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Review

Current Applications and Future Trends of Dehydrated Lactic Acid Bacteria for Incorporation in Animal Feed Products

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Abstract: Several lactic acid bacteria (LAB) species have been recognized as probiotics and are of considerable interest due to their potential ability to confer health benefits upon consumption. In the animal feed sector, probiotics offer an alternative to the use of antibiotic growth promoters. The preservation and incorporation of probiotics into dry products requires carefully meeting several criteria and overcoming technological challenges to maintain their functionality. Drying is a crucial step in the process, but the probiotic properties of the resulting powder and the final cell viability in the food product are significantly influenced by the type of protective compounds and drying techniques employed. In light of the growing demand for functional animal products, this review focuses on the damages incurred during microorganism dehydration processes for food incorporation, and explores strategies to minimize such damages. It provides an overview of the effects of probiotic products in the animal feed industry, including their incorporation in low-moisture food matrices and key considerations for success. Additionally, it highlights postbiotics as an attractive alternative for live probiotic cells with many technological advantages.

Keywords: lactic acid bacteria; dehydration; probiotics; animal feed; postbiotics

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1. Introduction

Lactic acid bacteria (LAB) have been the subject of extensive research for many decades, making them one of the most studied microorganisms. LAB play a crucial role in various biological processes and ecosystems, particularly in the realm of fermented foods. The science of fermentations has been thoroughly explored for over a century, and the utilization of LAB to transform raw materials into safe and palatable food products has been practiced for thousands of years as a means of preservation [1]. The primary focus of studying food-associated LAB has traditionally been on their fermentation capabilities and observable characteristics. However, recent advancements in the genome sequencing of LAB strains have significantly contributed to our understanding of their phenotypic properties and expanded our knowledge in this area [2–4].

In addition to their role in enhancing food quality and safety, LAB have garnered significant attention for their potential to impart functional properties to specific foods as probiotic supplements. Numerous LAB species and strains have been identified as probiotics, defined as "live microorganisms that provide a health benefit to the host when administered in sufficient quantities" [5]. The viability of probiotic LAB is a crucial aspect emphasized in the definition of probiotics. Therefore, ensuring the preservation of probiotic cultures is essential during the manufacturing process of probiotic products. Dry powder formulations offer numerous advantages over liquid cultures, such as room temperature storage, extended shelf life, ease of transportation, and suitability for incorporation into

complex matrices or supplements. When considering the factors that influence probiotic functionality and the selection of a food matrix as a delivery system for probiotics, several critical points or challenges need to be addressed (Figure 1). These include: (1) strain selection (choosing appropriate LAB strains with documented probiotic properties); (2) strain production (developing efficient methods for the production and cultivation of probiotic LAB in large-scale fermentation processes); (3) inoculation into the food matrix (determining the optimal dosage and technique for incorporating LAB into the chosen food matrix to ensure uniform distribution); (4) survival during processing (implementing processing techniques that minimize the detrimental effects on probiotic viability, such as exposure to heat, pH changes, or mechanical stress); (5) viability during storage (implementing proper packaging and storage conditions to maintain probiotic viability and prolong shelf life); (6) functionality in the gastrointestinal tract (evaluating the ability of probiotic LAB to survive passage through the harsh conditions of the GIT and exert their beneficial effects) [6,7].

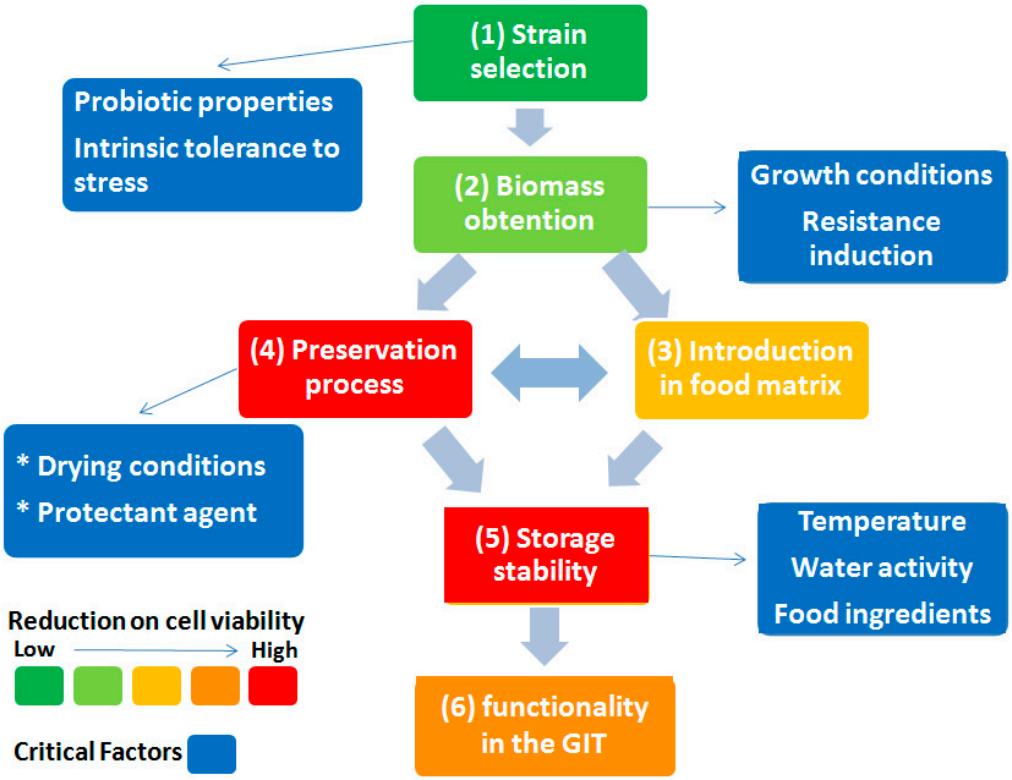

Figure 1. Critical points during the production of dried probiotics LAB. Different colors represents susceptibility to damage.

Extensive research and literature reviews have thoroughly examined the individual aspects mentioned [8–10]. However, in recent times, there has been a remarkable transition towards interdisciplinary collaboration, integrating fields like microbiology, biotechnology, biochemistry, and physics. This interdisciplinary integration has brought about notable progress in improving the survival of dried LAB and promoting collaboration between food processors and probiotic suppliers. As a result of these interactions, customized LAB cultures, designed for specific applications, have been developed, further advancing the field.

LAB have also garnered significant attention in animal applications, offering health benefits and improved production outcomes [11–13], thus emerging as a viable alternative to antibiotic growth promoters in animal husbandry. The use of antibiotics in many countries has become restricted or prohibited due to concerns over the effects of residual levels in food, and the development of microbial resistance, which poses risks to both

human and animal health [14,15]. Intensive farming practices expose animals to various factors that can disrupt their gut microbiota, including medication, stress, and dietary changes. LAB probiotics are extensively used in diverse animal species such as poultry, cattle, swine, ruminants, fish, and pets, among others [16–18]. These probiotics play a crucial role in enhancing animal health, promoting growth, boosting disease resistance, improving reproductive performance, and modulating the immune system.

The projected global revenue for the market of probiotics in animal feed is estimated to reach USD 7.3 billion by 2026 [19]. Factors driving this growth include continuous innovation efforts within the animal feed sector, increasing consumer awareness of the benefits associated with probiotics and prebiotics, rising concerns about zoonotic diseases, and the growing disposable income of major companies. However, the transition from laboratory-scale to industrial-scale production is a complex process. To effectively develop commercial and functional ingredients, it is crucial to design technologies that ensure efficiency and robustness. Probiotics, when incorporated into food and feed products, encounter various stress factors such as heat, cold, acidity, oxidation, high hydrostatic pressure, starvation, and osmotic stress during processing, transportation, and storage [20]. There are three major ways to ensure that probiotic cells retain their efficacy upon consumption: the cells can possess intrinsic resistance mechanisms [20], they can undergo adaptations during the production process [21], or they need to receive adequate protection through appropriate dehydration processes and storage conditions [22].

These strategies are essential for maintaining the viability and functionality of probiotics, enabling them to withstand the challenges encountered throughout the production and distribution chain (see Figure 1).

Enhancing the understanding and awareness of both policy makers and consumer groups regarding the advantages of probiotic products is crucial in order to foster the growth of the probiotics feed industry. Innovation plays a key role in developing functional probiotics for animal feed applications. Among the preservation methods, the drying of LAB emerges as the most effective approach to maintain their viability and activity when incorporated into food and feed matrices. However, optimizing the dehydration process remains a significant challenge that needs to be overcome to achieve desirable outcomes. In light of this, the objective of this review is to present the latest research findings pertaining to the incorporation of probiotic bacteria in animal feed. We aim to highlight the key beneficial effects of probiotics, explore the various dehydration conditions, including methods and protective compounds, and discuss their administration and inclusion in food matrices. The ultimate goal is to elucidate the impact of probiotics on the intestinal microbiota and their potential to enhance health and performance in the animal industry. Furthermore, this review encompasses the emerging paradigms concerning the use of inactivated lactic acid bacteria and their metabolites, expanding the scope of research beyond live probiotic cells. By considering these advancements, we hope to contribute to the current understanding of probiotic applications in animal nutrition and provide insights for future research and development endeavors in this field.

2. Lactic Acid Bacteria

Lactic acid bacteria (LAB) are a group of microorganisms that exhibit specific characteristics. They are non-sporulating and non-motile, and they possess acid tolerance while being non-respiring but aerotolerant. Gram-positive cocci or rods are common morphologies observed in LAB. One of their distinctive features is the production of lactic acid as the primary end product during the fermentation of carbohydrates, which distinguishes them from other microbial groups [23]. As probiotics, LAB are capable of enhancing host health under various conditions. Consequently, they hold significant potential as natural microecological preparations in intensive animal husbandry settings. Moreover, many LAB species have attained a status of generally recognized as safe, further supporting their suitability for use [5,24].

LAB have been extensively studied for their beneficial effects on animals, which are mediated through various mechanisms. Notable among these is the production of bacteriocins, which are antimicrobial peptides that inhibit the growth of other microorganisms [25]. LAB also exhibit an anti-mycotoxigenic effects, protecting animals from the harmful mycotoxins produced by fungi [26]. Another important attribute of LAB is their ability to produce acids, creating an acidic environment that inhibits the growth of pathogenic bacteria [27]. LAB are also capable of producing exopolysaccharides, which are complex carbohydrates that contribute to the formation of biofilms, provide protection against environmental stresses and play a role in enhancing the immune response and promoting gut health in animals [28–30]. Their multifaceted effects make them valuable candidates for the development of functional animal feed and probiotic supplements. In the quest for alternatives to antibiotics, the inclusion of probiotic LAB as feed additives has emerged as a promising strategy with positive impacts on animal performance and welfare, leading to a robust defense mechanism against the colonization of pathogens and stimulating the immune system, thus contributing to overall animal health and productivity [30].

It is essential for LAB to survive the passage through the physical and chemical barriers of the gastrointestinal tract, so they may successfully compete with various resident species and exert their beneficial effects. As part of the transient gut microbial community, LAB originate from the external environment, with food being a major source. This interaction between LAB and the established members of the gut microbiome occurs continuously, influencing the overall composition and function of the gut ecosystem [31].

Certain specific LAB have demonstrated potential as probiotics for various purposes, as summarized in Table 1. The sources of LAB with potential applications as animal probiotics are diverse, primarily encompassing the intestinal system [14,32]. However, unconventional sources such as fruit and vegetable juices [33], kefir grains [34], and fermented cereal grains [35] have also been explored. One of the key advantages of LAB species derived from normal intestinal microflora is their inherent resistance to low pH and bile, genetic stability, and ability to colonize the intestinal mucosa, which are essential features for ensuring probiotic viability and functionality [36]. Moreover, it should be noted that the selection of probiotics for animal use may differ from those for human use. While probiotics for human consumption are commonly derived from dairy products, the sources of probiotics for animals are often the animal's own digestive tracts [37]. The health-promoting effects of probiotics encompass their immunoregulatory properties, their ability to maintain a favorable balance of intestinal microbiota, and their interactions [38]. However, these physiological characteristics can vary among different species [39,40], thereby influencing the effects of probiotic administration. Nevertheless, several researchers have successfully isolated probiotics from various sources and utilized them in animal feed [41–43].

These probiotics have exhibited multiple positive effects in poultry, swine, and ruminants, and include direct-fed microbes such as genera belonging to *Lactobacillus*, *Pediococcus*, and *Enterococcus* species (Table 1). The incorporation of probiotics into animal feed has been assayed in different delivery media, including powders or suspensions, and at varying dosages. Strong evidence supports the beneficial effects of probiotic supplementation in animal diets, particularly concerning gastrointestinal health. Probiotics have been found to enhance the metabolic utilization of dietary nutrients and improve feed efficiency, which are critical factors for optimizing livestock and poultry productivity.

In recent years, there has been a notable rise in the popularity of probiotic products targeted towards pets, particularly dogs and cats. Pet owners are increasingly drawn to these products as scientific research supports their efficacy [18]. Consequently, the supplementation of animal diets with both defined and undefined probiotics [5] has emerged as a crucial approach to support and enhance the gastrointestinal tract health of companion animals, promoting their overall well-being. By incorporating probiotics into the diet of pets, owners can actively contribute to the maintenance and optimization of their pets' gastrointestinal health. This approach acknowledges the significance of probiotics in supporting the digestive system and overall health of companion animals. As the demand

for pet probiotics continues to grow, further research is necessary to better understand the specific benefits and optimal usage of probiotic supplements for different types of pets.

The use of LAB as functional starter cultures or additives in animal feed requires the enhancement of their performance and the optimization of microbial cell density. In recent years, various strategies have been developed to achieve high-density cultures, outperforming conventional methods and improving resistance to preservation techniques [44]. This approach allows for an increased production of target bacteria at a reduced cost, enhancing species-specific productivity. High-density cultures create a hypertonic, yet less inhibitory, environment through continuous alkaline supplementation to regulate pH and enhance microbial density [45]. Studies have demonstrated that high-density cultures exhibit improved stability and reproducibility across multiple growth cycles, as well as enhanced resistance to freeze-drying among LAB strains [46,47].

The preservation of LAB probiotic properties after the dehydration process has been widely demonstrated in in vitro models [34,48–50]. However, the evaluation of the effects of dried LAB on the health of pets or livestock under intensive farming remains limited [51–53]. This scarcity of research can be attributed to the existing challenges associated with obtaining and incorporating these bacteria into animal feed, primarily due to technological obstacles. Subsequent sections of this paper will delve into a comprehensive examination of the pertinent issues surrounding this topic.

Table 1. LAB as potential animal probiotics for different uses.

Used Group	LAB Species	Main Effects	Addition Method	Ref.
Poultry				
Broiler chicks	*Lpb. plantarum*	Improved growth performance, intestinal morphology and immune response in broiler chickens under heat stress.	Sprayed on the feed (postbiotic)	[54]
1-day-old chickens	*Lgb. salivarius*	Improved growth performance (weight and longer shank length), increased relative weights of the immune organs and decreased concentrations of odor-causing compounds.	In diet (10^7, 10^8, and 10^9 CFU/kg of feed)	[55]
Broiler chicks	*P. acidilactici*, *Lmb. reuteri*, *Enterococcus faecium* and *Lb. acidophilus*	Modulates the activation of the innate immune response and inhibits the activation of standard *C. perfringens* immune responses.	Water (postbiotic)	[56]
Broiler chicks	*Lgb. salivarius*	Improved body weight of broiler under low ambient temperature and a trend in reducing the mortality rate.	Mixed in feed	[57]
Broiler	*Lb. acidophilus*, *B. subtilis*, *S. cerevisiae*, *A. oryzae*	Improved overall weight gain and CP retention.	Mix of probiotics added in basal diet (0–30%)	[58]
Swine				
Weaned piglets	*Lpb. plantarum*	Increases diversity and richness in the microbial community, promoting intestinal development.	Liquid probiotic via feed (1.25×10^9 CFU/kg of diet).	[59]
Weaned piglets	*Lpb. plantarum* and *P. acidilactici*	Reduced impact of enterotoxigenic *E. coli*, being associated with decreased *E. coli* detection; modulation of the cytokine response, reduction in intestinal damage and clinical signs, and improved growth performance.	Microencapsulated probiotics suspended in sterile peptone water, given orally via sterile syringe (10^9 CFU/mL)	[60]

Table 1. *Cont.*

Used Group	LAB Species	Main Effects	Addition Method	Ref.
Weaned piglets	*Lb. Johnsonii* *Lb. mucosae*	Higher ($p < 0.05$) body weight gain, feed intake, and gain/feed ratio than weaned piglets fed basal diet. Probiotic feeding also increased the numbers of lactobacilli and decreased the numbers of *E. coli* in the feces of weaned piglets.	Probiotic freeze-dried and mixed into the basal diet	[61]
Pig farm	*Lpb. plantarum*	Improved meat quality and physicochemical characteristics.	Drinking water (2.5×10^7 CFU/mL)	[62]
Pigs	*Lb. acidophilus*, *B. subtilis*, *S. cerevisiae*, *A. Oryzae*	Improved overall performance. The overall gain and apparent total tract digestibility of CP were greater in pigs fed substrate fermentation (SF) diets than in pigs fed a liquid diet (LF).	Basal diets supplemented with 0.30% LF and 0.30% SF multi-microbe probiotic products	[63]
Ruminants				
Post-weaning lambs	*Lpb. plantarum*	Promotes the development of rumen papillae, enhances the immune status and gastrointestinal health.	In diet (0.9% *v*/*w*, CFS, Postbiotic)	[64]
Neonatal calves	*Lpb. plantarum*	Improves gut health to increase growth performance.	Drinking water (probiotic powder, 1.20×10^9 CFU/g)	[65]
Preruminant calves	*Lb. acidophilus*	Improved gut health. Lower incidence of diarrhea and higher cell-mediated immunity in probiotic fed groups.	Fermented milk, microencapsulated and FD (10^8 CFU/calf/d) were added in the milk or calf starter, depending on calf's age.	[51]
Others				
Rainbow Trout	*Ltb. Sakei*	Positive effect on growth, immunity, serum enzyme activity, gut microbiome, and resistance to *Aeromonas salmonicida*	Commercial diet coated in probiotic (1.0×10^7 CFU/g)	[66]
Common carp	*E. casseliflavus*	Improved growth and non-specific immune responses of common carp fingerlings (highest weight gain and specific growth rate at 10^{12} group, lowest feed conversion ratio at 10^{12} group)	In diet (10^{10}, 10^{11}, 10^{12} CFU/kg feed)	[67]
Rainbow trout	*Lmb. fermentum*	The encapsulated *L. fermentum* plus lactulose improved growth performance and avoided the absorption and accumulation of heavy metals in rainbow trout liver and gills	Encapsulated in diet (10^7 CFU g^{-1})	[68]
1 month old puppies	*Lcb. rhamnosus* and *Lpb. plantarum*	Significantly increased *Lactobacillus* and *Faecalibacterium* detection in fecal matter. Increased short-chain fatty acids (acetate, propionate and butyrate) concentration in feces. Prevented gastrointestinal infection.	In diet (10^9 CFU/day)	[69]
Young, training and elderly dogs	*Lactobacillus casei*, *Lpb. plantarum* and *B. animalis*	Promoted the average daily feed intake of elderly dogs. Improved average daily weight gain in all dogs. Enhanced the level of serum IgG, IFN-α, and fecal secretory IgA (sIgA), reducing the TNF-α. Increased beneficial bacteria and decreased potentially harmful bacteria.	In diet, 2×10^9 CFU/g (2 g for young, 4 g for training, 10 g for elderly dogs)	[70]

Table 1. *Cont.*

Used Group	LAB Species	Main Effects	Addition Method	Ref.
Kittens	*E. hirae*	Promoted intestinal colonization and fecal shedding of live *E. hirae* during administration. Ameliorated the effects of atypical enteropathogenic *E. coli* experimental infection on intestinal function and water loss	Probiotic powder ($2.85–4.28 \times 10^8$ CFU/day) mixed with 100 µL of sterile water and inoculated into canned cat food	[52]
Healthy adult cats	*Lb. acidophilus*	Improved fecal quality parameters, increased *Lactobacillus* count and decreased total coliform bacteria counts	In diet (5×10^9 CFU/kg of food)	[71]
Adult cats	*Lb. acidophilus*, *Lcb. casei*, *Lb. lactis*, *B. bifidum*, *E. faecium* and *S. cerevisiae*	Probiotics and synbiotics positively modulated ($p < 0.05$) the fecal microbiota of cats, increasing the lactic acid bacteria counts	Commercial kibbles coated with probiotics, supplemented with freeze-dried probiotics and fructooligosaccharides	[72]

3. Drying of Lactic Acid Bacteria

Preservation techniques, such as freeze-drying and spray-drying, are widely recognized for their ability to reduce water content in samples. This reduction in water content is essential for preventing deteriorative reactions and ensuring prolonged food storage without compromising microbiological and nutritional properties [73]. However, it is important to consider that each dehydration treatment requires the careful adjustment of several variables to achieve optimal water content reduction while preserving the structural and functional integrity of the food matrix. In the case of dehydrating LAB cultures, it is crucial to maintain their viability and/or activity throughout the manufacturing process and consumption. Thus, a thorough understanding of the specific requirements for each dehydration treatment is necessary to ensure the successful preservation of LAB cultures while retaining their desired functionality.

3.1. Drying Techniques

Freeze-drying (FD) is the primary preservation method employed for lactic acid bacteria (LAB) [74,75]. However, the high production cost associated with this method has led to exploring alternative preservation techniques, such as spray-drying, vacuum-drying, fluid bed-drying, and others [76]. While these methods are commonly used for dried food production, their application may result in greater temperature-induced damage compared to freeze-drying. Additionally, they may be less efficient in maintaining cell viability or activity, and their use requires the adequate selection of strains, drying parameters, and the incorporation of protective compounds [77,78].

During FD, cell cultures or other materials are frozen at temperatures below −20 °C, followed by the removal of ice-water through sublimation under high vacuum conditions and the subsequent desorption of the remaining water. This drying method offers several advantages, such as volume reduction and the ability to transport and store samples at room temperature. These benefits make freeze-dried products more practical to handle and reduce the costs of storage when compared to frozen samples [75].

While FD is the established method for probiotic and starter culture production in human food, the incorporation of these cultures into pet food and animal feed requires more cost-effective preservation methods. Hence, spray-drying (SD) has been extensively researched for LAB dehydration, and comprehensive reviews on this topic have been published [79]. During the spray-drying process, the LAB suspension or solution is atomized into microdroplets within a chamber with the controlled circulation of heated air. The rapid dehydration of the droplets results in powderization within a short period of time, allowing for the drying of large sample volumes. Numerous studies have reported the

successful application of spray-drying for LAB [79]. Several parameters can be adjusted in this technique, including the inlet and outlet temperature, which is controlled by modifying the airflow. The outlet temperature, typically recommended to be below 75 °C, is crucial for ensuring the survival of LAB after drying [80]. Although the cells may be exposed to higher temperatures during the process, the short duration of drying (seconds) helps minimize thermal damage. Conversely, excessively low outlet temperatures can be detrimental due to the high residual humidity of the sample, which may promote ongoing deteriorative reactions [81–83].

3.2. Alternative Drying Processes

Vacuum-drying (VD) is a well-established method for drying sensitive materials, as it allows for the removal of water at low temperatures under a vacuum, minimizing oxidative reactions. It has been found to be particularly suitable for drying sensitive LAB strains, such as *Lb. bulgaricus* [84]. Positive results have also been reported for drying *Lb. acidophilus* [85], *Lcb. casei* [86], and *Lb. helveticus* [87,88]. However, VD typically requires longer drying times, and the resulting water content may be higher compared to other drying methods, which can decrease stability during storage [89].

Fluid bed-drying (FBD) involves passing an air stream through a bed of solid particles, causing the particles to behave as a fluid and facilitating the heat-mass transfer required for water removal [90]. The cost of this method is comparable to spray-drying, and it can be scaled up for industrial production. Although the dehydration time is longer compared to spray-drying, the temperature is easily controlled. However, the use of FBD is limited by the physical characteristics of the particles, such as the irregular size and the tendency of granular materials to become sticky, which can result in heterogeneous or agglomerated particles that may affect drying rates [91].

Furthermore, air heat-drying (AHD), employing various methods such as convective drying or traditional oven drying, has been employed as a simple and cost-effective approach to incorporate dried probiotic microorganisms directly into animal feed [58,63,92,93].

A comparative summary of different parameters of drying methods is shown in Table 2, also listing the main benefits and drawbacks of each technique.

Table 2. Comparative advantages and disadvantages of different drying methods applicable to LAB cultures.

Drying Method	Production Cost *	Thermal Stress	Oxidative Stress	Large-Scale Production	** Final Humidity
FD	↑↑	↓↓	↓↓	↓	↓↓
SD	↓	↑	↑	↑↑	↓↓
VD	↓↓	↓	↓	↓	↑
FBD	↓	↑	↑	↑	↑
AHD	↓↓	↑↑↑	↑↑	↑↑	↓

* Cost of equipment and energetic cost of the process. ** Low humidity favors the long-term storage of samples (see Section 3.4). Blue arrows correspond to positive and red arrows to negative properties of the methods.

Table 3 provides examples of the different drying methods discussed in Table 2 that are commonly used for incorporating probiotics into animal products. Among these methods, FD and SD have been extensively studied. FD serves as the gold standard for microbial preservation and is widely employed to produce probiotic supplements for livestock and powders for pet food. On the other hand, SD offers the advantage of low production costs, enabling the large-scale production of dried probiotic products within a short time frame. However, it should be noted that SD is more suitable for strains that exhibit higher resistance to thermal stress, as indicated in Section 3.5 of this review. An emerging alternative is fluid bed-drying (FBD), which involves coating the animal feed with a probiotic suspension feed during the drying process. Although FBD shows promise, its application in animal feed research remains relatively limited [90,94].

Table 3. Methods, protectant compounds, and carriers for drying probiotic cultures in animal feed supplementation.

Drying Method	Strain	Protectant/Carriers or Feed Matrix	Animal Target	Storage	In vivo Study	Ref.
Freeze-drying	*Lgb.agilis*, *Lgb.salivarius*	SM/Suc/Tre	Broilers	4 °C and RT	No	[95]
	Lb. acidophilus, *Lcb. casei*, *Lb. lactis*, *B. bifidum*, *E. faecium*, *S. cereviceae.*	Tre/FOS Arabic gum SM	Cats	RT	Yes	[72]
	Lb. Johnsonii, *Lb. mucosae*	n.d.	Pigs	n.d.	Yes	[61]
	Lcb. casei	SM/Tre/SM/ Phytoglycogen	n.d.	4 °C 12 days	No	[92]
	Lgb. salivarius	SM	Broilers	n.d.	Yes	[57]
	Lmb. Fermentum	SM/lactulose	Fish	n.d.	Yes	[68]
	Lb. acidophilus	SM/Suc/starch	Calves	n.d.	Yes	[51]
	Lpb.plantarum, *Lgb. salivarius*, *P. acidilactici*	SM/MD/FOS /lactose	n.d.	4 °C 60 days	No	[96]
Spray-drying	*Lpb. plantarum*	Arabic gum/gelatin/ Coconut oil/MD	n.d.	25 °C	No	[97]
	Lpb. plantarum, *Lgb. salivarius*, *P. acidilactici*	NFSM/MD	n.d	4 and 30 °C 60 days	No	[98]
	Lb. acidophilus, *Lcb. casei* *Lb. lactis*, *B. bifidum*, *E. faecium*, *S. crevisiae*	Trehalose/FOS Arabic gum SM	Cats	RT	Yes	[72]
	Lpb. plantarum, *P. acidilactici*	double-coating with alginate and chitosan	Piglets	6 months Temp.: n.d.	Yes	[60]
	Lpb. plantarum	On feed	Fish	25 °C	No	[99]
	Lpb. plantarum *Lpb. paraplantarum*	Arabic gum/gelatin Coconut oil (SD)	Pig	4 °C	No	[100]
Air heat-drying	*Lcb. casei*	SM/Tre/SM/ Phytoglycogenon feed	n.d.	4 °C 12 days	No	[92]
	Lpb. plantarum	n.d.	Fish	26 °C–75% RH	No	[93]
	Lb. acidophilus, *B. subtilis* *S. cerevisiae*, *A. oryzae*	Growth in Solid state fermentation	Broilers	n.d.	Yes	[58]
	Lb. acidophilus, *B. subtilis* *S. cerevisiae*, *A. oryzae*	Solid state fermentation	Pigs	n.d.	Yes	[63]
Fluid bed-drying	*Lcb. brevis*	Mixed with feed	Fish	4 and 20 °C 42 days	No	[90]
	Lb. lactis	SM/MD/acacia gum MSG	Fish	4, 30 °C 12 months	No	[94]
Vacuum-drying	*Lcb. brevis*	Mixed with feed	Fish	4 and 20 °C 42 days	No	[90]

Strains: *Aspergillus oryzae*: *A. oryzae*; *Bifidobacterium*: *B. bifidum*; *Bacillus subtilis*: *B. subtilis*; *Enterococcus*: *E. faecium*; *Lactiplantibacillus*: *Lpb.* (plantarum, paraplantarum); *Ligilactobacillus*: *Lgb.* (salivarius, agilis); *Limosilactobacillus fermentum*: *Lmb. fermentum*; *Lactobacillus*: *Lb.* (acidophilus, Johnsonii, mucosae, lactis); *Lacticaseibacilluscasei*: *Lcb. casei*; *Pediococcusacidilactici*: *P. acidilactici*; *Sacharomyces cerevisiae*: *S. cerevisiae*. Protectants—SM: skimmed milk; NFSM: Non-fat skimmed milk; Suc: Sucrose; Tre: Trehalose; MD: maltodextrine; FOS: fructooligosaccharides; MSG: monosodium glutamate. RT: room temperature; RH: relative humidity; n.d.: no data.

In addition, Table 3 provides a comprehensive overview of the main protectant compounds and carriers utilized in the drying of probiotic cultures for feed supplementation purposes. This table also includes information on storage stability analysis and/or in vivo studies evaluating the probiotic properties post-drying. Section 3.3 below presents a de-

tailed discussion on the protectant action, advantages, and disadvantages of the most commonly used protectant compounds.

3.3. Protectant Compounds

Dehydration is known to inflict significant damage, and not all microorganisms can withstand its effects. Water is a vital component of life, constituting approximately 70% of cell composition. It serves not only as a solvent within the cytoplasm but also plays a crucial role in the structure and functionality of essential cell macromolecules, such as proteins and membranes. Consequently, the loss of water molecules during dehydration induces alterations in membrane integrity and protein structure, leading to compromised cellular activity and cell death [22,101]. The survival of microorganisms following the drying process is influenced by multiple variables. To enhance survival rates, the addition of protectant agents has emerged as a primary strategy. Among these agents, sugars are widely used, although polyols and amino acids have also been reported as effective alternatives [74]. While all sugars possess protective properties, it is fundamental to consider the chemical and physical characteristics of the specific sugar employed.

The protectant hypothesis proposes a mechanism known as "water replacement", in which the hydroxyl groups of sugars (or amino groups from amino acids) establish hydrogen bonds with polar groups present in the macromolecules of cells, such as membrane lipids, proteins, and cell surfaces. This interaction allows the sugars or amino acids to effectively substitute the water molecules lost during the drying process, thereby preserving the structural integrity of these macromolecules and, consequently, maintaining the viability of the microorganisms [102–104].

The alternative protectant hypothesis is known as vitrification, in which the dried sugar forms an amorphous solid matrix, referred to as the glass state, characterized by an extremely high viscosity. This glass matrix effectively halts most deteriorative reactions, such as precipitation, crystallization, denaturation, oxidation, and others [105]. These sugar matrices exhibit a glass transition temperature (*Tg*), which represents the point at which the sample transitions from a glassy state to a rubbery state. The *Tg* plays a crucial role in the protection and survival of dried cultures, as it can influence their stability and performance.

In both protectant hypotheses, the molecular weight (MW) of the sugars (or other protectant compounds) is closely associated with their protective ability. Low-molecular weight compounds, such as mono and disaccharides, exhibit a greater capability to replace water molecules within the cellular macromolecules, both inside and outside the cells. On the other hand, oligosaccharides and high-molecular weight compounds possess higher *Tg* values, allowing them to maintain their vitreous state at higher temperatures and humidity levels. This property has a positive impact on the storage stability of dried cultures [106].

In that regard, trehalose has been identified as a highly effective protectant molecule, exhibiting favorable properties such as low molecular weight (MW) and a high glass transition temperature (*Tg*), surpassing other similar sugars in terms of protection capability. Another emerging approach involves the use of protectant composites, where mono or disaccharides are combined with polysaccharides or other high-MW compounds such as maltodextrin, inulin, whey protein, or starch. These combinations aim to enhance the protectant action and overall stability of the dried cultures [106–108]. Moreover, the utilization of protectant composites is particularly relevant in the SD method, where certain sugars can form a rubbery product (e.g., sucrose and glucose) that adheres to the surface of the drying chamber, resulting in sample loss [109].

Skimmed milk (SM) is a widely utilized carrier in the drying of LAB through FD and SD methods. It primarily contains lactose, a disaccharide with a low MW and a high *Tg*. Although pure lactose is not commonly used as a protectant due to its tendency to crystallize, the presence of impurities in SM, such as mineral salts, proteins, and milk fats, helps prevent crystallization [110]. Additionally, when LAB is subjected to SD in a mixture of lactose and galacto-oligosaccharides (obtained from whey permeate), improved survival over time is observed compared to the use of pure lactose or whey permeate alone [111,112].

Regarding SD, it is worth mentioning that in recent years, protectants have also been employed as carriers. LAB can be encapsulated using techniques like the layer-by-layer method, where materials such as gelatin, Arabic gum, and coconut oil serve as carriers (Table 3) [60,97,100].

Table 3 shows that trehalose and MD are the primary protectant compounds used. However, trehalose is associated with high costs, whereas MD offers a more cost-effective alternative. Consequently, MD is extensively incorporated into commercial dried feed formulations. It should be noted that the use of milk as a carrier in FD or SD may be limited due to lactose intolerance in dogs and cats [113]. Lastly, FOS, inulin, and lactulose are utilized as protectant compounds. These oligosaccharides not only serve as protectants but also act as functional ingredients, serving as prebiotics, as further explained in Section 4. Particularly, FOS and inulin are excellent alternatives to MD and trehalose for preserving LAB through SD [107].

3.4. Storage Stability

Storage conditions play a key role in the survival and functionality of bacterial cells [114]. Even without affecting cell viability, storage conditions can impact the probiotic's stress resistance and ability to adhere to epithelial cells [23]. Therefore, after optimizing cell adaptation, drying processes, and protectant compounds, it is essential to establish appropriate storage conditions.

One critical parameter is the storage temperature [83,115,116]. Reddy et al. [96] shows that the storage of a *Lpb. plantarum* strain, at low temperatures (4 °C), improves cell viability and the conservation of its technological characteristics. In Piyadeatsoontorn et al.'s study [100], LAB isolated from pig fecal samples were studied. It was observed that all the isolates stored in an FD form had a higher survival capacity when they were stored at 4 °C for 28 days. As we explained above, the ability of sugars to form glassy matrices serves as a protective mechanism during storage. Therefore, when considering the long-term storage of dried products, it is fundamental to optimize the storage conditions based on the *Tg* at different water contents [117,118]. Maintaining the storage temperature below the *Tg* restricts molecular mobility, controlling the rate of physical, chemical, and biological changes and improving the preservation of microorganisms [106,119].

In addition, the storage stability can be analyzed by the Arrhenius equation [106]. Although this concept is well known for storage in pharmaceutical products, its application on dried cell cultures have been used only in the last few years, and could prove to be useful for estimating viability at different temperatures for long-term storage [94,96,106].

The preservation of viability during storage is also influenced by the water content of the sample, the storage environment and the presence of oxygen. A lower water content leads to an increased *Tg*, as water acts as a plasticizer that favors the transition from a vitreous to a rubbery state, thereby initiating deteriorative reactions [120]. Regarding the presence of oxygen, Brizuela et al. (2021) [121] proved that the storage of an *Lpb. plantarum* strain at low temperatures and under vacuum conditions improves cell viability and preserves its technological characteristics. Vacuum storage reduces oxygen levels, minimizing lipid membrane oxidation, which can negatively affect cell survival [74,122,123].

3.5. Intrinsically Resistant Microorganisms

It has been well established that different microorganisms possess varying abilities to tolerate environmental stress factors [20]. One of the most recognized mechanisms of resistance is sporulation, wherein certain bacteria, yeasts, and fungi form spores in a dormant form to survive unfavorable physicochemical conditions or nutrient depletion. Another tolerance mechanism is known as Anhydrobiosis, which involves the accumulation of high concentrations of protective compounds like trehalose, FOS, inulin, etc., fostering resistance to freezing and desiccation conditions [124].

LAB are non-sporulating microorganisms and must tolerate stress in their vegetative form. Certain species exhibit higher resistance than others, and even within the same

species, specific strains may display varying levels of resistance. This variation can be attributed to factors such as the fatty acid composition of their membrane lipids [125], the presence of heat shock proteins (HSPs) [126], and transmembrane proteins (e.g., aquaporins, aquaglyceroporins, and mechanosensitive channels) that facilitate the release or uptake of compatible solutes (sugars, polyols, salts, cations) to maintain osmoregulation and cell volume, thereby preventing lysis or plasmolysis [127]. Additionally, a proteolytic system can help maintain osmolarity by hydrolyzing proteins into peptides or amino acids, as extensively reviewed by Gao et al. [20].

Exposing bacteria to sub-lethal stress conditions, such as low pH, high osmolarity, or heat, is a strategy employed to enhance their resistance to dehydration. As can be seen in item 2 of Figure 1, growth conditions can influence the development of stress adaptation mechanisms. This mechanism, often referred to as "cross-protection", activates metabolic pathways in microorganisms [20,128]. For susceptible strains like *Lactobacillus bulgaricus*, Ma et al. [129] proposed dormancy induction to increase their resistance to spray-drying.

It has been observed that the original properties of bacteria with probiotic potential can be influenced by production methods, manufacturing processes, and the culture media employed [130]. Variations in strain properties among different sources of the same probiotic raise concerns about the reliability of intervention studies. Furthermore, research has shown that the ability of probiotic LAB to adhere to mucosal surfaces in dogs can be significantly affected by the growth media used for cultivation [131]. This impact extends to the attachment of enteropathogens to canine mucus, which varies depending on the specific growth media used for probiotic cultivation [132]. Thus, ensuring quality control in existing probiotics and identifying new ones for companion animals needs careful consideration in terms of the growth conditions and media. Even slight alterations in these factors can profoundly influence outcomes and subsequently impact the health of the host. Additionally, exploring the storage stability of non-viable forms of microorganisms opens new possibilities for developing nutritional supplements for pet food and feed (see Section 4.2).

4. Dehydrated Lactic Acid Bacteria in Animal Food

Functional foods have attracted significant interest in the food animal industry, with a growing focus on their use as carriers for probiotic cultures. The remarkable advantages offered by probiotics, prebiotics, and synbiotics in intensive farming, particularly in their potential to replace antibiotics, have been extensively documented [133,134].

In addition to functional and safety considerations, technological criteria associated with feed production and processing play a crucial role in the selection of probiotics. The incorporation of live microorganisms into animal feed poses significant challenges due to the exposure of probiotic bacteria to high temperatures during production and their vulnerability to adverse conditions such as low water activity, which can negatively impact bacterial viability.

The preservation of probiotic viability and functionality has been discussed in detail in part 3, focusing on different techniques and protective compounds. However, incorporating probiotics into dry products entails several challenges and requires the careful consideration of various criteria to maintain their functionality. Due to these challenges, many studies have explored the incorporation of probiotics in drinking water or mixed in food (as shown in Table 1), primarily due to the advantages of better concentration control and availability in small-scale investigations [135].

The matrices serve as the substrate for probiotic microorganisms, providing essential growth nutrients and acting as delivery vehicles. In the international market, several non-dairy food products intended for human consumption have been commercialized, incorporating probiotic LAB [136,137]. These non-dairy food products have been formulated to support the survival and functionality of probiotic strains, offering a diverse range of options for consumers seeking probiotic benefits [136,137].

Currently, commercial animal supplements are formulated with blends of various LAB species, often accompanied by enzymes or prebiotics [138]. These supplements are available as liquid additives for mixing into the feeding water or in solid–dry form to be incorporated into animal feed. They are typically administered during times of stress for the animals, such as ration changes, weaning, climate variations, transportation, and post-antibiotic treatment. Although there is limited research on incorporating probiotic LAB in solid–dry form into the food matrix for intensively reared animals, studies on incorporating probiotics into non-dairy matrices for human foods provide valuable insights into current advancements in this field.

Pelleting is a widely used thermal treatment method in the manufacturing of animal feeds [139]. The pelleting process involves various combinations of conditioning temperature and retention time in commercial feed mills. It should be noted that in some feed mills, conditioner temperatures may reach extreme levels of 90 °C, which can significantly impact cell viability [140]. As mentioned earlier, the use of protective compounds and encapsulation technologies offers new possibilities for customizing feed additives to withstand specific requirements and improve the survival and functionality of probiotics in animal feed. The successful incorporation of probiotics into such matrices requires the careful consideration of several factors. In addition to the previously mentioned criteria of safety and efficacy during selection, other aspects such as marketing, regulatory compliance, and technological considerations must also be taken into account [141].

4.1. Dosage of Probiotics

Information regarding the appropriate dosage of probiotics and legislative requirements for the concentration of live probiotics in food at the time of consumption is currently inadequate. The food industry generally considers an amount of 10^6 CFU/mL or g (as indicated by the FDA, Food and Drug Administration, of the United States of America) for human consumption [8]. In 2014, the International Scientific Association for Probiotics and Prebiotics reached a consensus that the daily intake of probiotics should range from 10^8 to 10^9 viable cells, equivalent to consuming approximately 100 g of probiotic-containing food per day [5]. For animal feed, the specific dosage is yet to be defined and will depend on the target animal species and growth stage. Probiotics exhibit diverse compositions, dosages, and delivery methods, making it challenging to discuss them comprehensively within a single study. Nonetheless, in recent years, research in the field of probiotics has significantly expanded, providing a growing body of knowledge that allows us to move beyond the uncertainties of empirical use.

Numerous factors can adversely affect the viability of probiotics in the food matrix. These factors include acidic or low pH conditions, hydrogen peroxide production, nutrient availability, dissolved oxygen levels, water activity, processing and storage temperatures, as well as potential interactions with other microbial strains and competitive inhibitors, among others [138]. To ensure consistency between batches and optimize the viability of probiotic strains in the final product, establishing and controlling the processing line and subsequent storage conditions is essential. Failure to address these factors can result in undesired interactions between bacteria and the food matrix, loss of probiotic viability during food processing and shelf life, and reduced viability of microorganisms as they pass through the gastrointestinal tract [141–143].

4.2. Incorporation in Low-Moisture Food Matrices

Ensuring the survival of probiotics requires the development of effective formulations and the careful selection of matrices or food vehicles [144,145]. Food matrices exhibit significant compositional variations, some of which may contain molecules that provide protective effects and stimulate the growth of probiotics upon reaching the intestine. Prebiotics, for instance, are non-digestible food ingredients that selectively stimulate a limited number of bacteria in the colon, thereby enhancing host health [146,147]. In animal feed, prebiotics can serve as substrates selectively used by microorganisms, conferring health

benefits and contributing to the viability and stability of probiotics within the food matrix [7]. Complex carbohydrates, such as β-glucans, fructans, arabinoxylans, and starches, can be exploited as functional prebiotic ingredients for animal health applications, offering a rich energy source [148]. It is important to highlight that the combination of appropriate prebiotics and food matrices has the potential to further enhance the survival of orally delivered probiotics. This should be considered when designing novel functional foods [149]. For instance, barley-derived β-glucans have been shown to provide tolerance to gastrointestinal transit stress for specific probiotic strains like *Lpb. plantarum* WCFS1, *Lb. acidophilus* LA5, and *Lb. johnsonii* CECT 289, while also reducing intestinal inflammation in in vitro studies [149].

The composition and diversity of food offered to livestock under intensive farming are highly variable, and each probiotic strain may respond differently. Therefore, it is key to conduct survival trials using different probiotic strains and the specific food matrix intended to contain the probiotic bacteria [142]. Factors such as storage temperature, the water activity of the food, and the type of container employed significantly influence the survival of probiotics, and should be regarded as essential considerations during the development of probiotic foods [8].

Whole grains present a promising option as vehicles for probiotics due to their rich content of complex carbohydrates, antioxidants, phytochemicals, and other bioactive compounds [150]. Incorporating probiotics into whole grain formulations can provide animals with the dual benefits of probiotics and additional bioactive components. The components of grains can serve as substrates for fermentation or act as encapsulation materials in probiotic feed formulations [151].

Food processing involves various technological steps, many of which can have detrimental effects on the viability of probiotic bacteria. Current research on probiotics has striven to evaluate the strain-specific effects of probiotic species in specific animal species. However, the impact of food matrices remains largely unexplored. This knowledge gap hinders the development of innovative probiotic products, hence this review highlights the importance of both probiotic strains (and their processing techniques) and food matrices, which are influenced by production and storage conditions, in determining the overall quality of a probiotic product. Pelleted feed is commonly used in intensive farming and is associated with higher feed efficiency. Incorporating dehydrated probiotics into such feed formulations could potentially reduce the susceptibility of these microorganisms to environmental stresses, including oxygen, pH, water activity, and temperature, during the dehydration process [152]. The inclusion of probiotic feed additives is expected to be a growing trend in the farming of intensively reared livestock, allowing for the large-scale incorporation of these beneficial microorganisms. Further research is needed to standardize the use of specific probiotic strains in the breeding of specific animals, while preserving their demonstrated properties.

5. Benefits as Postbiotics

Considering the challenges associated with the inclusion of live microorganisms in animal feed, postbiotics emerge as innovative supplements to enhance animal health. According to Salminen et al. [153], the emerging concept of postbiotics refers to "preparations of inanimate microorganisms and/or their components that confer a health benefit on the host". In scientific works, the focus has predominantly been on postbiotics derived from bacteria, particularly those produced by LAB species. However, it is noteworthy that additional bacterial and yeast species possess the ability to produce bioactive metabolites [154]. Postbiotics encompass a range of cell metabolites and cell wall-derived substances that are either secreted by live bacteria or released following bacterial lysis. These substances include enzymes, teichoic acids, glycolipids, peptides, polysaccharides, cell surface proteins, organic acids, and peptidoglycan-derived muropeptides [154,155]. Bacterial cell inactivation can be achieved through various processes, either physical (mechanical disruption, heat treatment, UV irradiation, high hydrostatic pressure, freeze-drying, spray-drying,

sonication) or chemical (acid deactivation), that can modify microbial cell structures or their physiological functions [156]. The cultivation of LAB in tailored culture media enables the synthesis of bioactive compounds within a defined matrix during microbial fermentation, employing precise and individualized control parameters for each microorganism [157]. Recent reports advance the idea of refining the traditional culture medium (MRS) to enhance the antimicrobial activity of postbiotics produced by *Lpb. plantarum* RS5 while reducing the cost of growth medium [158]. Subsequently, a functional formula is obtained as the final product, comprising bioactive ingredients derived from the microbial fermentation.

Recent studies suggest that postbiotics may serve as suitable alternative agents to live probiotic cells and can be employed in food applications for the prevention and treatment of certain diseases, animal health promotion, and the development of functional foods [159]. For instance, Zheng et al. [160] showed that the addition of inactivated *Lpb. plantarum* (achieved through heat and sonication) significantly improved weight gain, feed conversion ratio, and specific growth rate in farmed white leg shrimp (*Litopenaeusvannamei*). Similarly, Loh et al. [161] reported a significant increase in daily egg production in hens supplemented with a mixture of postbiotics derived from specific strains of *Lpb. plantarum*. Kareem et al. [162] conducted a similar study in broiler chickens and found that the inclusion of postbiotics obtained from *Lpb. plantarum* resulted in significantly higher final body weight and total weight gain compared to broilers fed a basal diet without postbiotics. Furthermore, Humam et al. [54] reported that the supplementation of postbiotics to broilers raised under stress conditions led to higher counts of beneficial microorganisms in the caecum and a significantly lower population of pathogenic bacteria, such as *Escherichia coli* and *Salmonella*, compared to the control groups. In the field of assisted reproduction in intensive livestock farming, a recent study evaluating the seminal quality of rabbits has shown that a significant improvement in semen characteristics and liver profile resulted from the administration of a postbiotic derived from lactic acid bacteria [163].

Postbiotics offer an attractive alternative as ingredients to produce functional foods, particularly when the characteristics of the food matrix are not conducive to hosting viable cells of probiotic microorganisms [164]. They possess several advantages over probiotics, as they exhibit greater thermal stability, are easily standardized, and are simple to incorporate into food products, while also having well-defined chemical structures.

To date, no studies have assessed the synergistic effects of probiotics and postbiotics in intensively reared animal studies. In recent mouse model studies, the interaction between a probiotic and a postbiotic (derived from *Lpb. plantarum* DSM33894) has been studied, revealing the synergistic effects of a combined probiotic and postbiotic treatment on enhancing lung health and mitigating allergic responses [165]. This represents a significant knowledge gap that warrants further research, and stands as a means to explore novel applications in which the effects can be further enhanced.

It is worth noting that postbiotics must originate from well-characterized microorganisms or combinations thereof, with known genomic sequences, and should be prepared using a reproducible and defined technological process for biomass production and inactivation [166]. The challenge lies in understanding the potential contributions of inanimate cells to the improvement of animal health. Consequently, postbiotics hold significant potential for the development of functional ingredients in the animal feed industry, allowing for easier large-scale utilization. However, it is imperative to ensure their uniformity during the manufacturing process and address regulatory and safety considerations [167].

6. Conclusions

The use of probiotics in animal feed holds great promise for improving animal health, enhancing feed efficiency, and reducing the reliance on antibiotics in livestock production. However, the incorporation of probiotics into the animal feed matrix presents significant challenges. Extensive research has been conducted on drying techniques, encapsulation, and storage conditions to address these challenges. These advancements have proven effective in protecting certain probiotic microorganisms from environmental stresses as-

sociated with dry food. Preservation techniques such as freeze-drying and spray-drying play a crucial role in reducing water content, dehydrating lactic acid bacteria cultures, and maintaining their viability and/or activity throughout the manufacturing process and consumption. Overall, the continued exploration of preservation techniques and advancements in drying technologies, encapsulation methods, and storage conditions will contribute to the successful incorporation of probiotics into animal feed, ultimately benefiting animal health, feed efficiency, and sustainable livestock production.

The successful incorporation of probiotics into dry products requires the careful consideration of various criteria and the resolution of technological challenges to maintain their functionality. Ensuring an adequate number of viable probiotic cells is a critical quality factor. It is important to note that the mere addition of a probiotic species does not guarantee a high viable content in the food product or throughout the storage period. The choice of food matrices not only affects the survival of probiotic microorganisms during production and shelf life, but also impacts their functional characteristics. For instance, the food matrix can influence the susceptibility of probiotics to harsh conditions in the gastrointestinal tract, such as low pH, bile salts, and various enzymes. Additionally, it can affect the immunomodulation activity of the probiotics.

Postbiotics represent a promising avenue for harnessing the beneficial properties of probiotics in food, offering a viable alternative to live probiotic cells. These nonviable probiotic products have the potential to promote animal health and facilitate the development of functional foods. Unlike live probiotic cells, postbiotics eliminate the need for costly measures to protect and store food and feed to maintain microorganism viability prior to consumption. The field of regulation specific to postbiotics is currently undergoing significant advancements, but collaborative efforts are necessary to develop guidelines and criteria for the safe and effective incorporation of postbiotics into animal feed.

The global market for probiotics in animal feed is expected to grow significantly in the coming years. This expansion can be attributed to several key factors. First, there is a growing focus on innovations within the animal feed sector, driven by the need to develop functional feed products. Additionally, consumer awareness regarding the benefits of probiotics, prebiotics and postbiotics in animal nutrition is increasing, leading to a higher demand for such products. Second, increasing resistance to antibiotics worldwide and the rise in zoonotic diseases, which can be transmitted between animals and humans, have also contributed to the growing interest in probiotics in the animal feed market. However, inadequate quality control regulations related to animal food present significant challenges for farm worker and animal safety.

The research, development and commercialization of probiotics as additives in animal nutrition has grown exponentially, and nowadays there are numerous commercial products available. Thus, the market for probiotics in animal feed is expected to continue expanding significantly in the near future, driven by the growing demand for functional and nutritionally enhanced animal feed products.

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