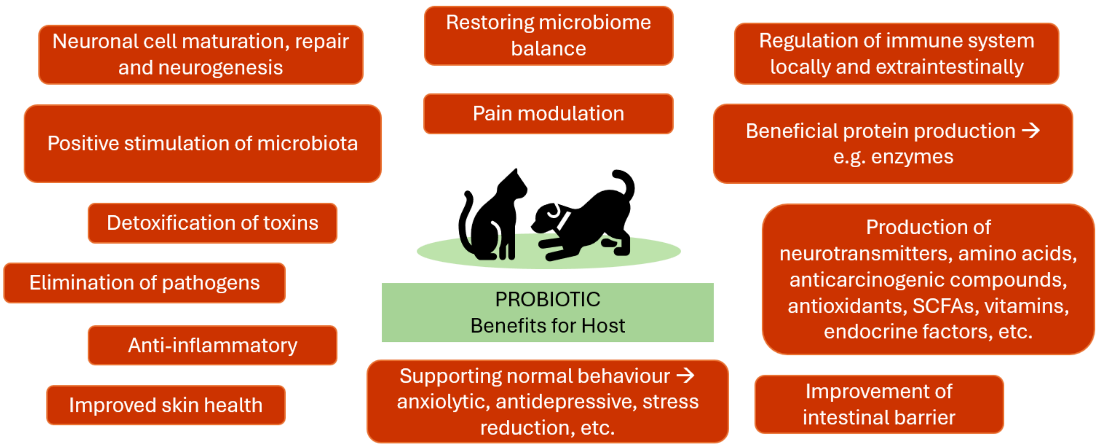

Figure 1 - Summary of the potential probiotic functions and their benefits on health in dogs and cats explained in chapter 3.1, 3.2, 3.3 and 3.4 [46, 53, 56, 57, 89–99]

3.1. Probiotics and the GI Tract

Today, in small animal practice, the trend when treating gastrointestinal disease is to avoid the use of antibiotics and moves in the direction of using probiotics, fecal microbial transplants (FMTs) and diet adaptation in the fight against gastrointestinal diseases. By avoiding antibiotics, a dysbiosis is treated not by potentially reducing the occurrence of pathogens alongside the diversity of the microbiome, but by reducing the occurrence of pathogens by strengthening those bacteria with positive effects, hoping to restore the balance of the microbiome. It is of utmost importance to maintain the diversity of microbiota to ensure the optimal function and health benefits of the microbiome.

The microbiome of the GI tract has various functions like synthetizing vitamins which have different vital functions. B vitamins take part in synthesis of nucleotides, other vitamins and amino acids, as well as DNA repair, methylation and replication. These microbial functions can strongly be influenced by the diet [18, 100]. As mentioned before, the mechanisms of action of probiotics are similar to microbiome functions ranging from the production of beneficial proteins like enzymes, production of essential amino acids, SCFAs, antioxidants, anticarcinogenic compounds and neurotransmitters, to decreasing inflammation, detoxifying toxins, improved intestinal barrier function, positive interactions with other gut microbiota, competitive exclusion of pathogens, and regulation of the immune system [46, 53, 56, 57, 89, 90]. The connection between the gut microbiome and the immune system called gut microbiome-immune axis is not the only connection to other organ systems. There is also a gut-brain axis, gut-gonadal axis, gut-kidney axis, gut-lung axis, gut-skin axis, etc. pointing out the impact and complexity of the microbiome and microbiome modulating probiotics on the general health [101–106].

A study involving healthy cats (n = 12) about oral application of multistrain probiotics (*Saccharomyces boulardii*, *Pediococcus acidiactici*) showed that those probiotics do not increase the microbiomes' diversity, but increase gut health, SCFA and antioxidant production, and decrease inflammation [89]. In 2011 a double blinded study by Bybee et al. was published, stating that the usage of probiotics in shelter cats with diarrhoea compared to a placebo (n = 217) group can significantly lower (by 13.3%) the incidence of diarrhoea lasting more than 2 days. The same study did not find any significant change in the incidence of diarrhoea in shelter dogs receiving probiotics, compared to the placebo group (n = 182), since they monitored the incidence of diarrhoea after 2 days, which was very low in both groups, meaning almost non of the dogs had diarrhoea for longer than 2 days [107]. This shows the interspecies differences between the reaction to this specific probiotic preparate.

Cao et al. (2023) tested the efficacy of different formulation containing artificial-enzyme-modified *Bifidobacterium longum* (*BL@B-SA*$_{50}$) in artificially induced Ulcerative colitis mice (UC-mice), which was administered for 4 days, showing a promising therapeutic effect like decreasing weight loss, suggesting possible reduction of ROS, Il-6, CD45, TNF-α, and increase in anti-inflammatory factors. The enzymes used to create *BL@B-SA*$_{50}$ are enzymes of single-atom catalyst (iron single-atom nanoenzyme treated with C18-PEG-B forming B-SA fused with *Bifidobacterium longum* (BL) through boronic acid vicinal-diol-based click reaction), protecting probiotics, having antioxidant effect relieving inflammatory symptoms, and increasing the colonization ability of the probiotics. It seems like especially the protective function increases the effectiveness of *Bifidobacterium longum* significantly. Also, the toxicity has been tested over 28 days in healthy mice, suggesting BL@B-SA$_{50}$ to be negligibly toxic. Compared with dexamethasone, methylprednisolone and 5-aminosalicylic acid, BL@B-SA$_{50}$ has increased efficacy [108]. A study was done with 30 mice in total, put into 6 groups of five, comparing artificially induced UC-mice, which were not receiving any treatment, receiving BL + 5-aminosalicylic acid, BL + dexamethasone, BL + methylprednisolone or BL@B-SA$_{50}$, to healthy mice. The use of BL@B-SA$_{50}$ in five UC-mice showed that BL@B-SA$_{50}$ can modulate the microbiome. Compared to non-treated UC-mice, the amount of Firmicutes increased, whereas Proteobacteria and Fusobacteria decreased in UC-mice treated with BL@B-SA$_{50}$, and after the end of BL@B-SA$_{50}$ treatment, pathogens were reduced and *Lachnospiracae* bacteria increased. Furthermore, the efficacy of BL@B-SA$_{50}$ in artificially created Chron's disease mice (CD-mice) was compared to *Bifidobacterium longum* alone, BL+B-SA and B-SA alone showed greater effectivity. Likewise, the effectivity of BL@B-SA$_{50}$ was also tested on artificially induced UC-dogs. The study size was small, containing only 9 subjects, which were divided into groups of 3 (healthy, control and BL@B-SA$_{50}$ treated dogs), evaluating the effectivity by performing a blood panel analysis and histological examinations as well as immunofluorescence staining of the GI tract and major organs showing a significant promotion of healing of colon in case of BL@B-SA$_{50}$ administration [108]. These new findings give hope for the treatment of severe microbiome related bowel diseases, however, due to the small study sizes, more studies must be done. Regarding these results, BL@B-SA$_{50}$ or maybe even other artificially-enzyme-modified probiotics could potentially replace other drugs like dexamethasone in IBD treatment, showing less side and more beneficial effects. The benefits of probiotic enzyme modification in this study seem promising and should also be studied for other probiotic strains.

Amit-Romach et al. (2015) found that the route of administration of probiotics in case of GI related problems like colitis can have an influence on the morphology, gene expression and microbial ecology of the colon of rats. Rectal administration of those tested probiotics shows a better result when treating colitis than oral administration, since they are targeted to the inflamed area, having a better adherence, and therefore reduce the inflammation more than when administered orally. Additionally, this study found that different probiotics which did not have the same mechanisms of action also showed different efficacy than anti-inflammatories, which is why the assumption arose in that paper that not every probiotic suits every individual, thus assuming that different individuals, diseases and disease stages require different probiotics which is why combination preparations could be advantageous [69, 109]. Lourens Baas Becking already said in *1934 "Everything is everywhere, but, the environment selects"* reminding us of how not every bacterium can colonize every individual [47, 76]. Already in 2013, Kamada et al. summarized in Nature Immunology that bacteria have multiple mechanisms (like change in pH, oxygen tension alteration, host immune system modulation, reduction of adhesion) influencing the growth and survival of other bacteria, which is why a temporary passage or short term colonization of probiotic bacteria through the gut could potentially be enough to eliminate certain pathogens [110]. Therefore, the question arises whether the efficacy of a probiotic is necessarily dependant on the ability of colonization.

Not all studies about probiotics show improvement of the clinical state of their patients undergoing probiotic treatment. There are risks and even contraindications using probiotics especially in critically ill patients. A study from 2005 showed that the usage of *Saccharomyces boulardii* against *Clostridium difficile* associated diarrhoea in critically ill patients can cause fungemia suggesting a careful reassessment of usage in all severely immunosuppressed and critically ill patients [111, 112]. Similar results regarding *Lactobacilli* in human ICU patients have been summarized in Yelin et al. (2019) [113]. 2021 Wombwell et al. found that the risk of treatment is not greater than the risk of any other bloodstream infection acquired in hospitals [114]. In addition to the potential risk of probiotic transmigration into the circulation, probiotics could replace useful microbes that are performing important functions or impact the function of other microbiota, altering their function, and therefore cause a decrease in health status. Those risks are low but should be considered before administration of probiotics [47]. Probiotics can therefore not only do good, but also harm our patients [115]. More about the risks regarding probiotic use can be read in chapter 7. Summarized Risks concerning the Use of Pro-, Pre-, Syn and Postbiotics.

Commonly, probiotics are used to prevent the overgrowth of harmful bacteria and therefore infectious complications. A double-blind, multicentred, placebo-controlled study from 2016 (PROPATRIA) showed that the use of multispecies enteral probiotics (three *Lactobacillus* spp., one *Lactococcus* sp. and two different *Bifidobacterium* spp.) in humans suffering from severe pancreatitis can increase the mortality rate from 6% to 16%. Out of 152 patients with pancreatitis that received probiotics, 16% died, whereas only 6% (9 out of 144 patients) of the placebo pancreatitis patient group died. The combination of probiotics and a high amount of proteolytic pancreatic enzymes can seemingly result in high mortality in addition to increasing levels of lactate caused by the bacterial transformation of carbohydrates, where the proteolytic enzymes cause intestinal epithelial cell damage. The authors suggest that probiotics may not be contraindicated in case probiotic therapy is started immediately after the first onset of disease symptoms while decreasing the patents polysaccharide intake, but more studies should be made. If treating acute pancreatitis with probiotics, the use of living probiotics in high doses immediately after the onset of symptoms in combination with protease inhibitors is advised [116]. Currently, the usage of probiotics in case of acute pancreatitis is not advised, and due to the failure of PROPATRIA study little to no further studies on humans or dogs are performed [117]. Rak et al. found that there is no benefit in using probiotics in case of steroid induced ulcerative gastric bleedings, even suggesting a harmful effect, but also mentions other papers stating the opposite [118]. In summary, probiotics are contraindicated in case of severe pancreatitis, severe immunosuppression and severe illness (where benefits are not exceeding the risk).

Pro-kolin Advanced probiotic oral paste for dogs containing *E. faecium* 4b1707, perplex prebiotic, combines kaolin and montmorillonite clay, pectin, psyllium, and beta glucan against simple acute diarrhoea was used in 148 dogs. Out of those 148 dogs 11 dogs were withdrawn from the study due to the worsening of the symptoms. Out of those 11 dogs, 9 were part of the placebo group and only 2 were part of the *Pro-kolin Advanced* receiving group, suggesting that treating dogs with this paste early in the course of the disease could prevent need for additional medication, like antibiotics, to treat acute diarrhoea. Those 2 dogs were screened for differences, compared to the other dogs where the *Pro-kolin Advanced* paste worked but no differences were found. The dogs that were treated with *Pro-kolin Advanced* recovered in general significantly faster than the dogs from the placebo group, however, all those dogs recovered [119].

Bacteria can not only be transferred using probiotics, but transferring bacteria by fecal transplant from a healthy patient to a sick patient is a new procedure (FMT) performed in critically ill dogs and cats. A similar method has been done for decades in cattle (ruminal transfaunation) to treat indigestion and support normal ruminal function after surgical interventions like left abomasum displacement or intoxications. Ruminal transfaunation is very effective when treating indigestion in cattle, suggesting the beneficial effects in other species, however, modes of actions and risks have not been widely studied [120].

For a small study (n = 18) with many limitations from 2023, dogs with moderately severe acute haemorrhagic diarrhoea syndrome (AHDS) were tested on the efficacy of probiotics vs FMT regarding duration and severity of clinical signs as well as fecal scores and serum LPS (marking the permeability of the gut). 9 dogs received PO probiotics daily for 14 days and a single sham enema, whereas the other 9 dogs received one FMT plus placebo oral capsules for 14 days. 4 of the FMT receiving dogs were withdrawn from the study, of which 2 were euthanized due to worsening of symptoms. This was most likely not due to a difference on severity of disease before the study started. The small study size, the lack of a placebo group and the withdrawal of 4 out of 18 candidates makes the results of the study less reliable, however, it seems like the treatment of AHDS with probiotics could be more effective than FMT (no difference in clinical disease course between both groups was detected), but more studies should be done. This study was performed on AHDS dogs, which have an increased risk of bacterial translocation, after screening the fecal donors for certain enteropathogens, which should have decreased the risk of bacterial translocation. Interestingly, however, euthanized dogs received fecal matter from different donor animals, compared to those animals which did not suffer any complications [84]. Extreme caution should be exercised when choosing and screening fecal matter donors, especially when treating animals suffering from AHDS.

For a different study, 84 AHDS dogs were treated with probiotics (*Lactobacillus plantarum* DSM 24730, *Streptococcus thermophilus* DSM 24731, *Bifidobacterium breve* DSM 24732, *Lactobacillus paracasei* DSM 24733, *Lactobacillus delbrueckii subsp. bulgaricus* DSM 24734, *Lactobacillus acidophilus* DSM 24735, *Bifidobacterium longum* 120 DSM 24736 and *Bifidobacterium infantis* DSM 24737) for 21 days as well as received additional therapy, consisting of crystalloid infusions, antiemetics, analgesics if needed and gastrointestinal diet. 59 patients were excluded from the study, however, the study still suggests that the application of probiotics reduces the enterotoxin concentration produced by *Clostridium perfringens* significantly in comparison to the placebo group, therefore suggesting the decreased occurrence of *Clostridium perfringens* due to probiotic use. Dogs treated with placebo or probiotics both recovered quickly, 4 and 3 days respectively [121]. The use of orally administered probiotics in combination with adsorption drugs like smectite, which can be used for treating GI disease, seems to be beneficial and showed promising results in treatment of type 2 diabetes in a small human study [122, 123]. Pieścik-Lech et al. found in 2013 that the combination of smectite with *Lactobacillus* GG compared to the use of

Lactobacillus GG alone in the treatment of acute gastroenteritis in children shows equal effectivity, which is why, according to this study, the combined use in this case is not justifiable [124]. This makes it seem like the combination of probiotics with absorption drugs must be studied for different health conditions, probiotics, adsorbents and animal species to be able to draw evidence-based conclusions, whether combined used of probiotics and absorbents is advisable or not.

In March 2023, a study was published by EFSA Panel on Additives and Products or Substances used in Animal feed (FEEDAP) et al., proving the safety and efficacy of a feed additive consisting of *Lactiolantibacillus plantarum* DSM 11520, which can be used not only for dogs and cats, but also for pet rabbits and horses. This bacterial strain does not contain any resistance to antibiotics of human and veterinary importance, is non-irritant to skin or eyes and can be fed to horses for meat consumption, not causing any harm for the consumers of horse meat. Shelf life and viability of bacterial strains after incorporation with certain feed materials were tested and are proper. The intended use of the product is to decrease the pH of certain feed stuff which was proven with in vitro studies only [125].

The study by Foongsawat et al. from June 2023 tested the 20 different Lactic acid bacteria strains originating from canine intestinal tracts (feces) in 30 dogs of different breeds for potential probiotic characteristics, like resistance to low pH, adhesion to epithelium, strain stability. antibiotic susceptibility and more, finding 2 strains (*L. fermentum* Pom5, *P. pentosaceus* Chi8) as potential probiotic candidates for canines. These probiotics also cause the production of essential amino acids [53].

The systematic review from 2019 by Jensen and Bjørnvad, evaluating 17 studies (of low sample size) on gastrointestinal disease in dogs, concluded that those studies about the clinical effect of probiotics did not show a clinically significant effect on the treatment of acute and chronic gastrointestinal diseases, and pointed out the need of much larger randomized controlled studies on this matter [126]. The currently available studies regarding feline probiotics as well as prospects and progress have been reviewed in Zah et al. (May 2024). This paper pointed out the sparse number of studies regarding feline probiotics in general and how more research must be done in this field, since commonly human and dog reports are extrapolated to design cat probiotic treatment [127]. Today (22.07.2024), when searching Google Scholar, more results can be found when searching for “probiotics cats OR cat OR felines OR feline -dogs -canines -canine -dog” (meaning articles on the topic of probiotics in cats without mentioning dogs), compared to “probiotics dogs OR canines OR canine OR dog -cats -cat -felines -feline”.

Table 3 - Summary of certain aforementioned study results of the chapter 3.1. Probiotics and GI tract

Predominant type	**Species**	**Potential effects**	**References**
Enterococcus faecium SF68	n = 217 cats with diarrhoea	Lowering incidence of diarrhoea	[107]
Enterococcus faecium SF68	n = 182 dogs with diarrhoea	No significant difference in diarrhoea incidence	[107]
artificial-enzyme-modified *Bifidobacterium longum* (BL@B-SA_{50})	artificially induced ulcerative colitis mice	Decreased weight loss, possible reduction of ROS, Il-6, CD45, TNF-α, increase in anti-inflammatory factors, antioxidant	[108]
artificial-enzyme-modified *Bifidobacterium longum (BL@B-SA_{50})*	n = 25 dextran sulfate sodium treated mice	Microbiome modulation, decreased pathogens in GI tract	[108]
artificial-enzyme-modified *Bifidobacterium longum (BL@B-*SA_{50})	n = 6 artificially induced ulcerative colitis dogs	Promotion of colon healing	[108]
Lactiolantibacillus plantarum DSM 11520	Dogs, cats, rabbits, horses	Potential decrease of pH of feedstuff	[125]
E. faecium 4b1707 containing *Pro-kolin Advanced* paste	n = 148 dogs with acute diarrhoea	Potential decreased need for antibiotics in the treatment of acute diarrhoea, faster recovery from acute diarrhoea	[119]
Streptococcus thermophiles, Bifidobacterium breve, B. longum, B. infantis, Lactobacillus acidophilus, L. plantarum, L. paracasei, L. delbrueckii bulgaricus	n = 18 dogs with AHDS	Probiotics could potentially be more beneficial than use of FMT	[84]
Lactobacillus plantarum DSM 24730, *Streptococcus thermophilus* DSM 24731, *Bifidobacterium breve* DSM 24732, *Lactobacillus paracasei* DSM 24733, *Lactobacillus delbrueckii subsp. bulgaricus* DSM 24734, *Lactobacillus acidophilus* DSM	n = 84 dogs with AHDs	Potential reduction in enterotoxin concentration produced by clostridium perfringens	[121]

24735, *Bifidobacterium longum* 120 DSM 24736 and *Bifidobacterium infantis* DSM 24737			
Saccharomyces boulardii, *Pediococcus acidiactici*	n = 12 healthy cats	Increased gut health, increased SCFA production, increased antioxidant production, decreased inflammation	[89]

3.2. Microbiome and the Immune System

The GI tract is the largest immune organ of the body [26]. It was shown that in infants early exposure to microbes as well as nutrient availability has a life-long effect on immunity and disease pathways influencing the susceptibility to intestinal disease [128]. The microbiome does not only influence the local immunity of the gut but also extra-intestinal immunity [129]. Despite the probiotics non-immunogenic functions, probiotics increase IgA (immunoglobulin A) secretion and stimulate antigen presenting cells, induce tolerance to food antigens, and regulate lymphocyte polarization [130]. An intact microbiome promotes the development of various immune cells like Treg, Th1, Th17 or TNF-α due to SCFAs function of promoting Foxp3^{+} Tregs proliferation [91]. *Bifidobacteria* take part in the maturation process of dendritic cells (DCs), and therefore influence the immune system. Depending on the type of bacterium leading to the maturation of the DC, the amount and quality (IL-6, IL-8) of cytokine production can vary [131]. If the gut microbiome is not intact, symptoms like leaky gut can occur, where we can see a translocation of allergens, proteins, bacteria, or endotoxins from the intestinal content to the rest of the body, causing inflammatory responses. This will lead to an increased infiltration of immune cells, which is for instance seen in CE [26]. Dysbiosis can therefore lead to a dysfunctional immune system and chronic inflammations.

Probiotics have been used in fish farming for over a decade already to improve their immune function as well as productivity and could potentially also be used in canine nutrition to increase immune function [132]. Probiotics can have an anti-inflammatory effect on the GI tract due to the alteration in microbiome, and therefore decrease the development of cancer, since cancer has been closely linked to inflammation. Probiotics can not only prevent the formation of cancer, but also stop tumors from growing and support cancer treatments in humans and canines. Probiotics can also restore the balance of the microbiome and decrease the viral integration into the host genome causing cancer [92, 133]. Sankarapandian et al. (2022) summarized the mechanisms of actions of probiotics against cancer formation in more detail and concluded that the use of probiotics in anticancer therapy still needs more research to assess proper concentrations, strains, risks and treatment duration to be used, however, current knowledge suggests that probiotics could one day potentially be effective anticancer drugs [90].

The gut microbiome can influence the development of immune mediated central nervous system (CNS) disease. A study from 2017, investigating 20 dogs diagnosed with Meningoencephalomyelitis (MUO), which is an immune mediated CNS disease, stated that there is an association between low *Prevotellaceae* abundance and MUO in dogs. Normal population of *Prevotellaceae* plays a role in reducing the risk for immune mediated CNS disease. This study also stated that dogs living in urban areas are at higher risk of developing MUO, but there is no proof that this is connected to a change in microbiome, and other factors may also lie in the background [134]. This does not only show the connection of the gut microbiome to the immune system but also to the brain.

3.3. Gut Microbiome, Probiotics and the Brain

The connection between the microbiome and brain has only been scarcely studied so far in dogs and cats, with newer dog targeted research arising in 2024, which is why this chapter lists research mainly done in humans and mice.

The brain and our behaviour can be influenced by the microbiome of the gut, which is called gut-brain axis, communicating via nervus vagus, the autonomic and enteric nervous system, hormones, tryptophan, immune system, short chain fatty acids and other microbial metabolites and metabolic pathways [93, 105]. The diet is one of the main factors influencing the gut-brain axis [105]. The key information from the gut is transmitted to the brain via endocrine factors like histamine, serotonin, melatonin, cholecystokinin and other mediators from the intestinal mucosa [93].

The microbiome influences the integrity of the blood-brain barrier (BBB), which is comparable to the effect of microbial produced SCFAs on the intestinal tight junction proteins. It has been proposed that the integrity of BBB can be restored by supplementation of SCFAs or introduction of SCFA producing bacteria [91, 93]. The microbiome influences the maturation of microglia (but not the number of total microglia), causing fewer mature microglia at the presence of dysbiosis as well as decreased anti-inflammatory effects due to decreased formation of Aryl hydrocarbon receptors (AHR) agonists and therefore decreased binding of AHR to astrocytes. Dysbiosis, germ free conditions or treatment with antibiotics can lead to abnormal neurogenesis in the hippocampus, which has been suggested to be linked to neurodegenerative disease like Alzheimer's and epilepsy in humans. It has also been suggested that maternal microbiome can influence the development of the foetal brain, impacting brain functions later in life. It has been put forward that microbiome regenerating treatments could improve diseases linked to abnormal neurogenesis [91, 94, 95]. Neuroinflammation, which supports normal reparative function and protection of the brain, is closely related to microbial composition and is bland in germ free mice [95].

Germ free mice showed decreased ability to recognition, memory, emotion, and learning, showing changes in many neurotransmitters like NMDA or 5-HT but increased exploratory behaviour [105, 135, 136]. The expression of synaptophysin and PSD-95 in certain regions of the brain are significantly lower in germ free (GF) mice compared to specific pathogen free (SPF) or conventionalized offspring mice (CON) (GF mice that were conventionalized with microbiota obtained by SPF mice), therefore gut microbiota can affect the normal brain development and behaviour of mice. Those proteins play a role in motor control and anxiety-like behaviour [136]. GF mice also show an increased hypothalamic-pituitary-adrenal axis response to stress in comparison to SPF mice, most likely due to downregulated expression of pituitary *Pomc* and *Crhr1* in SPF mice [137]. Hence, a dysbiosis of the gut can not only lead to digestive problems, but also conditions like increased stress, anxiety or depression [105]. Ait-Belgnaoui et al. found that *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175 combination can have anxiolytic and antidepressant activity by modulating members and pathways involved in stress response, whereas *Lactobacillus salivarius* was ineffective in the treatment of anxiety and depression [138]. A study in laboratory rats with stress induced "leaky gut" (translocation of proteins, bacteria, allergens or endotoxins) showed that *Lactobacillus farciminis* does not only suppress stress-induced hyperpermeability of the gut, but can also decrease the HPA response to acute psychological stress [139]. Ma et al. (2024) suggested that probiotics like *Bifidobacterium pseudolongum* may potentially improve canine memory during puppy development, pointing out the limitations of the studies and the need for more research in this field [140]. It has been suggested that probiotics and prebiotics might aid in the treatment of anxiety-related disorders in dogs. Certain probiotics have already been proven to decrease anxiety and stress in humans [96]. Probiotic anxiety relieving veterinary supplements are available on the market, like “Purina Pro Plan Veterinary Supplements Calming Care Canine Probiotic Anxiety Supplement”, containing *Bifidobacterium* longum BL999, or “Relaxing Pet dog” by NBF LANES, containing *Limosilactobacillus reuteri NBF1* [52, 96]. A study from 2022 demonstrated on 45 dogs with behavioural problems that *Lactiplantibacillus plantarum* PS128 alleviates separation anxiety and aggression and improves compulsive disorders. Although this result seems promising, due to the small sample size and imprecise study design it is not enough to draw conclusions and further studies should be done [141].

Recently, there have been studies considering the possible involvement of gut microbiome in the etiology of epilepsy of humans and dogs, however, due to the topic’s complexity, further studies should be performed to prove its connection [93]. Surgeries can negatively affect the gut microbiome due to the administration of medication, anaesthesia, mechanical bowel preparation or surgical manipulation, and operation stress, which is why probiotics are indicated after certain surgeries. Probiotics have been shown to alleviate post operative pain by decreasing inflammation, decreasing abdominal distension and ischemia and increasing opioid receptor expression [97].

This chapter shows the extent and versatility of the microbiome and probiotic effect on the brain. As mentioned before, little to no research has been done regarding the probiotic use in dogs and cats for these described purposes. Especially the potential risks are still unknown.

3.4. Microbiome, Probiotics and the Skin

The skin is the largest organ of the body, carrying out various functions, like maintaining body homeostasis, influencing the hosts immune functions, and protecting the body from the external environment like pathogens and therefore cutaneous disease [22, 142, 143]. The composition of microbiome is diverse, being influenced by all the previously mentioned factors. A study from 2013 found that adult humans living together with dogs share more microbiota with their dogs and household members than other households without dogs, suggesting that direct and frequent skin contact with cohabitants changes out microbial skin composition [144]. Pereira and Clemente (2021) summarized that *Proteobacteria*, *Actinobacteria*, *Firmicutes*, *Bacteroidetes*, *Tenericutes* and *Cyanobacteria* are the main bacteria comprising the skin microbiome, changing in occurrence and amount depending on the skin section [21, 145].

The dermal microbiome can not only be influenced by topical contact to other microbiomes, but also by feed changing the dermal fat microbiome. A very small study by Leverett et al. suggested that the quantity and quality of bacterial types can change when a dog (n = 8) is fed fresh feed compared to dry feed. This knowledge was obtained by feeding *Freshpet Select Sensitive Stomach and Skin roll* fresh diet to healthy dogs for 30 days and then their regular dry food of differing composition to the same group of dogs for another 30 days after a 4-day transitional period, followed by taking bacterial swabs from the interdigital area, internal ear and goi area. This study has been limited by the small participant size and the influence of environmental factors like the change in weather, nevertheless the authors suggest an small influence of the weather, since those dogs were indoor dogs [143]. Low gut microbial diversity can lead to allergic skin reactions. This connection between the gut and skin is called skin-gut axis [104]. Probiotics can therefore be used orally, but also topically, to manage certain skin disorders [98]. Transdermal delivery of probiotics into the skin using microneedles might also be beneficial, especially in the treatment of chronic infectious skin wounds [146].

The dermal microbiome influences our immune system by causing increased gene expression of antimicrobial peptides in keratocytes or increased recruitment of mast cells in case of viral challenges on the skin [142]. The dermal microbiome has an important role in skin wound healing. Disruption of the microbiome can decrease the immune system of the skin, disrupting the interaction between skin cells and therefore decreasing wound healing. An in vitro keratocyte scratch test study by Barthe et al., testing a topical mixture of probiotics as prevention of wound infections, found that the cell viability was only slightly affected by the probiotics at lower concentration, however, higher concentrations decreased the cell viability, nevertheless still being less toxic than commonly used cytotoxic disinfectants. Despite the promising results, Barthe et al. suggest further studies before being used in veterinary praxis for cleaning dermal wounds, and suggest to regard these results not as definitive results, but rather one of the first studies leading this field into a new direction [147]. In veterinary surgery probiotic-sorption drugs can be used to treat purulent-inflammatory processes in wounds, giving promising results in cats, like reduced time until beginning epithelization, better wound cleaning and faster complete healing compared to animals only receiving surgical treatment of wounds. Prevention of formation of surgical infections as well as faster healing was also noted using antibacterial therapy in combination with probiotic-sorption drugs [82].

Multiple studies have shown that dogs with canine atopic dermatitis (CAD) have a disturbed microbiome like decreased diversity and richness of microbiota [22, 148]. When treating CAD with a topical antimicrobial treatment like "Malaseb shampoo", the diversity of microbiome only increased until the termination of the 4 week long treatment, and decreased again in the following weeks after the treatment, showing no difference to the initial diversity, however, it is known to mitigate the clinical symptoms in case of CAD [148]. In 2019, topical therapies like moisturizers anti-inflammatories and antipruritic and skin barrier repairing agents like topical fatty acids were suggested as main treatment options for CAD, mentioning the possible topical use of probiotics in the future [149]. In 2010, López et al. suggested the usage of probiotics like *Bifidobacterium animalis* or *Bifidobacterium longum* prenatally as prevention of formation of atopic disease in human [131]. Probiotics like *Lactobacillus sakei* Probio-65*,* which are given orally, can lead to a slight improvement of symptoms in case of CAD, suggesting a combination treatment strategy for the treatment of CAD [150, 151]. Early treatment with *Lactobacillus rhamnosus* GG (LGG) can prevent the worsening of symptoms of CAD by decreasing allergen specific IgE [152]. A small study (n = 10) showed that fecal transplants in puppies with digestive problems can resolve all digestive symptoms and reduce the occurrence of CAD later in life [104]. A study was performed on 10 dogs with CAD, receiving 5 different nutraceutical preparations (4 rectally and one orally) one after the other, administered 15 minutes apart. First polyethylene glycol was administered, followed by isotonic silicone dioxide powder, multistrain probiotic powder plus multi-polyphenol ingredient nutraceutical, then hemp oil, lactated ringer solution plus butyrate paste, and lastly, altered calendar probiotic was given PO, leading to complete recovery of all subjects. All 5 preparations contributed to the recovery, establishing a novel treatment protocol. *Streptococcus thermophilus,* which was given orally, increased the production of lipids like ceramides in the stratum corneum, whereas bacillus subtilis prevented T cell-mediated disease and allergic eosinophilia [153]. A study from 2024 suggested that dietary probiotic supplements (multistrain probiotic nutraceutical blend containing *Lactobacillus rhamnosus, Bifidobacterium bifidum, Bifidobacterium infantis, Bifidobacterium animalis, Lactobacillus acidophilus* and *Lactobacillus casei* as well as vitamin E, B3, B6, B5, inositol), choline, lutein, mannanoligosaccharides, fructooligosaccharides and a *Saccharomyces cerevisiae* based postbiotic) may be used as antipruritic, antiallergenic therapies, potentially reducing skin reddening and improving life quality in dogs. This study is one of the first ones regarding this topic in dogs [99]. The role of topical and orally applied probiotics in human skin health was summarized by Gao et al.

2023, called in the review “The Role of Probiotics in Skin Health and Related Gut–Skin Axis: A Review” [154].

4. Prebiotics

The definition of prebiotics changes several times since 1995, when the first definition was established: *"non digestible food ingredient that beneficially affects the host by selectively stimulating growth and/or activity of one or a limited number of bacteria already resident in the colon"*. After that, new definitions were proposed several times, leading to the definition established by International Scientific Association for Probiotics and Prebiotics (ISAPP) in 2017: *"a substrate that is selectively utilized by host microorganisms conferring health benefit"* [155]. To radically simplify this definition, one could say that prebiotics are the "food" of beneficial microorganisms. They are non digestible carbohydrates which are fermented by microbiota, leading to the reduction of pathogens, regulation of pH, and production of vitamins, SCFAs, and other products that are absorbed, exerting a positive effect on the host [18, 46, 155]. Prebiotics are commonly used to stimulate the growth of certain beneficial bacteria like *Lactobacilli*, *Bifidobacteria*, *Roseburia*, *Fecalibacteria* and many more [46].

Examples for prebiotics commonly used in humans would include fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), CLA, conjugated linolenic acid, polyunsaturated fatty acids (PUFA), mannan-oligosaccharides (MOS), inulin, lactulose, psyllium husks and xylo-oligosaccharides (XOS). One shall keep in mind that, depending on the species, different substances are considered to be prebiotics, considering that cellulose is a beneficial prebiotic for ruminants, but barely utilizable by human microbiota [155–157]. Prebiotics can be classified based on solubility, fermentability, function, viscosity and chain length. Based on their different properties, these fibers will bring different health effects [158].

Prebiotics have various mechanisms of actions and health benefits. Bon et al. (2023) showed that a prebiotic mixture containing sugar beet pulp, GOS and cellulose can improve gut health, showing improved fecal scores of healthy senior dogs after administration for 21 days. It changes microbiota composition of feces and microbial metabolite profile in a positive way, reduces fecal pH, therefore enhancing gut pathogenic colonization resistance, and reduces fecal branched-chain fatty acids, which indicates a decrease in proteolytic degradation [159]. GOS can improve the immune system of dogs, however, if administered in too low doses, certain positive effects, like change in apparent digestibility coefficients of nutrients or increased microbial material in the feces, can be absent [160]. Supporting a pregnant bitch with prebiotics and probiotics can strengthen the immune system of her puppies [15]. Rousseaux et al. concluded in 2023 that prebiotics given to humans during pregnancy can cause “B immune imprinting”, meaning that prebiotics influence the transmission of specific B immune factors from mother to child by modulating the expression of genes encoding that function, therefore influencing the immune system of the offspring. Prebiotic supplementation during adulthood, however, does not effect the gene expression of genes encoding for specific B immune factors, but it can increase the Ig secretion in secondary lymphoid tissue from already existing B cells of animals [161]. FOS in the combination with *L. reuteri* and butyric acid can normalize stress response by supporting a normal hypothalamic-pituitary-adrenal axis response to stress [52]. Ephraim et al. (2022) indicated that fibers (tomato pomace), polyphenol sources and omega-3 fatty acids can influence anxiety-related metabolites, uremia-related metabolites and gut bacteria in a positive way [162]. In mice, FOS and GOS can increase the production of SCFAs, decrease depression-related behaviour, corticosterone levels, stress induced hyperthermia, stress induced defecation, increase serotonin levels in the brain, and affect the gut microbiota, but do not affect locomotor activity and nociception. FOS and GOS combined reduce or prevent psychosocial stress induced behavioural changes after 3 week long chronic stress [163]. Pecan shells, flax seed, powders of cranberry, citrus and beet in healthy cats can positively shift fecal bacterial composition and metabolites in a 31-day trial. Studying the effect on sick patients or long-term effects would be beneficial to evaluate its effect on diseases in cats. So far, the number of studies regarding cats is scarce and more separate studies for separate diseases should be done [164].

Another important health benefit of prebiotics is the production of SCFAs. SCFAs are VFAs mainly produced by bacterial fermentation of dietary fibers. [165] Psyllium husk and lactulose influence bacterial fermentation in dogs (n = 30), increasing the amount of volatile fatty acids (VFAs) in fresh feces after fifteen days of ingestion. The proportion of increase of different VFAs, as well as the amount and timing until an increase was noticed, differed in the 2 treatment groups. It was suggested that psyllium husk and lactulose therefore have a positive effect on the health of dogs by aiding the proliferation of beneficial intestinal bacteria as well as the maintenance of intestinal flora balance, increasing the production of VFAs, with lactulose increasing the total VFA content more rapidly than psyllium husks [157]. VFAs play an important role in maintaining gut health by providing energy to colonocytes, therefore supporting cell proliferation and metabolism, improving intestinal barrier function by inducing the expression of tight junction proteins and regulating permeability to prevent bacterial translocation, modulating immune and inflammatory response, regulating endocrine functions and gut motility, but also influencing the gut-brain axis, altering brain functions. VFAs may even slow down chronic kidney disease progression and ameliorating kidney damage [55, 93, 165–167]. Psyllium can act as an antioxidant, hepatoprotective, and reduce serum lipid level in hyperlipidaemia. It is commonly used against constipation and diarrhoea and increased peristaltic movement of the bowel [168, 169].

A study from 2020, containing 30 dogs with chronic colitis, suggested that treating those animals with a hypoallergenic, high fiber and therefore prebiotic containing diet in combination with probiotics will restore a healthy gut microbiome and cause the vanishing of clinical signs by reducing inflammation within one week on average [170]. A study from 2024 found that GOS has different effects in cats and dogs, being that in dogs GOS leads to an increase in VFAs, as explained earlier, whereas in cats GOS resulted in increased amino acid fermentation, and therefore increase in isovaleric acid as well as no change in α-diversity of cat fecal microbiota. GOS influences the amount of both Lachnospiraceae spp. and Bifidobacteria spp. in dogs and cats, therefore having positive effect on stool microbiota in both species [171].

The use of prebiotics does not come without any side effects. However, those side effects are mild compared to the side effects and risks of using probiotics. More about the risks and side effects of prebiotics can be read in chapter 7. Summarized Risks concerning the Use of Pro-, Pre-, Syn and Postbiotics.

5. Synbiotics

Synbiotics are defined by ISAPP as *"a mixture comprising microorganisms and substrate(s) selectively utilized by host microorganisms that confers a benefit on the host"*, and not just as a mixture of prebiotics and probiotics, since the individual pre- and probiotics mixed in a synbiotic could, in combination, be administered in lower doses, still exerting a health benefit compared to pre- and probiotics alone. Additionally, this definition keeps the possibility open to invent synbiotics comprised of microorganisms that do not have probiotic functions by themselves, or substrates that do not have prebiotic effect by themselves, however, excreting health benefits on the host in the combination with prebiotics and vice versa. There are two types of synbiotics, complementary synbiotics and synergistic synbiotics. Regarding synergistic synbiotics, the substrate must be utilized by the microorganism itself, having a synergistic effect, and therefore enhancing the health benefit of the microorganism, however, the individual microorganism or substrate must not exert a health benefit on the host when given alone. Complementary synbiotics consist of a prebiotic plus a probiotic together, achieving one or more health benefits. The prebiotics and probiotics used in a complementary synbiotic must also exert a health benefit in the absence of the co-administered part [172]. Certain drug combinations allow a reduction in dose given of each individual drug without reducing the wanted effect, therefore potentially having additive, synergistic or even potentiating effects [173]. Due to the difficulty of quantitatively measuring and comparing the health effects of synbiotics compared to their single ingredients, a superadditive effect is not a prerequisite in the definition of synbiotics. However, when designing and testing synbiotics, it must be made sure that there is no antagonistic effect. If the health benefit of a synbiotic containing a probiotic and prebiotic mixture is not proven, it must be labelled as probiotic and prebiotic containing, but may not be marketed as synbiotic. The effectivity of a synbiotic depends on similar things as the effectivity of pro- and prebiotics, i.e. host species, individual difference, dose, time and way of administration, host target site, environmental factors (like diet) and potentially genetic factors [172].

Synbiotics have similar functions as probiotics and prebiotics by positively altering the gut microbiota composition, improved immune system, increased production of SCFAs and lactate, however, the need for more studies in this field regarding dogs is higher than in the field of pro- and prebiotics [174]. Synbiotics may potentially be used in the treatment of acute diarrhoea [175]. It has been suggested that the administration of prebiotics in addition to probiotics to bitches during gestation and after parturition can influence the functionality of the immune system of the offspring via colostrum, since the gut microbiome has an influence on the development of gut associated lymphoid tissue (GALT) which is responsible for the production of plasma cells that release immunoglobulins, which are transferred via the mammary gland (entero-mammary link) into the milk and therefore strengthening the immune system of offspring. A trial from 2020 (n = 20), where bitches were supplemented with pre- and probiotics (mannan-oligosaccharides (MOS) and fructo-oligosaccharides (FOS) plus *Enterococcus faecium* and *Lactobacillus acidophilus*) from different time points during the gestation, showed significant differences between the IgG, IgM and IgA in the serum and colostrum, suggesting that a supplementation of pre- and probiotics from day 35 of gestation increased the amount of all three immunoglobulins significantly, whereas a pre- and probiotic supplementation from day 49 of gestation can increase the IgA concentration significantly [176]. In addition to the immunoglobulins, colostrum plays a role in the development of microbiome due to bacterial transmission from the mother to the offspring [19]. The administration of the same prebiotics (MOS and FOS) in combination with the same probiotics (Enterococcus faecium and lactobacillus acidophilus) during pregnancy of bitches also decreases the occurrence of gastroenteritis in neonates, probably due to the offspring's strengthened immune system [15].

Studies have suggested that CAD can be treated using synbiotics. Kawano et al. showed in a small single-center non-randomized study (n = 35) that the treatment of CAD with *Lactobacillus paracasei* M-1 and trisaccharide kestose can improve CAD. This could mean that in the future the long-term use of glucocorticoids, calcineurin inhibitors and immunosuppressants in CAD treatment could be reduced or even replaced, therefore decreasing the risk of liver failure, diabetes or other side effects [151].

Due to the recent (2020) change in definition of synbiotics it is difficult to find statistically relevant studies to this subject concerning dogs or cats. There is a big lack of research especially concerning synergistic synbiotics. The combination of probiotics and substrates could potentially mean that a dose reduction of each ingredient is possible, achieving a similar effect to both products given individually in higher doses, potentially decreasing the occurrence of side effects.

6. Postbiotics

The term postbiotic has only been defined in 2021 by ISAPP in Europe as *"preparation of inanimate microorganisms and/or their components that confers a health benefit on the host"* [177]. Considering this definition, metabolites of a microorganism alone (fully purified and not originating from a microorganism) do not officially qualify as postbiotics, which is why, for instance, short chain fatty acids (SCFAs) or other chemically synthetized compounds like enzymes (even if those also exist as microbial derived enzymes) alone are not considered postbiotics and shall be named by their exact chemical name rather than as a postbiotic. To qualify as a postbiotic, there has to be a detailed description of preparation of postbiotic, the progenitor microorganisms have to be screened for safety concerns and fully characterized molecularly, for example by genome sequency, the inactivation procedure has to be described in detail and has to be confirmed, there has to be evidence of health benefit to the host, and the safety has to be assessed for use in the target species [178].

To produce a postbiotic, the microorganisms used must be inactivated. This could be done by pasteurization, thermal sterilization, high pressure, ionizing radiation, pulsed light, magnetic field heating, X-rays, high-voltage electrical discharge, or spray drying. However, these treatments can influence the health benefit and function of a postbiotic, which is why studies should be done for each new postbiotic formulation [177]. Postbiotics are commonly used in foods and feeds, not only to exert health benefits but also to prevent spoilage. They can be classified into various classes, ranging from substances released after cell lysis, metabolites generated by bacteria, structural properties, elemental composition to a classification based on physiological function (like anti-inflammatory or antioxidants). Examples for postbiotics are bacterial derived SCFAs, enzymes, proteins, peptides, peptidoglycan derived muropeptides, polysaccharides, teichoic acids, organic acids, vitamins, lipids, and cell surface proteins [179–182].

ISAPP summarized the 5 possible mechanisms of action of postbiotics as beneficial modulation of microbiota by postbiotic products containing SCFAs, lactic acid or adhesins, the strengthening of epithelial barrier function by postbiotics containing proteins having growth promoting or antiapoptotic effect on enterocytes (like Msp1/p75 or Msp1/p40 produced by *Lactobacillus rhamnosus* GG) or SCFAs, modulation of local immune responses by postbiotics causing MAMP-PRR interactions (microbe-associated molecular pattern – pattern recognition receptor interactions), modulation of systemic metabolism by postbiotics containing vitamins, SCFAs or succinate and systemic signaling via the nervous system by postbiotics containing serotonin, acetylcholine, dopamine, GABA or SCFAs. The precise mechanisms of action are partially still unclear [11, 177, 183]. Depending on the postbiotic chosen, they can have many different effects, ranging from immunomodulation to anti-inflammatory, hypocholesterolemic, anti-obesogenic, antitumor properties, against constipation and to improve absorption of beneficial elements. They can also reduce the risk of infection prevention by sealing the intestinal barrier, accelerate wound healing and decrease the risk of cardiovascular disease [181, 184].

Advantages of postbiotics over probiotics are a potential decreased risk of transmission of antibiotic resistance genes and no risk of colonization of gut, making them safer. Postbiotics have the big advantage of usually being more stable than probiotics, prolonging their shelf life and being easier to store and transport. They can also be used in patients with leaky intestinal barrier without the risk of inducing bacteriaemia and potentially closing the intestinal barrier [178, 182, 184]. It seems like postbiotics such as SCFAs are important in maintaining a healthy intestinal barrier function [177]. A study with 122 dogs found that SCFAs (acetate, butyrate and propionate) concentration in dogs with chronic enteropathy was significantly lower than in healthy dogs, in addition to increased dysbiosis indexes [185]. Orally applied heat-killed Enterococcus faecalis FK:23 seems to be a good adjuvant therapy for treating atopic dogs [186]. Heat-killed *Lactobacillus fermentum* and *Lactobacillus delbrueckii* and its fermentation products can potentially have a positive effect on immune function, decrease oxidative stress and improve stool quality of adult dogs [187].

This makes it seem like postbiotics are a safer alternative to probiotics with similar effectivity, and they might even be suitable to replace antibiotics, due to their microbiome modulating effects. However, unlike some probiotics, postbiotics do not have a sustained effect if the application is stopped. More research must be done in this field regarding the effect of specific postbiotics on specific dog or cat diseases, but the present results seem promising.

In animal production, postbiotics are used to promote health, and therefore increase productivity and decrease the prevalence of certain pathogens, therefore improving food safety [188]. There is high potential using postbiotics not only for therapeutic reasons, but also for commercial reasons. The market and research done in this field is growing quickly. Postbiotics can be used in the food industry for preservation, to increase shelf life and to combat biofilms, which is critical for food safety and therefore human health. Postbiotics have also been proven to support human mental health [180]. Postbiotics are also commonly given in combination with other biotics. Postbiotic (indole-3-proprionic acid) and prebiotic (alginate sodium, resistant starch, chitosan) mixtures in microcapsules have been investigated on their synergistic effect, suggesting a positive effect on improving colitis in mice. The microcapsules used lead to fast release of IPA in the lower GI tract and slow release in the upper GI tract, making a targeted delivery possible. This specific combination of probiotics and prebiotics showed a significant increase in effectivity of treating colitis in mice, compared to mice that only received one of the two preparations [189].

Delivery routes of postbiotics have not been well researched yet. Since the definition of postbiotics was only established in 2021, some papers regarding this topic seem to be using an outdated definition or are only covering theories (see [11]), which is why one must be careful when evaluating publications on this topic.

Many studies concerning postbiotics research its use via oral delivery [186, 187]. Topical application of certain postbiotics regarding certain diseases in certain species has been researched to be effective and safe [190]. The intradermal and intravenous delivery routes of postbiotics could potentially also be used, however, more research must be done in this field. There are many positive aspects regarding PO delivery of postbiotics for the treatment of gastrointestinal disease, compared to other potential delivery methods comprising direct delivery to the target site, high local concentrations, smaller doses needed and reduced unwanted systemic effects. Other delivery systems circumventing the GI tract could potentially be delivered to the target site before enzymatic degradation, therefore potentially showing higher bioavailability. Postbiotics can be protected from this degradation by nanoparticles like lipids [11]. Rectal delivery of other medical products is commonly done and could potentially also be an advantageous delivery route for postbiotics [177]. The question arises whether intravenous, intradermal or rectal delivery of postbiotics is safe or not, and whether those delivery systems are more advantageous than oral or topical delivery.

7. Summarized Risks concerning the Use of Pro-, Pre-, Syn and Postbiotics

PROBIORICS:

Nowadays, due to the increasing research that is done in this field, it seems that scientists and doctors all over the world have high hopes regarding the positive effects of pro-, pre-, syn- and postbiotics, as well as other microbiota modulating treatments in treating, improving, or curing disease that have yet been non curable. Like any medical treatment, the use of biotics does not come without risks. This must be considered before prescribing biotics to a patient.

Studies have shown that many probiotics contain antibiotic resistance genes, which can be transferred to other bacteria of the gut microbiome by horizontal gene transfer. The existence of antibiotic resistance genes does not automatically mean that a probiotic is not used in practice anymore. There have been probiotic strains like *Bifidobacterium lactis* that are considered safe to use in infants by FDA, even though that strain contains antibiotic resistance genes [47, 191]. In this case, those genes are located in the chromosomes, which are not mobilizable horizontally to other bacteria, and they are resistant to an antibiotic that is classified as not clinically relevant since it is not used in children. The risks for the health of the patient receiving this probiotic are therefore according to Gibson et.al negligible [192].

The risk of transferring pathogenic bacterial strains should also be considered, as well as the chance of contamination of the products [47]. Choosing an incorrect route of administration also poses its hazards, which is why certain products should only be administered in certain routes, and one should pay attention to the correct preparation of the drug before administration for the safety of the patient. Correct preparation is also of importance, regarding contamination of other objects in the health care facility via air, surfaces, or hands [47, 193]. It has been suggested that the use of probiotics in critically ill patients can lead to a translocation of bacteria, potentially causing sepsis [47, 111, 113]. Translocation of fungi, causing the development of fungaemia has also been reported in human infants [194]. Yelin et al. studied the risk of *Lactobacillus* bacteraemia in human intensive care unit (ICU) patients, finding that the administration of probiotics to those patients can increase the risk of bacteraemia (about 1,1% increased risk), however, it remains unsure if the probiotics given are the true cause or just a correlation, since *Lactobacillus* bacteriaemia was also noted in non-ICU patients who did not receive probiotics (0,01% increased risk) [113].

Considering that this study compared ICU patients to non-ICU patients, which are usually in a better health condition, the difference of 1% incidence increase for bacteriaemia is most likely negligible and might not be due to the use of probiotics. However, we should keep in mind that there is a possibility that probiotics could potentially cause an increased risk for bacteriaemia, and therefore only use them in those vulnerable patients (e.g. ICU patients) where the use is indicated, and the potential benefit seems higher than the risk.

Studies have shown that certain bacteria found in probiotics (*Limosilactobacillus reuteri*) can also lead to raised autoimmune markers in mice, potentially leading to autoimmune disease like Lupus erythematosus [50]. In case a patient receives other drugs in addition to probiotics, the interactions between those drugs must be considered since the microbiota have an effect on the metabolism of drugs [47]. Long term potential benefits but also risks in some cases are the possible lifelong influence of the probiotic strain on other microbiota [47]. The close interaction between humans and their pets also impacts their microbiomes, having a positive or negative effect. An alteration of a dogs' or cats' microbiome using probiotics can therefore also alter the owners' microbiota [9, 195]. There are many studies testing the risks of probiotics before they are used in humans, however, not all probiotics for dogs are tested which can also be a risk to humans, due to the close lifestyle of humans and their dogs. In 2018 a study was performed, testing 17 non-pathogenic Enterococcus strains on antimicrobial resistance to 19 antimicrobials (of which 9 were proposed by European Food Safety Authority (EFSA)) to assess their potential use as probiotics. All strains were susceptible to the 9 antibiotics proposed by EFSA, except 6 probiotics, which were excluded for further testing due to antimicrobial resistance to EFSA recommended antimicrobials. Interestingly, many of these probiotic strains showed antibiotic resistance genes, but were not resistant against those antibiotics in practice [109]. The spread of antimicrobial resistance genes can also be a problem in the case of fecal microbiota transplantation (FMT), which is why the fecal matter that is transferred should be screened for resistance genes before application. The use of FMT could further decrease the use of antibiotics, and therefore, if used carefully, even reduce the spread of antimicrobial resistance genes [196]. It has been suggested that the resistome is primarily located in the large intestine, since fewer resistance genes were detected in the small intestine compared to fecal samples. This, however, is not proven and needs further investigation [33].

Long term colonization achieved by probiotics can be a goal when using probiotics, however, there is limited data about long term safety regarding the colonization of the host posing a potential risk. More research must be done in this field [47]. One has to consider that short term colonization of the gut can potentially be enough to eliminate certain pathogens and exert a beneficial impact on the host, therefore long term colonization is not necessarily our goal during treatment [110]. Ineffectivity of the probiotic treatment is a risk that can be due improper dose, improper selection of bacterial strain for a given species, or non-uniform shelf life/improper quality of probiotic products [55, 155]. Lack of effectivity or even adverse effects are possible if the list of ingredients and its allergens were not considered carefully [197]. Allergic responses or bowel ischemia due to bacterial strains have been reported in humans [198]. Probiotics in food can produce biogenic amines which might potentially cause various side effects in sensitive humans, like headaches [199, 200]. One shall not forget that probiotics can also cause gastrointestinal side effects like flatulence, abdominal pain due to bloating or cramps, nausea, diarrhoea and many more [201]. It is the responsibility of the veterinarian to choose a probiotic suitable for the patient.

The review by Liu et al. from march 2024 is highly acknowledgeable for more detailed information about the risks of probiotics in humans [202]. Still today, there is a lack of studies examining the risks of probiotics, most likely due to its common classification into food supplement category (regulated by EFSA in Europe) rather than medicinal products, making it inadequate to protect consumers suffering from severe disease and to guide doctors [197]. It seems to be advisable for doctors to prescribe probiotics approved as drugs rather than food supplements to their patients.

PREBIOTICS:

In general, side effects in case of prebiotic intake can be bloating, flatulence, increased borborygmi, cramps and abdominal pain, or, if consumed in larger doses, diarrhoea or constipation can be an issue. The severity and presence of side effects depends on the dose chosen, type of prebiotic (length of prebiotic chains) and individual sensitivity. Long chain prebiotics like inulin tend to have fewer side effects compared to short chain prebiotics [203].

SYNBIOTICS:

In synergistic and complementary synbiotics, the side effects are similar to the aforementioned probiotic and prebiotic risks, however, in case a lower dose of probiotic or prebiotic is used, the side effects and risks are potentially lower [172]. Not many studies have been done focusing on the risk and side effects of synbiotics until this point, and more research is needed on the safety and toxicity of synbiotics.

POSTBIOTICS:

Postbiotics are considered to be significantly safer than probiotics, due to their inactivation of microorganisms leading to the lack of replication, and therefore a deceased risk of bacteraemia or fungemia after administration, however, one has to bear in mind that the risk for those side effects is relatively low in healthy patients even in probiotics, but can be a substantial safety issue in case of severely sick patients [177, 182]. Postbiotics produced from gram negative bacteria could potentially cause endotoxic shock due to lipopolysaccharide release after bacterial death [177]. In general, postbiotic safety, toxicity and immunogenicity has not been well researched and more studies should be done [204].

Even though we know the overall potential risks of pro-, pre-, syn- and postbiotics, safety assessment must be done for each product at the prescribed amount in the indicated species and patient population and in case of overdosing.

Table 4 - Summarized potential risks and side effects regarding pro-, pre-, syn- and postbiotics

	Potential risks and side effects
Probiotic	• Antibiotic resistance • Contamination of medical instruments • Bacterial translocation and bacteriaemia • Fungemia • Increased risk of adverse reaction development in case of vulnerable population/severely ill → potential high-risk groups • Probiotic to drug incompatibility • Long-term colonization • Dog-human microbial interaction • Autoimmune modulation • Ineffectiveness • Allergens • Allergic reactions to probiotic bacteria • Bowel ischemia • Side effects induced by biogenic amines • Bloating, flatulence, cramps, abdominal pain, nausea, diarrhoea, etc.
Prebiotic	• Bloating, flatulence, increased borborygmi, cramps, abdominal pain • Diarrhoea • Constipation
Synbiotic	• Potentially similar risks as pro- and prebiotics • More research needed to assess risks, side effects and contraindications
Postbiotic	• More research needed to assess risks, side effects and contraindications

Table 5 - Suggested contraindications regarding probiotic use

Probiotics: suggested contraindications (see chapter 3.1 Probiotics and the GI Tract)	• Severe pancreatitis • Severe immunosuppression • Severe illness (where benefits are not exceeding the risk)

Method

This literature review aims to give a better understanding for pro-, pre-, syn- and postbiotics and its newest findings regarding risks, application routs, advantages, and disadvantages in the veterinary field. PubMed® and Google Scholar were used to search key words, including probiotics, prebiotics, synbiotics, postbiotics, probiotics delivery, probiotics risks, probiotics dogs, dysbiosis index, microbiome cats, probiotics skin, etc. Choosing sources of peer reviewed nature was of importance. It was important to select reviews, books, research articles and internet sources that were released recently, with a few exceptions, to prevent the use of outdated information due to the rapid development of this field. Even though the selection of larger studies would have been preferred, it was not possible to do so for many topics regarding dogs and cats, since studies commonly were performed on less than 30 subjects. Studies with subject numbers of less than 30 were mostly used to highlight newest theories. In these cases, the small study sizes or the need for more studies that should be done to prove its statements have usually been mentioned. Double blinded placebo studies were also preferred over other studies to assure a decreased occurrence of biased results, however, it was not possible to solely make use of those types of studies, since most studies were placebo studies but not double blinded. Sample or selection biases were commonly pointed out, and review papers, as well as research papers, were only selected if there was no strong conflict of interest.

Results

In total, 204 documents were included in this review to give a profound overview of this topic. The objective of this review was to summarize the risks, advantages, disadvantages, new findings and possibilities, regarding the use of pre-, pro-, syn- and postbiotics in the veterinary field, and therefore to provide a foundation of knowledge on this topic.

Surprisingly, it was found that overgeneralisation and extrapolation of results is commonly done in this research field, however, due to the differences in the composition of microbiome in different species, the extrapolation of scientific results is extremely complex and not recommended. Lack of literature has been identified regarding the function of specific bacterial strains or specific pre-, syn- or postbiotics on specific diseases in different species at different concentrations and durations of application. This is not an easy nor quick task, needing many resources. However, the numbers of publications and research published has been rising over the past years, enabling a quick advancement in this field. There is especially a need for much larger studies regarding acute and chronic gastrointestinal disease in dogs and cats. To decrease the occurrence of unwanted side effects and risks when using pro-, pre-, syn- and postbiotics, it is advisable to conduct studies regarding each product in the advised dose, species and patient population, as well as in case of overdosing. Explored generalized risks that have been identified to this point have been summarized in chapter "7. Summarized Risks concerning the Use of Pro-, Pre-, Syn and Postbiotics". Advantages and disadvantages of different administration routes regarding probiotics have been summarized in "Table 2 - Advantages and disadvantages regarding common routes of administration of probiotics in case of gastrointestinal diseases". More research must be done regarding the administration routes of postbiotics, to clarify, whether intravenous, intradermal or rectal delivery of postbiotics is safe or not, and whether those delivery systems are more advantageous than oral or topical delivery.

The results regarding the composition of gut microbiome of cats and dogs at different sections of the gut have been inconsistent. Many rather small studies have produced different results on the composition of the gut microbiome of dogs and cats, which is why large studies in this field should be done. It is important to know the composition of a healthy gut microbiome to be able to evaluate the functionality of pro-, pre-, syn and postbiotics. There was a discrepancy in results regarding studies concerning the applicability of DI in different age groups. Different reference values might be needed for a proper applicability of DI in different age groups. More studies need to be done in this field. It has to be clarified whether different probiotics are needed in different age groups.

It was noted that the drug interactions of commonly combined drugs, like adsorbents, and probiotics in GI disease should be studied in more detail. Probiotic modification using artificial enzymes to improve efficacy has shown promising results. More studies should be done regarding synbiotics, especially to find out whether certain synbiotics possess an additive or superadditive effect. Conflictive information was found regarding *Lactobacillus reuteri* being recommended by EFSA, but also being suggested to have autoimmune stimulating effect in mice.

Discussion

Today we know that the gut microbiome can influence many different organ systems and therefore our overall wellbeing. Influencing an individual's microbiome bears the risk of destroying the natural balance and symbiosis of that system. Pro-, pre-, syn- and postbiotics are potentially prudent ways of influencing and restoring the microbial balance of dogs and cats suffering from medical conditions like dysbiosis.

To put it simply, probiotics are beneficial microorganisms, and their health benefits range from regulating the immune system to production of beneficial proteins like enzymes, production of neurotransmitters, amino acids, anticarcinogenic compounds, antioxidants, SCFAs, vitamins, endocrine factors, etc., improvement of intestinal barrier, decreasing inflammation, supporting normal behaviour, improving skin health, elimination of pathogens, detoxification of toxins, positive stimulation of gut microbiota, neuronal cell maturation, repair and neurogenesis, pain modulation, and, last but not least, restoring the balance of microbiome [45, 46, 53, 56, 57, 89–99]. When choosing the correct probiotic for a patient, it is important to remember that there are interspecies differences in reactions to a specific probiotic preparate. The treatment success depends on disease, disease stage, bacterial strain, concentration, time of application and individual response [54, 69, 107, 109]. Especially when using probiotics together with antibiotics to prevent or reduce antibiotic associated diarrhoea, one shall remember that probiotics do not increase nor maintain the microbiomes' diversity [66, 89]. Combination preparations could be beneficial in treating individuals that do not respond to single strain probiotic treatment. However, increased concentration and number of different bacterial strains also means an increased risk of experiencing unwanted side effects [69, 109]. Enzyme modification of probiotics could potentially increase their colonization ability, effectivity, and protect probiotics from the environmental factors [108]. Treatment success does not depend on long term colonization in every patient, where the wanted effect is the elimination of pathogens, which in certain cases is possible after short term colonization [110]. Whether probiotics are indicated or not can be assessed using the dysbiosis index to prove a suspected dysbiosis or to compare the severity of dysbiosis between patients [34].

The use of probiotics is contraindicated in the case of severe pancreatitis, severe immunosuppression or severe illness, where benefits are not exceeding the risk [111, 112, 116]. AHDS patients are considered a vulnerable patient group, which is why veterinarians must be more cautious when choosing suitable probiotics or donors for FMT [84]. Risks of using probiotics can vary, depending on health status of the patient and probiotic strain used. Risks of using probiotics connected to improper production and licensing are contamination of products and existence of horizontally transmissible resistance genes against clinically relevant antibiotics [47, 191, 192]. Risks of using probiotics connected to user mistakes are choosing the incorrect way of administration or preparation, or contamination of objects endangering the health of other patients [47, 193]. Potentially, translocation of probiotics causing bacteriaemia or fungaemia could be a risk in vulnerable patient populations [113, 194]. Other potential risks would be the influence of probiotics on drugs or on the microbiome of humans living closely together with the animal patients, induction of autoimmune disease, long term colonization, ineffectiveness, allergic response, bowel ischemia, and the occurrence of side effects like abdominal pain, flatulence, diarrhoea, nausea and more [9, 47, 50, 55, 155, 195, 198, 201]. Certain probiotics show beneficial effects in the combination with adsorption drugs in certain health conditions, whereas others do not [122–124]. Probiotics marketed as drugs (regulated by FDA or EMA) undergo more strict safety, efficacy and quality checks before approval on the market. Probiotics marketed as food supplements are not strictly regulated. Probiotic food supplements underly the regulation of EFSA in Europe, simply regulating that any health claim for food marketed in Europe must be evidence based [32, 47, 48]. When choosing a probiotic, veterinarians must inform themselves about the potential health effects and side effects or risks for each individual patient. To minimize the risks, veterinarians are advised to choose probiotics marketed as drugs rather than food supplements, and to choose suitable probiotic preparations, based on the patient's needs, where benefits outweigh the risks. A prudent use of probiotics is advised, which is why they should not be administered if they are not indicated. The route of administration can also change the effectivity of a probiotic, which is why all advantages and disadvantages of each administration route listed in "Table 2 - Advantages and disadvantages regarding common routes of administration of probiotics in case of gastrointestinal diseases" shall be considered.

Prebiotics are the feed nourishing beneficial members of the targeted microbiome [155]. Health effects vary, based on the prebiotic chosen from the effect on the GI tract, like improved fecal scores, changing the microbial composition of feces in a positive way, providing energy to colonocytes, influencing gut motility (commonly used against constipation or diarrhoea) improving intestinal barrier function, reducing fecal pH and increasing pathogenic colonization resistance, to improving the immune system, modulation of inflammatory response, normalizing stress response, decreasing anxiety and depression related behaviour, increasing metabolite production like SCFAs and serotonin, decreasing corticosterone levels, regulating endocrine functions, altering brain functions, reduction of hyperlipidaemia, fungate as antioxidant, hepatoprotective and potentially slowing kidney disease progression [15, 52, 55, 93, 157, 159–163, 165–169]. Like in probiotics, administration in correct doses is important to see the desired effect [160]. One must remember that only certain prebiotics have certain effects in certain species. This must be considered when choosing a prebiotic. It is the duty of the veterinarian to choose a suitable prebiotic product, dose and treatment duration, fitted to the patients needs, to prevent side effects as much as possible. The prevention of mild side effects is not always possible, which is why prebiotics should only be administered if the benefits outweigh the risks. In general, to our current knowledge, side effects of using prebiotics are commonly less severe than side effects of probiotics, making it seem to be a more prudent treatment option.

Synbiotics are a health beneficial mixture of substrate and microorganisms, where the substrate is utilized by host microorganisms [177]. In general the health benefits of synbiotics, as well as risks, side effects and factors influencing its effectivity, are similar to those of probiotics and prebiotics [172, 174]. MOS and FOS together with *Enterococcus faecium* and *Lactobacillus acidophilus* supplementation of pregnant bitches can increase the amount of immunoglobulins in the colostrum, therefore improving immune functions of newborn puppies in addition to decreasing the occurrence of enteral diseases in the first weeks of life [15, 176]. Synbiotics can potentially have a potentiating or additive effect, allowing administration of the probiotic and substrate (prebiotic) in smaller doses. This dose reduction might potentially lead to decreased side effects. Therefore, it seems that synbiotics with potentiating or additive effect, should be the center of future research in the field of synbiotics. It has been noticed that less dog and cat specific research has been done regarding synbiotics compared to pro- and prebiotics.

Postbiotics are preparations beneficial to health containing bioactive compounds, produced by microorganisms, in combination with those inanimate microorganisms or by itself. An ideal inactivation method for the production of postbiotics not influencing the health benefit and function of postbiotics has not been found yet [177]. Postbiotics are not only used to exert health benefits, but also in the food industry, preventing spoilage and combating certain pathogens [179, 188]. Mechanisms of actions are diverse, ranging from positive stimulation of microbiota, improvement of intestinal barrier, immune system regulation, modulation of nervous system, and systemic metabolism via compounds like VFAs or neurotransmitters, proteins having antiapoptotic effects on enterocytes, increasing binding of MAMP to PRRs and organic acids, therefore depending on the chosen postbiotic product for a given species potentially exerting anti-inflammatory, antimicrobial, anticarcinogenic, hypocholesterolemic or anti-obesogenic effects. Postbiotics can also be used against constipation, to accelerate wound healing, to decrease risk of cardiovascular disease, improve absorption of beneficial elements and even for supporting human mental health [11, 177, 180, 181, 183, 184]. Postbiotics have some advantages over probiotics. Postbiotics seem to be safer, having fewer risks and side effects compared to probiotics, due to their inactivated nature, however, postbiotics are also underresearched, which is why less research has gone into safety, immunogenicity and toxicity research compared to probiotics. The theory has been established that postbiotic products produced from gram negative bacteria might lead to an endotoxic shock [177, 182, 204]. Postbiotics have a decreased risk of antibiotic resistance gene transmission or gut colonization, no risk of inducing bacteriaemia or fungaemia, and are more stable, having prolonged shelf life [178, 182, 184]. Many different delivery routes and methods for postbiotics have been studied, and theories have been made to improve effectivity and bioavailability [11, 177, 186, 189, 190]. However, delivery routes are insufficiently studied, especially regarding safety, advantages and disadvantages compared to each other. It seems that postbiotics, due to their better safety profile, are a superior choice compared to probiotics in the treatment of vulnerable patient populations, having similar health benefits, however, those health benefits are only exerted during the course of treatment, since postbiotics cannot colonize the host. Postbiotics production technological methods still show potential for improvement.

It was important to conduct this work to get a general understanding and overview about the most important aspects of pro-, pre-, syn- and postbiotics in the veterinary field, including newest research. The lack of literature has limited the ability of drawing conclusions in many cases, which has been pointed out. We know now that pro-, pre-, syn- and postbiotics can replace antibiotics to some extent in certain diseases, being an equally efficient or even better treatment option. In practice, it is important to use pro-, pre-, syn- and postbiotics marketed as medication rather than food supplement. These products should not be prescribed if not indicated or if contraindicated for a patient group. If veterinarians decide to prescribe pro-, pre-, syn or postbiotics, benefits must outweigh the risks and attention must be paid to choosing the best treatment plan for each individual patient.

One thing we certainly know about the effect of pro-, pre-, syn- and postbiotics is that this complex topic needs to be researched further, since it holds utmost potential for improving the health of dogs and cats as well as humans. One shall not glorify the known and theoretical positive effects of biotics and keep the possible negative effects in mind. The transfer of antibiotic resistance genes is one of the biggest risks, which must be prevented at all costs. The assumption that pro-, pre-, syn- and postbiotics can assist in the reduction of antibiotic resistance formation by using it alternatively to antibiotics is true, however, only if those products undergo strict safety evaluation regarding the possession of antibiotic resistance genes. Pro-, pre-, syn- and postbiotics can only replace and reduce the use of antibiotics in certain cases, however, if faced with such a case, veterinarians must prefer biotics over antibiotics. In the field of microbiota and their modulations and functions, many things are still unclear, however, what we believe to know until this point underlines the extreme importance of our microbiota, especially regarding the gut microbiome and health, leading to the conclusion that maybe a content life is more about gut feelings than we thought it was.

The time has come for the medical field to move away from using destructive medication, like antibiotics, towards a more sustainable, less destructive solution, which could potentially be biotics. As always, the progress in medical field means that more research must be done, and alternatives must be found to those medications that we once thought to be good treatment options. Today, we think we know about the great dangers of using antibiotics, which is why it seems natural to substitute them with biotics, however, even though the reduction and prudent use of antimicrobials is of the highest priority, one shall never forget the risks we think biotics might have and especially the dangers of the unknown risks. Biotics hold a great potential to be a reasonably good solution to many problems regarding the medical field, which is why this should allow veterinarians to look optimistically into the future.

Summary

The research and use of pro-, pre-, syn- and postbiotics has increased in the least years in the veterinary field. They have diverse therapeutic local and systemic health benefits, being commonly applied in the health sector and food production. Commonly, those products are used in case of gastrointestinal dysbiosis, which can be quantified by assessing the dysbiosis index. Healthy gut bacterial composition changes depending on the section of the gut as well as host species and may be influenced by other factors like age or living environment.

Oversimplified probiotics are beneficial microbiota, whereas prebiotics feed the beneficial microorganisms shifting the microbiota towards a healthier state. Synbiotics combine the desired functions of pro- and prebiotics into one product and postbiotics, being inanimate, give the benefit of bioactive substances produced by probiotics but with decreased risks and side effects. All these products have similar beneficial health effects ranging from modulation of different microbiomes, immune system, pain, intestinal barrier to influencing the production of beneficial proteins, vitamins, antioxidants, fatty acids, endocrine factors, amino acids, anticarcinogens, and improving overall health of many organs like skin, gut, CNS, kidneys or brain. The list of risks and side effects when using probiotics is long, ranging from the spread of antimicrobial resistance to drug interactions or sepsis, whereas prebiotics only cause gastrointestinal side effects like abdominal pain. Little is known about the risk and side effects of postbiotic and synbiotic use. Synbiotics consisting of a combination of pro- and prebiotics might have the risks of both, however, if both components are used in lower doses than they would be if used alone, there will potentially be decreased risks and side effects. Those beneficial effects and risks must be assessed for each species, patient group, biotic active ingredient and health condition to allow the drawing of accurate conclusions. To make sure the maximal health effect has been achieved, correct administration route, time, concentration, duration and product must be assured for each individual patient. Pro-, pre-, syn- and postbiotics marketed as drugs are to be preferred over nutritional supplements due to stricter regulations.

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Statements

UNIVERSITY OF VETERINARY MEDICINE, BUDAPEST

founded in 1787, EU—accredited since 1 995

INTERNAT NAL STUDY PROGRAMS

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Thesis progress report for veterinary students

Name of student: Schafzahl Lena Maria

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Department: Department and Clinic of Internal Medicine

Thesis title: Pro-, Pre-, Syn- and Postbiotics

Consultation — 1st semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2022	10	15	Overview of the topic	
2.	2022	12	14	Correction of structure, removing some sections and bring them in order	
3.	2023	02	27	Preparation of the first draft version. Adding transitions to each section	
4.	2023	03	11	Add further literature. Correction of the draft version	
5.	2023	05	06	Correction of the draft version	

Grade achieved at the end of the first semester: ...**5 (excellent)**...

Consultation — 2nd semester

	Timing			Topic / Remarks of the supervisor	Signature of the supervisor
	year	month	day		
1.	2023	11	13	Correction of some sections	
2.	2024	03	04	Correction of some sections	
3.	2024	04	22	Edited version from consultation 1	
4.	2024	08	28	Edited version from consultation 2	

1

5.	2024	09	03	Correction of the final version	

Grade achieved at the end of the second semester: **....5 (excellent).......**

The thesis meets the requirements of the Study and Examination Rules of the University and the Guide to Thesis Writing.

I accept the thesis and found suitable to defense,

signature of the supervisor

Signature of the student: ....

Signature of the secretary of the department: ....

Date of handing the thesis in...5th of October 2024....

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