Chapter

A Review of Application Strategies and Efficacy of Probiotics in Pet Food

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Abstract

In companion animal nutrition, probiotics (direct-fed microbials) are marketed as functional ingredients that add value to pet foods due to the impact they have on gastrointestinal and immune health of dogs and cats. The nature of the beneficial effect each probiotic strain exerts depends on its metabolic properties and perhaps most importantly, the arrival of a sufficient number of viable cells to the large bowel of the host. Pet food manufacturing processes are designed to improve food safety and prolong shelf-life, which is counterproductive to the survival of direct-fed microbials. Therefore, a prerequisite for the effective formulation of pet foods with probiotics is an understanding of the conditions each beneficial bacterial strain needs to survive. The aims of this chapter are: (1) To summarize the inherent characteristics of probiotic strains used in commercial pet foods, and (2) To review recently published literature on the applications of probiotics to pet foods and their associated challenges to viability.

Keywords: probiotics, viability, pet food, commercial processing, formulation

1. Introduction

Recent U.S. pet ownership statistics estimate that 70% of U.S. households own at least one pet, accounting for nearly 90.5 million homes [1]. Collectively in 2021, Americans invested $123.6 billion in their pets by purchasing pet foods, veterinary care, supplies, and non-medical pet care services, a clear indication that pets have become highly valued members of society. Over the past two centuries, the societal role of dogs has evolved from predominantly labor (i.e., guardianship, transportation, herding, and hunting), to a range of special operations (i.e., rescue, police, and military), therapeutic care (i.e., disease detection, assisting the sensory impaired, emotional support), and general companionship, deepening the reaches of the human-animal bond and a rising anthropomorphic view of companion animals [2]. Considering their increasing prominence in American lives, many pets today are viewed as members of the family and as such are being fed and nurtured with the goal of improving their wellness, longevity, and quality of life instead of solely production and performance.

A shift in feeding strategy for companion animals is perhaps most evident in the emerging market of functional foods and treats, which are foods considered to offer a positive health outcome that extends beyond providing essential nutrients [3]. Functional ingredients may include plant extracts, fibers with varying degrees of fermentability, joint supplements, non-essential nutrients, or microorganism and yeast-derived products, which can add value to pet foods by serving a preventative or therapeutic role [4]. Among these, direct-fed microbials (DFM) (commonly referred to as "probiotics") have been used for centuries to ferment staple human food products such as yogurt, cheese, wine, and bread and have only recently been embraced as health-promoting supplements [5]. The efficacy of probiotics in pets is a relatively new area of research, and innovations in the form of new application strategies, unique probiotic strain selection, and substantiating the potential health benefits is necessary to ensure the efficacy of products containing these beneficial microorganisms. The objectives of this chapter are to summarize the various sources and applications of probiotics to pet foods and their associated challenges to viability.

1.1 Historical highlights of probiotics

Probiotics have been present in food since early human civilization. It is presumed that our knowledge of bacteria in our food began when instances of spoilage and poisoning were encountered as early as 8000–10,000 years ago [6]. It wasn't until the mid-nineteenth century, however, that Louis Pasteur made the scientific community aware of acid-forming microorganisms and their role in the souring of milk and fermentation of wine [7]. This discovery prompted a succession of experiments aimed at identifying other microorganisms and uncovering their invisible but significant role in our food system. Nearly a half-century later in 1907, Nobel prize-winning scientist, Elie Metchnikoff, proposed that lactic acid bacteria in fermented milk were responsible for certain health benefits, particularly in delaying the onset of aging [8]. This came about from observing Bulgarian centenarians, who consumed the curdled milk ("yogurt") regularly. In one of his books, "The Prolongation of Life," Metchnikoff proposed that *Lactobacillus* might have a part in counteracting the putrefactive waste products of metabolism that contributed to disease and symptoms of aging, and thus the notion of consuming certain bacteria for promoting health was born. This intriguing theory inspired researchers over the next several decades to turn their focus to the health-promoting mechanisms behind the consumption of microorganisms.

Besides *Lactobacillus*, bacterial spore-formers were also discovered in the same time period. In 1876, Ferdinand Cohn recognized and named the bacterium *Bacillus subtilis* and shortly after Robert Koch described the life cycle of *Bacillus anthrax* [9]. *Bacillus coagulans* (originally named *Lactobacillus sporogenes*) was later described by the Iowa Agricultural Experiment station in curdled milk, and the organism was successfully isolated in 1932 [10, 11]. The unique sporulated condition of *Bacillus* microorganisms was credited with allowing them to survive in the environment as well as endure certain industrial processes such as the vacuum drying of evaporated milk. This provided early evidence that sporulated bacteria have the potential to survive an industrial food production process.

At the turn of the twenty-first century, the passing of the Dietary Supplement Health and Education Act of 1994 led to exponential growth in the sales of products marketed as probiotics for humans [12]. The global market of probiotic-fortified foods is expected to grow from $48 billion to $94 billion with a 7.9% compound annual growth rate between the years 2020–2027 [13]. This surge in interest in

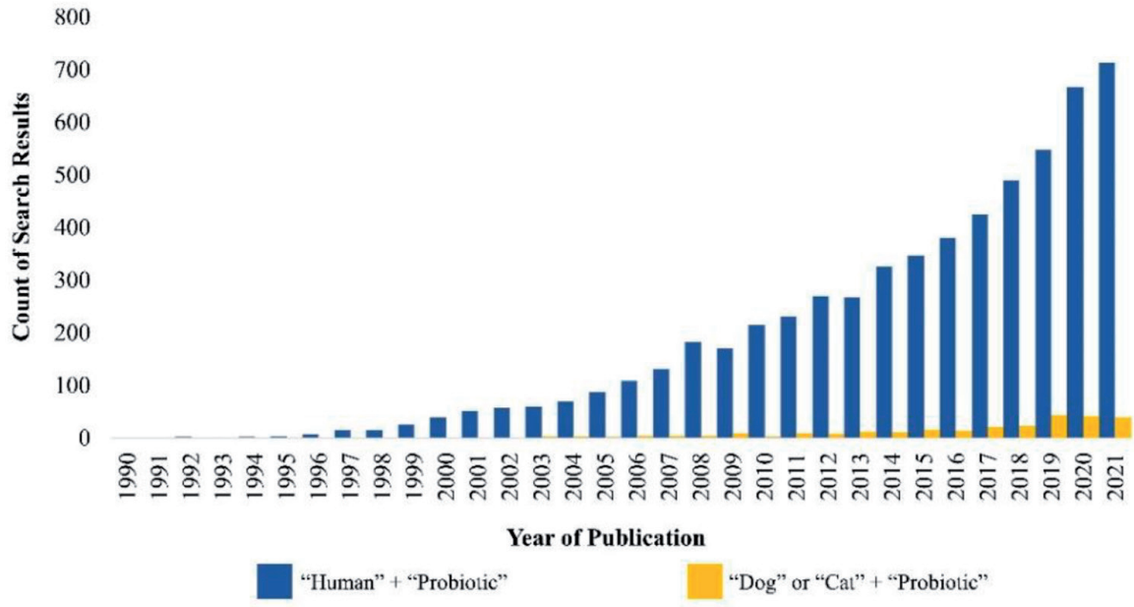

Figure 1.
Number of research publications returned by the PubMed database for search terms "human" or "dog" and "probiotic" between 1990 and 2021. Data presented for 2021 represents year-to-date publication counts available as of march 2021.

functional foods for humans inspired similar developments in the pet food industry, although far less research is available for the use of probiotics for dogs. For example, the PubMed open-access database returns >20,000 publications for "human" and "probiotic" between 1990 and 2021, whereas <250 publications are returned for "dog" and "probiotic" (**Figure 1**). Despite the small body of research available relative to that of humans, probiotics are still promoted for dogs in pet supplements, foods, and treats, and have garnered some support by veterinarians for use in clinical practice [14–16]. This rapidly growing market warrants a closer evaluation of novel probiotic strains, their viability through processing, as well as their ability to deliver similar health benefits as has been observed in humans.

1.2 Definitions and regulatory status

The term "probiotic" is derived from the Latin preposition "pro," which means "before, in front of" and the Greek word "biōtikós" meaning "of life" [17]. Over the last several decades, the definition of probiotics has been refined to incorporate various aspects of a probiotic's intended use and benefits (**Table 1**). The term "probiotic" is often used interchangeably with "direct-fed microbial" when referring to pet foods. However, the most current definition, and that which is used as the context for this chapter, is "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" [24].

The criteria for receiving approval as an acceptable probiotic strain in animal feeds involves a framework for verifying the ingredient's compositional analysis, toxicological potential, and evaluation of animal exposure with a focus on potential adverse health effects [25]. The Food and Drug Administration's Center for Veterinary Medicine along with the Association of American Feed Control Officials (AAFCO) first issued a list of bacterial and yeast organisms for use in animal feeds in 1989 that has been revised over the years to include new organisms based on available research

Term	Definition	Year proposed	Reference
Direct-fed microbials	Live microorganisms that, when provided in adequate amounts in the diet, can improve gut microbial balance; the anaerobic bacteria that are able to produce lactic acid and stimulate the growth of other organisms	1965	[18]
Probiotics	Tissue extracts which stimulated microbial growth	1972	[19]
Probiotics	Organisms and substances which contribute to intestinal microbial balance	1974	[20]
Probiotics	A live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance	1989	[21]
Direct-fed microbial products	Products that are purported to contain live (viable) microorganisms (bacteria and/or yeast)	1995	[22]
Probiotics	Live microorganisms which when administered in adequate amounts confer a health benefit on the host	2001	[23]
Probiotics	Live microorganisms that, when administered in adequate amounts, confer a health benefit on the host	2014	[24]

Table 1.
Published definitions of probiotics and direct-fed microbials.

mainly in swine and poultry. Today, there are 41 non-toxigenic bacteriological species that have been deemed safe for use in companion animals [26]. These microorganisms can be further classified based on physiological characteristics such as the structure of their cell wall, oxygen tolerance, and whether or not they are spore-forming (**Table 2**). Which traits these microorganisms share in common, and which make them unique, are important for the assessment of their potential use in specific food applications.

1.3 Strain selection criteria

In addition to meeting safety and regulatory guidelines, in general a probiotic candidate should have some degree of resistance to acid and bile salts, which are two principal chemical stressors that will be encountered in the gastrointestinal tract [27–29]. The canine digestive system has evolved with mechanisms to effectively inactivate pathogenic microorganisms and extract nutrients from a broad assortment of ingested materials. Comprehensive reviews of canine gastrointestinal tract physiology are available and serve as a useful reference for identifying the conditions that would exert the most stress on a potential probiotic microorganism (i.e., lowest gastric pH, and longest gastric and upper intestinal transit times [30]. For example, conditions mimicking gastric transit (1 h at pH 2.0), small intestinal transit (4 h at pH 6.80), and colonic transit (6–10 h at pH 5.6–6.9), with simultaneous exposure to other relevant biochemical components (i.e., digestive enzymes and bile salts) have been used in the development of *in vitro* canine gastrointestinal models [31, 32]. These conditions could also be applied for the screening of microorganisms intended for use in the diets of dogs.

In addition, any strains intended for application in commercially processed foods pet foods should exhibit high resiliency to process-related stresses, such as heat, prolonged shelf-life, and chemical composition of the food itself (i.e., matrix acidity, oxygen presence, water activity, or presence of microbial inhibitors [33]).

Taxonomic classification		**Physiological characteristics**		
Phyla and genus	Species	Gram +/−	Spore-forming	Oxygen tolerance
Firmicutes				
Bacillus	*amyloliquefaciens, coagulans, lentus, licheniformis, pumilus, subtilis*	+	yes	microaerophile and facultative anaerobe
Enterococcus	*cremoris, diacetylactis, faecium, intermedius, lactis, thermophilus*	+	no	facultative anaerobe
Lactobacillus	*acidophilus, animalis, brevis, bulgaricus, casei, cellobiosus, curvatus, delbrueckii, fermentum, helveticus, lactis, planatarum, reuteri*	+	no	microaerophile and facultative anaerobe
Leuconstoc	*mesenteroides*	+	no	facultative anaerobe
Pediococcus	*acidilactici, cervisiae, pentosaceus*	+	no	facultative anaerobe
Bacteroidetes				
Bacteriodes	*amylophilus, capillosus, ruminocola, suis*	−	no	obligate anaerobe
Actinobacteria				
Bifidobacterium	*adolescentis, animalis, bifidum, infantis, longum, thermophilum*	+	no	obligate anaerobe
Propionibacterium				
Propionibacterium	*freudenreichii, shermanii*	+	no	obligate anaerobe

Table 2.
Taxonomic classification and physiological characteristics of direct-fed microorganisms approved for use in dog and cat foods.

For pet owners, feeding probiotics as part of a food offers the convenience of daily administration to the pet while increasing perceived value of the product compared to conventional foods [34]. However, when probiotics are selected without consideration for these characteristics, the resilience of individual strains in commercial food applications is still open to question. In a study investigating the probiotic integrity of pet foods obtained from the marketplace, 53% of the sampled commercial products were found to be severely inadequate with respect to strain identity and colony-forming unit guarantees on the labels [35]. This highlights a need for validation of probiotic strains to ensure viability at the time of consumption by the animal.

When an organism can be guaranteed to be safely delivered to the gut, the metabolic activities of a bacteria are strain specific. Not all species of bacteria nor strains with a species favor the same metabolic pathways [36]. *Enterococcus*, *Lactobacillus* and *Bifidobacterium* are the most commonly used probiotics for animals, which produce lactic acid as a primary end product. Traditionally, lactic acid producing bacterial strains are Gram-positive anaerobes or facultative anaerobes, and non-spore-forming [37]. These strains also produce other substances such as hydrogen peroxide and bacteriocins which can influence the host microbiota [38]. The health benefits conferred to dogs have been summarized in several recent reviews, and include improvements to stool quality and mixed effects on apparent total tract digestibility, microbial fermentation end products, as well as immune system responses [39–41]. However, as vegetative cells intended for food applications, they are more susceptible to injury and death

from the stresses associated with cooking and gastrointestinal transit. The survival of these microorganisms may be enhanced by the use of cell protection technologies, such as microencapsulation [42]. This is a growing area of research that is critical for the future of functional foods incorporating non-sporulating probiotics.

1.4 Inherent probiotic survival mechanisms

Many bacterial species have the ability to cope with rapidly changing and sometimes hostile conditions to protect themselves [43]. One of the most effective adaptations is forming spores in response to a nutrient-deficient environment, low water activity, unfavorable temperatures, or extremes in pH [44]. From the sporulated form, microorganisms regress to a state of dormancy characterized by low metabolic and respiratory activity [36–46]. Gram-positive bacteria, such as *Clostridia* and *Bacillus* species, can form thick protective barriers within the bacterial cell. The main layers of the spore include the core, peptidoglycan-rich germ cell wall and cortex, proteinaceous coats, and exosporium (**Figure 2**). Environmental sensing mechanisms allow the spore to germinate when favorable growth conditions are detected, such as the activation of nutrient and non-nutrient receptors located on the outer spore membrane [47, 48]. A metabolically dormant microorganism can be advantageous with regard to survival in prepared foods due to an increased tolerance to processing conditions and shelf-life during storage [49]. In addition, spores exhibit higher thermo-tolerance compared to vegetative cells and persist under conditions of low pH and in the presence of external proteases. Once the bacteria reach a suitable environment, the spores will initiate the germination process and be restored to a metabolically active state [50].

Bacillus spp. are a sporulating genus that has been evaluated in the diets of calves, broilers, and piglets over the past decade [51–53]. Key findings of these works include validating spore survival through the ruminant digestive tract, improvements to growth performance, and increases in apparent total tract digestibility. There is only one documented reports of *B. coagulans* in the diets of companion animals, despite this strain being included on the approved microorganisms list [54]. Even so, products containing *B. coagulans* are available nationally in stores for consumers to purchase. For example, *B. coagulans* GBI-30, 6086 is a lactic-acid producing, Gram-positive,

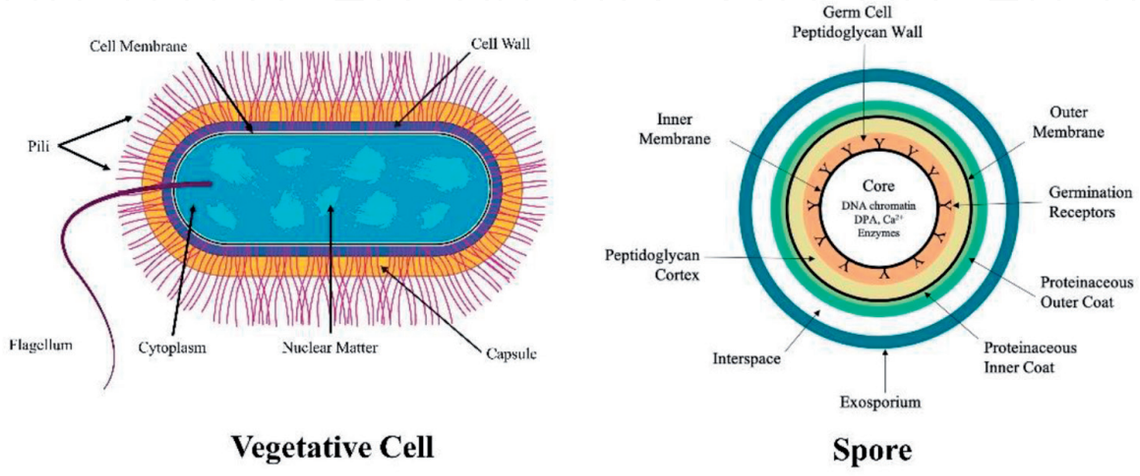

Figure 2.
Stylized illustration of vegetative cell and spore structural layers of probiotic bacteria.

spore-forming rod-shaped bacterium that is microaerophilic. This strain was developed by researchers at Ganeden Biotech (now a subsidiary of Kerry, Inc., Beloit, WI), under U.S. Patent No. 7,713,726. It was granted generally recognized as safe (GRAS) status in 2012 and became the first probiotic strain to receive a published monograph in the Food Chemical Codex (USP Monograph FCC 10). The isolate name GBI-30, 6086 signifies an optimal growth temperature of 30°C with an American Type Culture Collection designation number of PTA-6086. The spores of this strain are resistant to temperatures of up to 90°C, able to germinate in the body while resisting damage by gastric acids and bile salts as determined by both *in vitro* and *in vivo* evaluations [55, 56]. In addition, the safety of this strain with regard to toxigenic and genomic properties is well-established [57–60]. Thus, making this strain and others like it compelling candidates for incorporation into pet food products.

1.5 Enhancing probiotic survival potential

The careful selection of suitable probiotic strains and validation of survival through process conditions may still leave manufacturers unable to guarantee viability claims through the end of a product's shelf-life. Uncontrolled circumstances such as the handling and storage of the foods throughout distribution, retail merchandising, and in consumers' homes can contribute to adverse conditions and subsequent losses in viability over time. Thus, additional steps may be taken to lend further support to the survival of direct-fed microbials for the duration of a product's intended shelf-life. Microencapsulation is a technique that physically enrobes probiotic cells with an additional barrier against adverse external conditions. Spray-drying is one method of encapsulation for large-scale production. This process involves the dispersion of the cells into a liquid polymer solution, homogenization of the mixture, and evaporation of the solvent (commonly water) to form a matrix of dried microcapsules. Microencapsulation can also be accomplished by coextruding a bacterial culture emulsion with an outer gelling agent such as pectate, kappa-carrageenan, locust bean gum, gellan gum, or agar-agar [61]. The co-extruded material is then broken up into droplets that form capsules once dehydrated and cooled [62].

The encapsulation material should be approved for use in food products, nontoxic for the microorganism, and suitable for the food matrix. For example, the presence of singly charged ions such as phosphates, acetates, and citrates, may lead to the premature destruction of calcium alginate capsules through ionic competition. Furthermore, alginate is generally very sensitive to low pH values and heat, and loses its crosslinked structure and thus impair its functionality as a protectant very easily under these conditions [63]. Since many pet food matrices contain inorganic mineral salts and tend to be slightly acidic, this could lead to inferior performance of alginate encapsulations in certain matrices. It has been proposed that combining alginate with chitosan and poly-L-lysine to create multi-component microcapsules may enhance the stability of probiotics, while also reducing the destructive effects of substances that disrupt the structure of the encapsulation [64]. Egg whites, lecithin, whey protein, and carboxymethyl cellulose have also been proposed as compatible substances that may enhance alginate scaffolding for probiotic encapsulation in food applications [65–67].

Starches have also been shown to serve as successful encapsulating substrates [68, 69]. When considering starches as encapsulants, the starch amylose: amylopectin ratio has been reported to influence the effectiveness. For example, high-amylose corn starch granules led to greater resistance to heat and digestive enzymes compared native cereal starches [70]. Innovations in encapsulation technology include

multi-component substrates, such as co-encapsulating prebiotics, probiotics, and other bioactive components to pet foods and treats [71]. Once in encapsulated form, the probiotic can be introduced into the food production process as discussed in the following sections.

2. Application of probiotics in commercially processed pet foods

After a desired strain and preparation is selected, probiotics have several hurdles to overcome before they can confer a benefit to the animal (**Figure 3**). For probiotics incorporated into food products, one of the most intense stressors is thermal processing. The vast majority of pet foods are cooked to some degree or commercially sterilized to extend shelf-life and reduce the risk of pathogenic microorganisms or their toxins from enduring in the finished, ready-to-feed product. The basic premise of thermal processing is to reduce or destroy microbial activity, which can be counterproductive to the inclusion of direct-fed microorganisms. Microbial eradication is enforced by federal regulations such as the Food Safety Modernization Act [72], the FDA's zero-tolerance policy for pet foods contaminated with Salmonella [73], and in 21 CFR Part 113 for thermal processing of low-acid canned foods packaged in hermetically sealed containers. As such, process controls are developed accordingly within food safety plans to ensure the target pathogenic species are effectively inactivated.

There are several mechanisms that have been proposed for the action of heat on vegetative cells, including damaging the outer cellular membrane and peptidoglycan wall, loss of cytoplasmic membrane integrity, and the denaturation of cellular organelles, RNA, DNA, and enzymes [74]. Depending on the organism and intensity of heat treatment, the action of heat may lead to one or more of these events, and the ultimate goal is to render pathogenic cells injured beyond repair. Spore-forming microorganisms are reported to exhibit greater wet-heat resistance compared to vegetative cells [75]. The mechanisms controlling heat resistance of spores have not been fully elucidated. However, known heat resistance factors include the accumulation of divalent cations such as Ca^{2+} and the dehydrated state of the spore core. Dipicolinic acid (DPA) also serves an important role by chelating the cations, which helps maintain a low moisture environment and high mineral density in the center of the core [76]. Microorganisms which possess genes encoding for DPA during the sporulation process tend to show increased heat resistance.

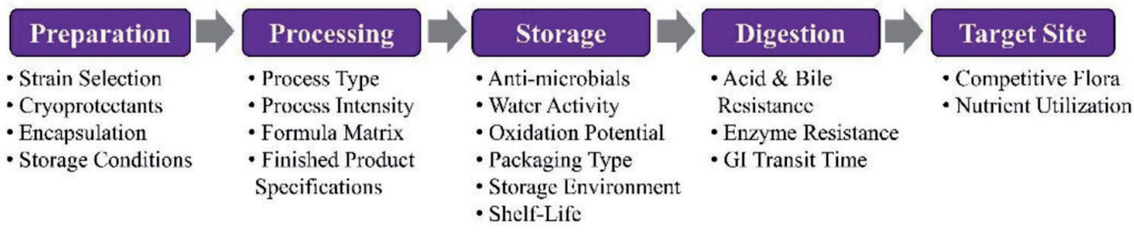

Figure 3.
Flowchart highlighting key considerations for the application of probiotic microorganisms into pet food products. Several variables are nested within each commercialization step, adding to the complexity of factors that influence probiotic survival and efficacy potential.

2.1 Extrusion cooking

Extrusion cooking is the most widely used technology in the commercial production of pet foods today, representing the largest category of pet food in terms of market share. Extruded pet foods are nutrient-dense, highly palatable, shelf-stable products which are produced in a continuous high throughput system. Extrusion is a high-temperature, short-time, high-shear process in which pre-conditioned raw materials are conveyed by a rotating screw through a barrel and forced through a small opening (a die) that results in vapor flash-off and expansion of the exiting product. Extruders are available as single- or double-screw configurations, and there are a variety of screw elements that can be combined to create a customizable screw profile in a given system. Throughout the conveying process, thermal energy (usually in the form of steam injected at the pre-conditioning step) and mechanical energy (generated by shear forces from the rotating screws contacting the material) cause the temperature inside the barrel to rise, which allows for the gelatinization of starch, cooking of the material, and serves as a key step in the destruction of spoilage and pathogenic microorganisms that may have been carried in with the raw materials [77]. It has been demonstrated that the ratio of specific thermal energy to specific mechanical energy applied to the food mass during extrusion influences the structural characteristics of pet food kibble [78, 79]. While thermal destruction of pathogens and surrogate microorganisms has been extensively studied, less is known about the effects of specific mechanical energy on microbes. It is possible that extrusion may influence microbial survival differently than other food processes.

Thermophilic organisms, such as *Bacillus* spp., are proposed as better suited for process validation studies since they would exhibit more thermotolerance and therefore be a reliable indicator for developing processes to achieve sterilization [80]. An experiment was carried out wherein different settings for the extruder barrel exit temperature, mash feed moisture content, and barrel retention time were combined to create 15 process combinations in order to compare the suitability of *Bacillus thermophilus* as a surrogate organism for Salmonella during single screw extrusion of animal feed. The results of the study indicated no survival of Salmonella when the feed was extruded at 24.5% moisture content, 3 s retention time, and 82°C or higher die temperature. On the other hand, *Bacillus stearothermophilus*, a spore-former, was detectable at all processing conditions in the range of moisture from 24.5–34.5%, retention times of 3–11 s, and extruder die temperatures of 77–100°C). This study demonstrates the potential for sporulated microorganisms to survive extrusion, while also allowing for destruction of pathogenic cells. Additional studies evaluating microorganisms of sporulating and non-sporulating taxa are summarized in **Table 3**.

2.2 Retort cooking

Retort cooking of most pet foods involves the heating of low-acid (pH >4.6) high-moisture (>0.85 a_w) products in hermetically sealed containers to a minimum of 121°C by injecting steam into a pressure vessel, with the goal of eliminating all vegetative pathogens and spoilage microorganisms as well as spores of *Clostridium botulinum,* rendering the final product commercially sterile. The retort is brought up to temperature during a 3–10 minute come-up period and held at 121°C for at least 2 min, depending on the food composition and packaging type. The hold time must be long

Microorganism	Food material	Process conditions	Viable cell loss	Reference
Bacillus cereus	commercial pet food diet	NR	1.08 log	[81]
Bacillus stearothermophilus	animal feed mash	Extruder: single screw RT: 3–11 s IBM: 24.5–34.5% Die Temp.: 110°C	1 log	[80]
Clostridium sporogenes	mechanically deboned turkey and white corn flour	Extruder: twin screw RT: 3.4 min IBM: 32% Die Temp.: 93.3°C	2 log	[82]
Clostridium sporogenes	mechanically deboned turkey and white corn flour	Extruder: twin screw RT: 3.4 min IBM: 32% Die Temp.: 115.6°C	4–5 log	[82]
Enterococcus faecium	dry dog food ration (corn flour, poultry by-product meal, corn gluten meal, rice meal, vitamins, and minerals)	Extruder: single screw RT: 71 s – 105 s IBM: 21.68% Die Temp.: 120–140°C	6 log	[83]
E. faecium	balanced carbohydrate-protein meal (chicken meal, rice, potassium chloride, ptoassium sorbate)	Extruder: single screw RT: NR IBM: 28.1% Die Temp.: 81.1°C	5 log	[84]
E. faecium	balanced carbohydrate-protein meal (chicken meal, rice, potassium chloride, ptoassium sorbate)	Extruder: single screw RT: 48–62.5 s IBM: 27.4–27.8% Temp 55.5–75°C	1.4–5.81 log	[85]
E. faecium	balanced carbohydrate-protein meal (chicken meal, rice, potassium chloride, ptoassium sorbate)	Extruder: single screw RT: 48–62.5 s IBM: 26.8–27.3% Temp: 80.3–100.5°C	2.3 to >5.87 log	[85]
Salmonella	oat flour	Extruder: single screw RT: 18–46 s IBM: 14–26% Die Temp.: 83–103°C	5 log	[86]
Salmonella typhiumurium	animal feed mash	Extruder: single screw RT: 7 s IBM: 28.5% Die Temp.: 83–103°C	8 log	[20]
Salmonella enterica	balanced carbohydrate-protein meal (chicken meal, rice, potassium chloride, ptoassium sorbate)	Extruder: single screw RT 48–62.5 s IBM 27.3–27.6% Temp 55.5–68°C	4–6.5 log	[85]
S. enterica	balanced carbohydrate-protein meal (chicken meal, rice, potassium chloride, ptoassium sorbate)	Extruder: single screw RT: 48–62.5 s IBM: 25.6–26.8% Die Temp.: 77–101°C	>6.86 log	[85]

Microorganism	Food material	Process conditions	Viable cell loss	Reference
Streptococcus thermophilus	whey protein isolate	Extruder: twin screw RT: 25 s IBM: 4–5% Die Temp.: 143°C	4.2 log	[87]
Streptococcus thermophilus	whey protein isolate	Extruder: twin screw RT: 35–40 s IBM: 4–5% Die Temp.: 133°C	4.9 log	[87]
B. cereus	commercial pet food diet	Coated on exterior of kibble after expansion-extrusion and drying; stored in commercial packaging at room temperature in a dry well-ventilated warehouse for 12 months	0.1–0.4 log	[81]

NR = not reported; RT = extruder residence time; IBM = in-barrel moisture content; and Die Temp. = maximum temperature measured at the die.

Table 3.
Summary of log reduction in microorganism viability under various extrusion processing conditions.

enough to achieve a 12-$\log_{10}$ reduction in the number of spores of this pathogen if it should happen to be present within the raw material matrix. The temperature inside the vessel is then cooled with injection of cold water until the pressure is reduced and the vessel can be safely opened. Steel or aluminum cans are the most common package used in pet food retort systems, however recent advancements in packaging technology have expanded into pouches, cups, and tubs made from a variety of starting materials (commonly polyethylene and its derivatives). Federal regulations have been established for manufacturers in 21 CFR Part 113 to mitigate the public health risk of botulism associated with past market recalls of foods processed using this method. Due to the intentionally severe conditions exerted on microorganisms present inside the food container during cooking, even the hardiest live microbials are not well-suited for retort applications. Opportunities for functionality do exist for the inclusion of pre-biotics and post-biotic ingredients, however.

2.3 Freeze-drying

Freeze-dried pet foods and treats have gained popularity in the past decade as the market demand for products with high bioavailability and less thermal processing has increased. Freeze-drying is considered a relatively gentle dehydration process due to the absence of heat and the slow rate of water removal using lyophilization, the phase transition of ice directly into vapor without passing through the liquid phase. This is achieved by first freezing the food preparation, applying a high vacuum to a sealed vessel to reduce the pressure, allowing the ice to sublimate from the product and collect on a condensing unit for removal from the system. Opposite to most pet food manufacturing technologies that aim to destroy viable microbes, freeze-drying is widely used as a preferred method for preservation of bacterial cultures. Cellular water can be removed to reversibly inactivate microorganisms to facilitate

their storage. This makes freeze-dried pet food applications a good candidate for the application of direct-fed microbials.

Since the product is dehydrated without the use of heat, freeze-drying is not considered a cooking process. However, the ingredients used in freeze-dried pet food formulations can be pre-cooked or raw depending on the product's design. Many probiotic preparations that are used in pet foods are initially preserved by freeze-drying with the aid of a protective medium that helps prevent damage of cellular membranes and proteins as water is removed from the core of the cells. This prolongs the shelf-life of the probiotic cultures and allows for their downstream incorporation into many shelf-stable food applications. When blended into a food matrix, previously dehydrated probiotics have an advantage over vegetative bacteria when subjected to freeze-drying since their cellular water content is already low. The bulk of the water removal from the food matrix is from water surrounding the cells, rather than water within the bacterial core. For vegetative cells, the primary mechanism of cell injury is disruption of the cell membrane structure during intracellular ice formation [88]. A lower survival rate of Gram-negative bacteria relative to Gram-positive strains has also been reported, and this is thought to be due to the thinner peptidoglycan layer and the presence of lipopolysaccharides within the cell wall of Gram-negative species [89]. However, the damaging effects of freeze-drying on live cells is not significant enough to mitigate the risk of food-borne pathogens. Therefore, many freeze-dried pet foods and treats, particularly those containing raw ingredients, may undergo additional processing such as irradiation or high-pressure processing independent of the freeze-drying cycle for food safety. Adjunct processing for pathogen control can present additional challenges to probiotic viability but is not covered within the scope of this chapter.

2.4 Baking

Baking encompasses a wide range of products and processes including bread, snacks, cakes, tortillas, pastries, pies, pet treats, pet foods, and more. Baked products are traditionally composed of cereal flours, but meat-based formulations are also common in the pet food industry. Baking for food preservation is regarded as one of the oldest cooking methods documented in human civilization and was in fact the first process used to commercialize the first dog biscuits in 1860.

At a basic level, the baking process consists of combining ingredients to form a dough, forming the product into the desired shape, cooking the raw dough using dry heat in an oven, and cooling the baked product at ambient temperatures before packaging. The types of ovens in industrial-scale settings are gas-fired, oil-fired, and electric, fitted with a single or multi-pass conveyance system that transports the dough on a wire mesh belt. The transport of heat to the surface of the dough occurs through conduction, convection, and radiation, allowing for the evaporation of water from the surface of the product followed by a formation of crust layer. Standard baking times for bakery products range between 2 and 30 minutes, dependent on the oven design, starting moisture content, dough density, temperature, and desired finished product characteristics (color, size, appearance, and texture). Baking is generally a lower throughput process relative to extrusion and canning-retort, however it offers advantages such as the development of desirable colors and flavors that result from Maillard reaction product formation.

The primary stressor live microorganisms encounter during baking is heat. The duration and high temperature of typical baking are usually sufficient to inactivate *E.*

coli or salmonella pathogens, however formal scientific validation of the diversity of commercial baking processes for the inactivation of pathogens or direct-fed microbials has not been thoroughly studied. Across available data, a ≥ 5 log CFU/g reduction in *Salmonella enterica* serovars was demonstrated by 17 min of baking, and a 6.1 log CFU/g reduction by 21 min of baking at 190.6°C in an electric oven in muffins [90]. Higher temperatures were needed to achieve >6 log CFU/g in hamburger buns baked in a conventional oven for 13 min at 218.3°C [91]. This demonstrates variability in microorganism survival that may be dependent on the properties of the dough matrix and type of oven in addition to the microorganism's inherent thermal-resistance properties. To our knowledge, no such studies have been conducted on the inactivation of pathogens in baked pet foods and treats. However, from learnings gleaned from other thermal process technologies such as extrusion, it is reasonable to expect that dormant and microencapsulated probiotic preparations and those with higher thermal resistance attributes would be better suited for the baking environment.

To circumvent thermal stress, entrapment of probiotic cells in edible films or coatings on the surface of baked products is a promising approach. Using film-forming solutions based on sodium alginate, whey protein concentrates to suspend probiotics in a gel that can be applied as a topical coating to baked goods. Functional starch-based coatings have been successfully implemented using microencapsulated *Lactobacillus acidophilus* achieved 63% survival when the coating was comprised of 94% water, 5% starch, ad 1% microencapsulated probiotic applied to the loaf and baking at 180°C for 16 minutes [92]. The survival of *Lactobacillus plantarum* (strain CIDCA 83114) was reported to have improved retention during baking at 30°C for 40 minutes when applied as a corn-starch-based film (4 log reduction in viable cell counts) compared to a sodium alginate film (6-log reduction in viable cell counts) [93]. This suggests starch-based suspensions may be more effective than other films at protecting probiotic viability under baking conditions. However, validation of probiotic viability should be included as part of the commercialization process because of the wide range of direct microbial preparations, raw materials used in pet food and treat formulations, application strategies, and processing conditions.

3. Conclusion

Probiotics are one of a growing number of functional ingredients that contribute to the advancement of companion animal health and wellness, but delivering viable microorganisms in commercially processed food products presents many challenges to ensure the viability and efficacy they are marketed for. Pet food manufacturing processes are designed to improve food safety and prolong shelf-life, which is counterproductive to the survival of direct-fed microbials. Thus, making the selection of appropriate strains critical for their intended application. Among the most important characteristics to consider when selecting of probiotic strains used in commercial pet food applications are the strain physiological attributes (especially thermal resistance, oxygen tolerance, acid and bile resistance), stabilization method (such as sporulation, freeze-drying, or encapsulation), processing conditions (including time, temperature, pressure, moisture, water activity, pH), application method, and packaging and storage conditions. Verification of probiotic viability should be performed when working with novel probiotic strains, and when any modifications are made to processing conditions, product formulations, or packaging designs.

Conflict of interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

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