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A COMPREHENSIVE REVIEW ON THE SYNERGISTIC EFFECTS OF SACCHAROMYCES CEREVISIAE AND BACILLUS LICHENIFORMIS AS SYNBIOTICS IN POULTRY PRODUCTION

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Abstract

Synbiotics have emerged as an alternative to antibiotics in poultry production, enhancing nutrient utilization, performance, and gastrointestinal health. Several probiotic strains and prebiotics, including Bacillus licheniformis and Saccharomyces cerevisiae, have been identified for their potential to reduce pathogenic load in poultry. Bacillus licheniformis, a lactic acid-producing bacterium, is highly stable in the gastrointestinal tract and produces digestive enzymes, vitamins, metabolites, and short-chain fatty acids. On the other hand, Saccharomyces cerevisiae contains β-glucans and mannan-oligosaccharides as the main components; these two have the potential to improve growth performance, intestinal development, and boost immunity, thereby protecting against pathogenic infections. As dietary supplements, both Bacillus licheniformis and Saccharomyces cerevisiae have been shown to modulate the intestinal microbiota and improve gut health in animal models. Although many studies have explored the systemic and immunological effects of these two, not all have established the synergy between them. This review summarizes the characteristics of both probiotics, the mechanisms by which they resist pathogens, and their synergistic effects in poultry production.

Keywords: Probiotics, prebiotics, Saccharomyces cerevisiae, Bacillus licheniformis, Synergy, Poultry production.

Introduction

Synbiotics are increasingly supported as alternatives to antibiotics for promoting animal health without targeting specific infections (Abd El-Aziz et al., 2023). Many probiotic strains have the potential to inhibit various animal pathogens, making them promising antibiotic alternatives in animal farming, including poultry, to enhance immune function and prevent disease (Rabetafika et al., 2023). Traditionally, antibiotics have been used in farm animals to boost growth, production, and disease control

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(Abreu et al., 2023). However, antibiotics can also destroy beneficial bacteria in the poultry gut. The ban on antibiotics as growth promoters or feed additives is due to the rise of antibiotic-resistant microbes that treat animal and poultry diseases (Abd El-Aziz et al., 2023). Synbiotics have become essential as the best alternative to antibiotics, contributing to growth promotion in poultry production. The efficiency of probiotics largely depends on selecting effective strains, gene manipulation, interaction of multiple strains, and combining prebiotics with synergistic components (Song et al., 2014). Synbiotics have gained significant attention in poultry production due to their potential to improve gut health, enhance nutrient absorption, and boost overall performance. The poultry industry has seen significant productivity increases, which have been accompanied by the emergence of various pathogens and bacterial resistance (Islam et al., 2022). Dietary and management constraints strongly affect gut microbial communities in livestock, leading to impaired health and performance (Ahmed et al., 2022). In this context, the poultry industry needs effective antibiotic alternatives to control outbreaks of necrotic enteritis (NE) due to *Clostridium perfringens* (Wu et al., 2018). Synbiotics have emerged as potential alternatives to antibiotics in poultry, with studies demonstrating their mitigating effects on subclinical necrotic enteritis (SNE) (Khalique et al., 2020). Furthermore, the use of these two feed additives, viz, *Bacillus licheniformis and Saccharomyces cerevisiae,* has shown promising results in improving gut health and performance in poultry. Additionally, dietary supplementation with *Bacillus licheniformis and Saccharomyces cerevisiae* will promote growth of poultry by improving intestinal structure and function, antioxidative capacity, and microbial composition. These findings underscore the potential of synbiotics as non-drug feed additives for enhancing the performance of broilers during a necrotic enteritis challenge (Calik et al., 2019). Moreover, the proper selection and evaluation of Bacillus spp. as potential multi-mode probiotics for poultry is crucial for delivering commercially relevant products to the industry (Ramlucken et al., 2020). This comprehensive review aims to explore the synergistic effects of *Bacillus licheniformis and Saccharomyces cerevisiae* as potential substitutes for antibiotics in poultry production, with a focus on their mechanisms of action, impact on gut microbiota, immunity, and overall benefits for poultry performance.

Probiotics in poultry production

Probiotics have gained attention as a potential alternative to antibiotics in poultry production due to their ability to modulate the intestinal microbiota and promote overall health and growth performance (Dong et al., 2024). The use of probiotics in poultry nutrition has been associated with improved nutrient utilization, growth, laying performance, and gut health. Furthermore, probiotics have been shown to have beneficial impacts on poultry production systems, including improving food safety, mitigating pathogenic organisms, and enhancing egg and meat quality and production (El Jeni et al., 2021). Spore-forming probiotics, in particular, have been utilized in poultry and aquaculture feeding to prevent oral and gastrointestinal infections (Popov et al., 2021).

The application of probiotics in poultry feed has been recognized as a means to improve poultry health, growth performance, and immune function, particularly in mitigating the adverse effects of heat stress on poultry (Ahmad et al., 2022). Additionally, probiotics have been found to have a positive impact on poultry immunity and production, making them a preferred choice over antibiotics in chicken production. The use of probiotics in poultry has also been associated with improved feed conversion efficiency, immune responses, and production performances (Ismoyowati et al., 2022).

Moreover, probiotics have been identified as a potential tool for reducing pathogen load in poultry, with the production of organic acids by probiotic lactobacilli being used to improve performance parameters in the egg and poultry industry (Neal-McKinney et al., 2012). The application of probiotics in poultry farming has been linked to increased resistance to bacterial and protozoal diseases, improved feed conversion, and better growth performance. Probiotics have also been demonstrated to promote growth, stimulate immune responses, and improve food safety in poultry (Tugiyanti et al., 2022). The use of probiotics in poultry production has shown promising results in improving nutrient utilization, growth performance, gut health, and overall poultry well-being. The potential benefits of probiotics in poultry production make them a valuable alternative to antibiotics, contributing to improved food safety and quality in poultry products.

The use of *Bacillus sp*., such as *Bacillus licheniformis*, in poultry production has been extensively studied. Research has shown that supplementation with *Bacillus licheniformis* in poultry feed can lead to increased body weight gain and improved feed conversion efficiency (Qin et al., 2024). Similarly, studies have demonstrated the positive effects of *Bacillus coagulans* on the growth performance and health status of swine and poultry (Zhang et al., 2021). Additionally, dietary supplementation with *Bacillus licheniformis* has been shown to suppress subclinical necrotic enteritis in broiler chickens in a microbiota-dependent manner (Zhao et al., 2022). Moreover, spore-forming *Bacillus* probiotic bacteria isolated from poultry microbiota have been found to have beneficial effects on broilers' health, growth performance, and immune systems (Popov et al., 2024). A recent narrative review has also highlighted the updated knowledge on the beneficial impacts of probiotics, including *Bacillus* species, on poultry (Al-Otaibi et al., 2023). These findings collectively support the potential impact of *Bacillus licheniformis* as a probiotic in poultry production, emphasizing its positive impact on growth performance, nutrient utilization, gut health, and immune function.

Table 1. Bacillus licheniformis as a probiotic in poultry production

Sources	Findings
Pan et al. (2022)	*B. licheniformis* DSM5749 can improve laying performance, promote intestinal health, affect the composition of cecal microorganisms, and regulate the intestinal microecological balance, making *B. licheniformis* a good probiotic candidate for application in the laying hens’ industry.
Kan et al. (2021)	Dietary supplementation of *B. licheniformis* alleviated the intestinal damage caused by the SNE challenge, which coincided with modulating the intestinal microflora structure and barrier function and regulating the intestinal mucosal immune response.
Qin et al. (2024)	The dietary *supplementation with B. licheniformis improved growth*

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Li et al. (2021)	*performance, immune and antioxidant functions, and altered the structure of the intestinal microflora and metabolites in yellow-feathered broilers, laying* a good basis for the application of probiotics in the future.
	The supplementation with different concentrations of *B. licheniformis* improved growth performance, antioxidant capacity, and modulated intestinal microbiota in Pekin ducks. The optimal dietary supplement dose is 400 mg/kg
Zhou et al. (2016)	Dietary *B. licheniformis* supplementation can enhance growth and antioxidant ability, as well as change the expression of genes related to fatty acid synthesis and oxidation in the livers of NE-infected broilers.
Chen and Yu (2020)	*B. licheniformis* fermented products can improve the growth performance and fecal microflora composition of broilers

Prebiotics in poultry production

Prebiotics are non-digestible food ingredients that aid the host by stimulating the growth and/or activity of one or a limited number of bacteria. Prebiotics serve as substrates for beneficial gastrointestinal bacteria. In the small intestine of a monogastric, a large number of bacteria can be found that have the potential to utilize these carbohydrates as a source of energy (Shehata et al., 2022). Traditionally, prebiotics were represented by a limited set of carbohydrates, which includes compounds with fructooligosaccharides (FOS), galactoologosaccharides (GOS), mannanoligoccharides (MOS), and beta-glucans, which are the most commonly employed in animals and poultry research (Ricke, 2018). These compounds are not utilized by the host but can serve as substrates by particular bacteria, viz., bifidobacteria and lactic acid. There are many commercial prebiotics that have been studied and utilized, including Biolex, MB40, and Leiber ExCel. These brands are brewer’s yeast cell walls made of MOS (Kim et al., 2019). These products were found to reduce Campylobacter concentrations and alter the microbiome, and there is an expectation of MOS-based products to reduce pathogens that utilize mannose-specific type fimbriae, such as *Salmonella*.

Prebiotics, which are natural feed supplements, help improve the feed efficiency, decrease mortality rates, and increase growth rates (Yaqoob et al., 2021). The addition of prebiotics in poultry diets can help minimize the use of antibiotics and drug resistance to bacteria due to their productive performance and health status. A study was conducted to investigate the influence of prebiotics on the performance and some physiological parameters of broiler chickens (Ibrahim et al., 2021). They found that the addition of prebiotics (mannan-oligosaccharides and β-glucan) can partially mitigate the adverse effect of high stocking density on productive performance and physiological and oxidative stress parameters. Feeding broilers with a protein diet with prebiotics had a beneficial effect on the growth performance, carcass traits, and economic value without adverse effects on broiler immunity (Ibrahim et al., 2021). Prebiotics used in chicken help increase amylase production in the gut and improve the general growth rate of broilers (Rehman et al., 2020). Some prebiotics aid in the protection against Salmonella by providing binding sites for bacteria to be flushed out of the gut. Prebiotics in various forms offer a means to modify the gastrointestinal tract microbiota to benefit the birds in multiple ways. In addition to improving the gastrointestinal tract and host health benefits, prebiotics give a dietary means to select the gastrointestinal tract bacteria that can potentially serve as a barrier for colonization by foodborne pathogens (Ricke et al., 2020). The use of prebiotics is a promising approach to enhance the role of endogenous beneficial microbes in the gut. Based on this, they can be used as a potential alternative to antibiotics. The use of prebiotics helps feed beneficial bacteria, depending on the type and concentration provided. In addition, prebiotics may affect gastric emptying, intestinal transit time, nutrient digestibility, fecal bulking, short-chain fatty acids, intestinal morphology, and the immune system (Procházková et al., 2023). Prebiotics have a protective effect not only on the gastrointestinal system but also on the parts such as the immune, central nervous, and cardiovascular systems.

Table 2. *Saccharomyces cerevisiae* as a prebiotic in poultry production

Sources	Findings
Soren et al. (2024)	*Saccharomyces cerevisiae* fermentation products and B*acillus subtilis* probiotics could be viable alternatives to antimicrobials in poultry production, considering beneficial impacts in broilers fed an antibiotic-free diet
Fornazier et al. (2024)	Using commercial *Saccharomyces cerevisiae* in diets improved growth performance in broiler chickens. Including the prebiotic for broilers promoted environmental benefits due to the reduction of phosphorus in the litter.
Mirza et al. (2020)	The baker’s yeast supplementation in the diet and drinking water of quails substantially improved production performance, gut microbiota, and hematology parameters of local quails
Tian et al. (2016)	Yeast beta-glucan supplementation improved the intestinal health of broiler birds challenged with *C. perfringens*-induced necrotic enteritis
Lin et al. (2023)	*Saccharomyces cerevisiae* hydrolysate supplementation in broilers could improve growth performance, intestinal morphology, and barrier function while regulating intestinal inflammation, which might be attributed to the enhancement of bacterial richness and alteration of

	microbial composition, particularly the enrichment of SCFAs-producing bacteria.
Al-Ali et al. (2023)	Using *S. cerevisiae* and probiotics as a safety material to the regular antibiotics used in the chicken industry, the study influenced the growth performance, immune organs activity, and hematological parameters of broilers

Mechanism of probiotics and prebiotics on the inhibition of pathogens

Probiotics

Probiotics have several modes of action against pathogens. Due to gaps in antibiotics, probiotics have positive effects on the gastrointestinal tract and the immune system. Probiotics exhibit compositional variability, which aids in eliminating pathogens. However, the composition of the microbiome is influenced by other factors, including the quantity and quality of nutrients and the composition and balance of the feed (Nova et al., 2022). Research has indicated that bacteria are competitive by nature, which enables them to suppress or fight harmful germs or microorganisms that could affect the intestinal system. This is termed bacterial interference/antagonism or competitive exclusion (Rajput et al., 2020). However, this concept describes the creation of pathogen-resistant bacteria in young chicks through the introduction of intestinal microorganisms (Abreu et al., 2023). The pathogens that dominate adhesion sites or mucosal surfaces cause intestinal infections by upsetting the microbiota balance in the intestine. Thus, these pathogens disrupt the equilibrium of microflora. Positively, probiotics are capable of adhering to mucosal surfaces or intestinal epithelium, hence their stay in the gastrointestinal tract (You et al., 2022). Probiotic bacteria and normal gut microbiota are reported to inhibit *C. difficle* growth by consuming nitrogen-containing amino acids, sialic acid, succinic acid, and host-derived glycans, which are nutrient sources for *C. difficle*, and by producing short-chain fatty acids (Spigaglia, 2024).

Probiotics are microorganisms that provide beneficial effects on modulating the gastrointestinal tract micro-ecology conditions by reducing harmful bacteria and favoring beneficial ones (Aleman & Yadav, 2023). A healthy GIT microflora provides effective protection against pathogenic microorganisms, ultimately resulting in improved performance in animals (Chandrasekaran et al., 2024). The prophylactic use of probiotics transpires through antagonistic actions on other microorganisms and in competition for the adhesion receptors or nutrients needed for their survival, and some mechanisms, like intestinal epithelial function and status (Krysiak et al., 2021). Animal health and production performances are also affected, which could lead to the development of probiotics. The antagonistic action or the inhibition of different pathogens is one of the essential properties of potential probiotics. Probiotic microorganisms have a distinctive capacity to produce substances that have a bacteriostatic or bactericidal impact on some microbes, viz., lysozymes, protease, siderophores, hydrogen peroxide, or bacteriocins (De Mandal & Bhatt, 2020). However, other probiotic microorganisms also produce volatile fatty acids and organic acids, which decrease the pH of the gastrointestinal lumen, aiding in preventing the proliferation of opportunistic pathogens (Markowiak-Kopeć & Śliżewska, 2020). Probiotics may be considered beneficial through a direct antagonist mechanism against specific organisms, which decreases the population, by an effect on their metabolism, the generation of essential nutrients, or by the stimulation of immunity (Plaza-Díaz et al., 2018). In recent years, there has been a rise in the knowledge of antiviral activities of probiotics. Many probiotics used in poultry production have been documented to exert direct antimicrobial activities against known pathogens (Dong et al., 2024). Probiotic organisms secrete various substances that can inhibit the multiplication of pathogenic microbes, which is essential for future consideration because of the safety of their consumption and associated health benefits.

Prebiotics

Prebiotics represent feed additives that would potentially select gastrointestinal bacteria that potentially benefit the host in several ways, including the health of birds, prevention of pathogens, and improvement of performance. Therefore, the impact of the individual probiotics may vary, as there are several chemicals and different sources among the prebiotics (Ricke et al., 2020). Recently, attention has been shifted to the use of prebiotics, since most of these are polysaccharides and can be metabolized by the intestinal microbiota, contributing to the production of short-chain fatty acids. These metabolites show anti-inflammatory and immunomodulatory properties, suggesting an essential role in the treatment of several pathological conditions (Yaqoob et al., 2021) suggested that when prebiotics are induced in the host, two major modes of action can potentially occur. The first mode of action occurs when the prebiotics reach the intestine of the chicken without being digested in the upper part of the gastrointestinal tract, but are selectively utilized by certain bacteria that are beneficial to the host. Secondly, gut activities occur due to the presence of prebiotics, including the generation of short-chain fatty acids and lactic acid as microbial fermentation products, a decreased rate of pathogen colonization, and potential health benefits. Numerous studies have seen a reduction in Salmonella population by increasing the concentration of short-chain fatty acids (Micciche et al., 2018; Ricke et al., 2020).

In the gut, microbial fermentation takes place, where they produce short-chain fatty acids. In the reflux process are not only nutrients for the intestinal epithelial cells but also control factors living in their microflora. The addition of prebiotics reduces the colonization of pathogens and their movement into the internal organs and eggs and increases the absorptive surface of the intestine (Shehata et al., 2022). In recent times, studies have highlighted the health benefits of prebiotics, which may include effects on the gastrointestinal tract, thus the prevention of pathogen damage or immune system modulation, the improvement of the gut barrier function, reduction in the pathogenic bacteria population, and production of short-chain fatty acids (Ricke, 2018; You et al., 2022). The potential for enhancing prebiotic-selected gastrointestinal microbial production of short-chain fatty acids antagonistic to feed-borne pathogens has been suggested as a beneficial mechanism. The complexity of the gastrointestinal tract microbial activities also contributes to the gut environment that would be considered hostile to foodborne pathogens. Similarly, the composition of the different compounds that exhibit prebiotic properties would represent a complex array of substrates for a physiologically diverse gut microbial population to metabolize (Barathan et al., 2024). Prebiotics that get to the ceca would likely be utilized by this cecal population in a variety of fermentation products. The quantities of cecal short-chain fatty acids fermentation products may vary depending on the diet; they consist of acetate, followed by lesser amounts of propionate and butyrate. The foodborne pathogens can also reside in the ceca, and the production of SCFA would presumably be an antagonist to their presence (Ricke, 2021). Prebiotics influence the potential effect on the gastrointestinal tract by deeply modulating the composition of the intestinal microbiota. The gut microbiota has been involved in the pathogenesis of a lot of gastrointestinal disorders (Olvera-Rosales et al., 2021). There is an increasing interest in dietary strategies to modulate microbiota. As a result, research has focused on the use of prebiotics, since many of these polysaccharides can be metabolized by the intestinal microbiota, leading to short-chain fatty acids (Guarino et al., 2020).

The synergy between probiotics and prebiotics as feed additives

Prebiotics are compounds that cannot be digested by the host but can be used and fermented by probiotics, to promote the reproduction and metabolism of intestinal probiotics for the health of the host (You et al., 2022). Probiotic strains, on the other hand, have a good adhesion ability and can block the adherence of pathogens by competing for the host cell binding sites. Probiotics and prebiotics have received major attention in recent years. The publicity around the microbiome research has helped in the awareness creation of microorganisms, beyond disease-causing agents that should be avoided, to a more critical view, integrating a better understanding of the beneficial roles of microorganisms (Cunningham et al., 2021). Probiotics and prebiotics play essential roles in promoting the nutrition and health status of poultry. According to (Wang et al., 2020) prebiotics were believed to favor certain beneficial gastrointestinal tract bacteria such as *Lactobacillus* and *Bifidobacteria*. Probiotics and prebiotics serve as promising candidate interventions with the potential to boost health status and generally maximize production in poultry. This is achieved by utilizing multiple modes of action. Both probiotics and prebiotics have advantages as natural supplements without any grace period. Prebiotics complement the function of the gut. The function of probiotics in the gut is complemented by prebiotics, which are an additional source of energy for intestinal microflora (Dahiya & Nigam, 2022).

The preparation of prebiotics effectively stimulates the activity and growth of probiotics. Between both the pro and prebiotics, there is an existing mutual correlation. The diets of animals may be supplemented with both of these feed additives by using synbiotics (Stasiak et al., 2021). Synbiotic combinations, which involve the simultaneous use of probiotic and prebiotic feed, may become possible as more is learned about the chicken's digestibility limits, and other nutrient candidates could be utilized to develop unique symbiotics with additional functions beneficial to the host (Di Gioia & Biavati, 2018). A study reported that poultry that consumed the yeast in combination with Aflatoxin B1 showed an absence of inflammatory infiltrate in the intestinal villi and improvement in the intestinal histomorphometry parameters (Poloni et al., 2020). The combining effect of fermentable substrates and live microorganisms is the blend known as symbiotic. Synbiotics may be complementary or synergistic in nature. The complementary synbiotics are made of a combination of an accepted prebiotic and probiotic, as their mechanism of action can be independent of each other, and both prebiotic and probiotic must have their benefits to the host (Cunningham et al., 2021). Alternatively, synergistic synbiotics contain a fermentable substrate for the co-administered live microbe, where the substrate may or may not elicit a health benefit independently of each other. In this case, the individual component did not necessarily need to be confirmed probiotics or prebiotics; however, they must have demonstrated health benefits in combination (Swanson et al., 2020). Similar to probiotic and prebiotic fields, the future of synbiotics will be influenced by the development of novel strains and substrates, informed by and targeted to vacant microbiome niches in individuals and subgroups, with the potential applications in both the gastrointestinal and ex-gut sites.

Probiotics and prebiotics function in the intestine through the crosstalk with the host and commensal bacteria in some ways, which are not fully understood regarding the specific molecules directly conferring the health benefits, the host targets of these molecules, and signal transduction pathways. Presently, the understanding of the interaction between probiotics, prebiotics, microbiota, and pathogens in the gut is limited, which affects the application of probiotics and prebiotics (Cunningham et al., 2021). The prospect of synbiotics will be influenced by the development of novel strains and substrates, informed by and targeted to vacant microbiome niches in individuals and subgroups with potential in both gastrointestinal and ex-gut sites. (Granstad et al., 2020) reported that the combination of *S. cerevisiae* and *Bacillus subtilis* proved a positive impact on intestinal health with a decrease in the population of *Clostridium perfringens* at the cecal level and improvements in productive performance.

Characteristics of *Bacillus licheniformis*

Bacillus species are saprophytic Gram-positive bacteria found in the soil, water, dust, and air (Liu et al., 2019). These bacteria are considered allochthonous and enter the gut by association with food. Since the spores of *Bacillus* species can readily be found in the soil, it might be assumed that live bacteria produced from these spores are soil inhabitants. However, the ability of the spores to be dispersed in dust and water implies that the spores can be found everywhere. *Bacilli* are aerobic and endospore-forming bacteria that have recently shown tremendous promise as probiotic candidates because of their survival through the digestive process and germination within the digestive tract (Lan & Kim, 2019). These bacterial species exist in an endosymbiotic relationship with their host, being able to temporarily survive and proliferate in the gastrointestinal tract. *Bacillus spp*., such as *B. licheniformis* and *B. subtilis,* have been used in competitive exclusion experiments.

Bacillus licheniformis is a gram-positive, endospore-forming, mesophilic bacterium belonging to the genus of firmicutes in the family of Bacillaceae (Olvera-Rosales et al., 2021). It plays an essential role in the biotechnology field as a strain for expression platform, a compound producer, an environmental applicant, and finally as a probiotic. *B. licheniformis* has shown that it's a multipurpose organism, which is mostly found in the soil and feathers of ground-dwelling birds (Ghani et al., 2013). The strain is used to produce polypeptide antibiotics known as bacitracin. *B. licheniformis* has the potential to produce bacteriocin under aerobic conditions, as well as under anaerobic conditions against anaerobic microorganisms (Shleeva et al., 2023). *B. licheniformis* is characterized by high temperature and stress resistance. *B. licheniformis* has been reported to produce digestive enzymes, including amylase, alkaline protease, keratinase, and mannanase, which enhance the digestibility of nutrients (Parrado et al., 2014). Additionally, *B. licheniformis* can produce bacitracin against pathogenic microorganisms. Among the *Bacillus* species, *B. licheniformis* was identified from the gastrointestinal tract of broilers to exhibit antipathogenic activity (Chen & Yu, 2020).

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Studies have reported that *B. licheniformis* could produce a variety of biologically active substances, such as digestive enzymes, lysozymes, bacteriocin, and antibacterial peptides, which promote animal performance by improving feed digestibility, stimulating the development of the immune system, enhancing intestinal mucosal barrier function, inhibiting the colonization of pathogenic bacteria, promoting the proliferation of potentially microorganism, and maintaining the balance of intestinal microflora (Chen & Yu, 2020; Kan et al., 2021). The dietary supplementation of *B. licheniformis* improves growth performance and alleviates *Clostridium perfringens-induced* necrotic enteritis in broilers (Xu et al., 2021). Some findings concluded that *B. licheniformis* can be a promising growth promoter of the intestinal balance of microbial population in broilers (Liu et al., 2012). It has remarkable potential in improving poultry production and meat quality.

Characteristics of *Saccharomyces cerevisiae*

Saccharomyces cerevisiae is a unicellular, eukaryotic, facultative yeast that ferments in high concentrations of glucose and aerobic media. Its pH is 6.6-6.7 and, in turn, is controlled by the vacuolar (V)-ATPase inactivation (Parapouli et al., 2020). *S. cerevisiae* contains a high level of digestible protein, vitamins, and essential elements; thus, it's noted to be a crucial protein and amino acid source for poultry growth. Baker's yeast is rich in thiamine, riboflavin, nicotinic acid, pantothenic acid, biotin, magnesium, and zinc (Perli et al., 2020). *S. cerevisiae* undergoes fermentation and mutation processes; however, when mixed with other components, it decomposes. *Saccharomyces cerevisiae* (baker's yeast), as an alternative to antibiotics, acts as a prebiotic and probiotic and has received significant attention. The addition of different levels of inclusion of *S. cerevisiae* as both probiotic and prebiotic corroborated a decrease in the prevalence of *Salmonella heidelberg* at the cecal level in poultries infected by direct contact with the bacteria; this led to less contamination of chicken meat in the processing plants, reducing the incidence of zoonotic transmission of *Salmonella heidelberg* (Ahiwe et al., 2021). *S. cerevisiae* contains β-glucans and mannan-oligosaccharides as the main components. Studies have shown that *S. cerevisiae* is an alternative protein source that positively affects poultry growth performance, blood parameters, and immune response (Qui, 2023). Yeast is an excellent source of small peptides containing free amino acids. This ensures the rapid rate of digestion and absorption, which could significantly enhance feed utilization. Yeast, which is utilized as a prebiotic and probiotic in poultry diets, acts as a stimulator of bile acid secretion. It is used in recovering acid bile, which results in more cholesterol as a precursor of acid bile. As a precursor, it helps in the reduction of blood serum cholesterol levels (Azrinnahar et al., 2021). Yeast has shown antimicrobial properties, which are accountable for the immunomodulatory response of the host. It protects against pathogenic bacteria by producing mycotoxins, secreting inhibitory substances that degrade the toxins, preventing the adhesion of pathogens to the epithelial cell surface, and creating competition for nutrition (Hatoum et al., 2012). Research demonstrated that the β-glucan and MOS from the yeast wall group recorded the highest carcass percentage as compared to the control (Van den Abbeele et al., 2020). Their study concluded that the combination of the application of MOS and β-glucan has the potential to improve growth performance, intestinal development, and boost immunity, which protects from pathogenic infection as well as establishes a stronger intestinal ecosystem in broiler chickens. When β-glucan is used in association with probiotic bacteria, the prebiotic effects have been demonstrated due to their ability to enhance growth, metabolism, and/or beneficial activities of probiotics. The emphasis is that the combination of microorganisms and β-glucans could synergistically modulate the expression of several immune-related genes, resulting in an overall enhanced anti-inflammatory effect of probiotics (Arena et al., 2016). The study of these two hosts deserves deeper research.

Mannan-oligosaccharides (MOS) and β-glucans are found in high concentrations in the yeast cell wall. MOS are found in the cell walls of numerous fungal species, including brewer's yeast (*Saccharomyces cerevisiae*) and *Saccharomyces boulardi*, and certain plants. One of its properties as a prebiotic is its resistance to pathogenic bacteria present in the intestinal microbiota. MOS acts as a ligand for type fimbriae, which results in the reduction of bacterial proliferation. MOS is capable of binding pathogenic bacteria to reduce pathogen infection in the intestines of animals. It has been reported that MOS could increase *Lactobacillus species*, enhance villus height in the intestine, and regulate gene expression of toll-like receptors and cytokines in the ileum and cecal tonsils (Teng & Kim, 2018). The mannan-oligosaccharides play a role in preventing pathogenic bacteria from attaching to the intestinal wall. Broiler chickens do not have enzymes to break down MOS; therefore, it is suggested that the bacteria in the gastrointestinal tract, like the ceca, are responsible for their digestion (Barbalho et al., 2023). One particular advantage of MOS as a prebiotic is its ability to pellet during steaming, which allows it to be easily added to feed. The yeast-derived MOS can directly decrease gastrointestinal tract pathogens by binding with the flagella of microorganisms such as *E. coli* and *Salmonella,* by decreasing the colonization of the gut through interference with their attachment to the gut epithelial cells (Ricke, 2018). MOS acts as a proinflammatory factor, inducing the immune response. However, MOS can enhance antibody production against infectious bursal disease virus, Newcastle disease virus, and avian influenza virus (Teng et al., 2021). The yeast mannans serve as immune adjuvants and directly initiate the immune response by binding macrophages and dendritic cells that contain C-type lectins of the mannose receptor.

The chemical structure of beta-glucan is a glucose polymer consisting of D-glucose core chains that can contain up to 250,000 glucose residues linked by beta-glycoside bonds (Arena et al., 2017). Such a core can branch by further side chains of sugar, whose characteristic branching depends on the source. The sources of beta-glucans, metabolites, and several industrial enzymes are also widespread in yeast. β-glucans are long-chain complex carbohydrates that can be found in cereals, seaweed, mushrooms, yeast, and some bacteria. According to Van den Abbeele et al. (2020), the source of β-glucan will influence the type of bond, structural branching, solubility, and molecular weight, which can affect their functional properties. Chitin, β-glucans, and mannan are the main polysaccharides in yeast cell walls, which constitute 90% of their dry matter weight. The yeast cells can detect and interact with each other by the carbohydrate component β-glucans of mannoproteins, which aids in the determination of the yeast's immunological specificity (de Macêdo et al., 2024). The presence of glucan rolls in the diet, and more specifically in the cell wall of yeast, might be responsible for lowering cholesterol levels. The fermentation process for glucan fiber occurs within the intestinal tract, allowing for the production of short-chain fatty acids (acetate, propionate, and butyrate), which are then taken into the liver through the portal vein (Portincasa et al., 2022). These fibers

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remain in the gut, resulting in reduced sugar absorption followed by a decrease in insulin levels in the blood. The biological properties, such as anti-infection, anti-inflammatory, glucose regulation, and immune-modulating activities, of β-glucan. The molecular weight, tertiary weight, and degree of branching of molecules influence the biological effects of β-glucans. Beta-glucan can boost the immune system, increase natural resistance, and enhance animal defense mechanisms. It helps in the prevention of diseases, which in turn increases poultry mortality. The increment in immune cell activation and migration to the intestine after chickens were fed a diet with β-glucan included (Amer et al., 2022). Yeast-extracted β-glucan can be used as a growth promoter and has the potential to substitute antibiotics in poultry against enteric pathogens by increasing the population of IgA secretory cells (Schwartz & Vetvicka, 2021). The research of (Amer et al., 2022) stated that the good benefits of beta-glucans on body weight were a result of the priming of the microflora in the gut, which promoted its development and contributed to its growth. It can also minimize the amount of competition for nutrients that occurs between the host and its microflora by decreasing the number of pathogenic bacteria that colonize the intestinal tract, which in turn improves intestinal health and mucosal integrity.

Role of *Bacillus licheniformis and Saccharomyces cerevisiae* in poultry production

Bacillus licheniformis

Most studies of *B. licheniformis* focus on animals and have proven to be safe for consumption, with the ability to resist the conditions of the entire gastrointestinal system since it's an organism that can form spores and, in turn, benefits its industrialization and handling in less-than-optimal conditions for its production, getting better proliferation without losing its vitality (Ramirez-Olea et al., 2022). *B. licheniformis*, which is generally recognized as a safe bacterium, has been extensively used in the poultry industry. These bacteria can serve as an alternative to antibiotics to enhance growth performance in poultry (Zhou et al., 2016). The supplementation of *B. licheniformis* with a broiler diet could improve body weight and average daily weight gain (Qin et al., 2024). Another study revealed that dietary *B. licheniformis* could improve egg production and feed intake in laying hens under heat stress (Ahmad et al., 2024). Furthermore, the study showed that 0.2% *B. licheniformis* fermented products supplemented had the best results in their study, this is characterized by an increase in gut morphology and integrity and lower inflammatory response in heat stress in laying ducks. The dietary *B. licheniformis* supplementation effectively alleviates the negative effects of necrotic enteritis infection, thus providing new insight into the prevention of necrotic enteritis in broilers (Zhou et al., 2016). Their study further demonstrated that the supplementation could reduce antioxidant stress, enhance growth performance, and adjust the expression level of certain key genes related to lipid metabolism. The use of *B. licheniformis* spores as direct-feed microorganisms or probiotics could be an alternative to antibiotics for preventing and aiding in the treatment of necrotic enteritis in broiler chickens under commercial-like conditions (Kan et al., 2021). The intake of *B. licheniformis* is related to a reduction of pro-inflammatory cytokines IL-8 and an increase in IgM, IgG, and IgA antibodies, and a higher concentration of total serum proteins and globulins (Qin et al., 2024). The application of *B. licheniformis* probiotics was successful in sustaining systemic mucosal immunity, as well as resistance to *A. hydrophila* (Gobi et al., 2018). In addition to modulating the immune response, it has been found that antimicrobial properties, improved enzyme secretion, and enhanced eubiotics contribute to increasing diversity in the microbiota (Ramirez-Olea et al., 2022).

Saccharomyces cerevisiae (baker's yeast)

Saccharomyces cerevisiae has been explored in various ways in scientific studies. As a potential feed additive, *Saccharomyces cerevisiae* plays a crucial role in poultry production by enhancing poultry performance through increased nutrient absorption and digestibility. It also elevates poultry health by improving the immune response and blood parameters (Qui, 2023). The application of *S. cerevisiae* in the diet could improve both production and reproduction aspects. The use of *S. cerevisiae* incorporated into the diet helps improve the morphological structure of the poultry's gut, facilitating an increase in growth performance. This, in turn, stimulates feed intake, enhances body weight gain, and improves feed conversion efficiency. A study by Abdelrahman (2013) demonstrated that supplementing yeast in the diet of broilers increased body weight gain and decreased feed conversion ratio during the finisher stage, but no alteration in the feed consumption was identified in broilers; thus, yeast improved digestibility in broilers. Furthermore, where live yeast was used as a substitute for antibiotics showed improvements in intestinal morphology, including an increase in villus height, which was beneficial for nutrient utilization, stress resistance, and gut barrier functions in animals (He et al., 2021). Some studies have demonstrated that the addition of yeast cell walls to the diets of laying poultry increased productivity while also improving egg quality both internally and externally, thereby contributing to increased profitability (Qui, 2023).

The hematological parameters are significant markers of pathological, physiological, and nutritional responses of animals (Etim et al., 2014). These parameters exhibit changes that can be interpreted as the impact of nutritional components and feed additives on animal performance. The effect of *S. cerevisiae* on blood parameters has been indicated through cholesterol, albumin, and hemoglobin concentration; however, the important effect of *S. cerevisiae* is the reduction of cholesterol in plasma and an increase in albumin (Abd El-Naby et al., 2024). The reduction effects are a result of various internal and external factors, including the breed, strains of yeast used, and the environment. The immune system is also enhanced by the reduction of harmful microorganisms, which effectively prevents poultry diseases and ultimately improves poultry health. The cell wall of *S. cerevisiae* has been shown to stimulate productive performance, exert an effect on the innate immune response, and have antimicrobial action (Lin et al., 2023). A study revealed that an increase in villi height, secretion of glycoconjugates, the number of calceiform cells, and a reduction in the number of CD45 cells led to a decrease in pathogens (Pascual et al., 2020). The inclusion of *S. cerevisiae* in the diet of poultry declared yeast improved feed efficiency, increased the number of *Enterococcus*, reduced the concentration of *Lactobacillus* in the ileal digesta, and *E. coli* in cecal digesta (Bortoluzzi et al., 2018). They further concluded that *S. cerevisiae* improved the productive parameters of broilers and modulated the intestinal microbiota and immune system.

Synbiotic supplementation in poultry production

In poultry farming, antibiotics and other traditional antimicrobials are commonly used to prevent and treat diseases. The misuse of these substances encourages antimicrobial drug resistance, posing major public health concerns (Abreu et al., 2023). With the rise in

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concern over antibiotic resistance and the ban on the use of antibiotics as growth promoters in several countries (Abd El-Aziz et al., 2023). However, the quest for alternatives in poultry production has increased. Synbiotics are gaining popularity and scientific credibility as functional feed supplements in poultry nutrition. The combination of prebiotics and probiotics, thus synbiotics, is theorized to act in synergy by improving animal health because prebiotics act as nutrients for probiotics, thereby increasing probiotic survivability (Matthew et al., 2022; Nawaz et al., 2025; Simon et al., 2021). Furthermore, the use of synbiotics produced a synergistic effect in broiler chickens because the prebiotics enhance the survival and multiplication of probiotics by increasing their tolerance to high temperature, oxygen, and low pH (Alloui et al., 2013). However, the synergistic effect of synbiotics in broiler chickens has not been reported consistently in some studies, which may be due to the variations in the compatibilities of the types of probiotics with prebiotics used in the study, followed by their evaluation in broiler chickens (Mookiah et al., 2014).

Poultry, in their quest for nutrients, unwittingly consume harmful bacteria from their host, potentially infecting their small intestine (Acharya et al., 2024). The blend of prebiotics and probiotics, which modifies the gut microbiota and immune system, can prevent pathogen colonization and thereby reduce enteric illness in poultry farming (Gaggìa et al., 2010; Yang et al., 2025). A synbiotic, rather than a probiotic, is more effective in enhancing growth and health in broilers (Al-Sultan et al., 2016). The addition of synbiotics to broiler diets enhanced various parameters, such as weight gain, FCR, villus height, and crypt depth, surpassing the control (Min et al., 2016). These studies demonstrated improved poultry performance and health from synbiotic administration (Markowiak & Śliżewska, 2018; Raut et al., 2025). Several studies analyzing probiotics supplementation on intestinal infection in broiler chickens reported that drinking water probiotic supplementation was more effective than in-feed probiotic supplementation in enhancing poultry health (Ritzi et al., 2014). However, the combined results of a study demonstrate that drinking water supplementation of synbiotics can significantly regulate the immune response and intestinal microbiota of laying hens with and without *Salmonella* challenge (Dev et al., 2020; Markazi et al., 2018). In poultry production, several options have been considered, such as prebiotics, probiotics, synbiotics, organic acids, essential oils, enzymes, and emerging novel compounds. The prebiotics, probiotics, and their combination have become an increasingly significant substitute. The enteric infections significantly impact poultry health by lowering productivity, increasing mortality, and potentially contaminating human meat consumption (Acharya et al., 2024).

In poultry, synbiotics can be administered through multiple methods; this includes in feed, drinking water, and *in-ovo* injection. The effectiveness of different synbiotic administration methods remains uncertain despite numerous discoveries detailing their health benefits. The synbiotic application methods vary, influenced by the intended product outcomes (Żbikowski et al., 2020). The in-feed or in-water supplementation provides ongoing exposure when used, but *in-ovo* injection allows for early life programming of the gut microbiome and immune system (Śliżewska et al., 2020). The effectiveness of synbiotics administration methods on poultry growth remains undefined.

Poultry gastrointestinal tract (Gut)

The gastrointestinal tract of the chicken is complex due to the bird's large energy requirement. It is a natural barrier between the host and the intestinal microflora. The chicken gut includes the crop, gizzard, duodenum, ileum, and cecum, which are microbiologically abundant with over nine hundred documented bacterial species (Micciche et al., 2018). The digestive enzymes and bile from the pancreas and gall bladder are added to the duodenum to further break down food, which will aid in proper absorption into the bloodstream through the villi (Ogobuiro et al., 2023). This process proceeds through to the ileum in the lower small intestine. The small intestine is dominated by anaerobic bacteria; however, the most densely colonized organ is the cecum, and its bacterial diversity is much higher than in the upper digestive tract of the birds (Yaqoob et al., 2021). The cecum is essential in water absorption and urea recycling, although the nutritional significance is unclear. The cecum has the longest transit period of 12-20 hours in the digestive tract. It is where fermentation of complex undigested components, including cellulose and other polysaccharides, takes place in two blind pouches (Álvarez-Mercado & Plaza-Diaz, 2022). The microbial species have several nutrient preferences for growth and maintenance; the digestive system's microbial profile, especially the cecum, is generally considered a reflection of the feed ingested and the nutrients absorbed in the small intestine (Yang et al., 2022).

The gut bacteria play an important role in the health of the host, including providing the host with a defense system and helping the gut to maintain normal function, while their composition can be influenced by the host. Commensal bacteria and feed additives can promote the integrity of gut barriers. The gut bacteria maintain resistance against the colonization of pathogenic bacteria by competing for nutrients and attachment sites on the mucosal surface in the colon, a phenomenon collectively known as colonization resistance (Kim et al., 2017). The invasion of pathogenic bacteria is prevented by commensal bacteria due to the reduction of intestinal pH by the production of lactate and short-chain fatty acids. Another way is by producing toxic and carcinogenic metabolites to inhibit the growth and kill potential pathogenic bacteria, together with the volatile fatty acids that can inhibit the colonization of pathogenic bacteria (Zhang et al., 2015). Gut bacteria benefit the host in a variety of ways, such as regulating gut motility, producing vitamins, transforming bile acids and steroids, metabolizing xenobiotic substances, absorbing minerals, activating and destroying toxins, genotoxins, and mutagens (Krishnamurthy et al., 2023). The gut resists pathogenic bacteria through two barriers, the mechanical barrier and the immune barrier. The mechanical barrier consists of a single layer of polarized intestinal epithelial cells, the enterocytes, and mucus; on the other hand, secreted immunoglobulin A (IgA), intraepithelial lymphocytes, macrophages, neutrophils, natural killer cells, Peyer's plaques, and mesenteric lymph node compose the immune barrier (Zhang et al., 2015).

Feed additives that can modulate the poultry gastrointestinal tract and provide benefits to bird performance and health have recently received more interest in commercial applications. Prebiotics potentially select gastrointestinal tract bacteria that potentially benefit the host in several ways, including health, prevention of pathogens, and improvement of chickens' performance. The use of prebiotics as feed additives to hasten the complexity of the gastrointestinal tract microbiota in newly hatched birds aims to

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decrease exposure to intestinal pathogens (Yaqoob et al., 2021). The most important impact of prebiotics is the selection of lactobacilli and bifidobacteria populations. These bacteria are considered an indication of healthy microbiota due to their inhibition of putrefactive proteolytic bacterial growth. Studies have indicated that broiler chicks' lactic acid and other short-chain fatty acids created by commensal bacteria prevented the growth of *S. typhimurium*, *C. perfringens,* and *E. coli* through the decreased pH and the bactericidal influence of the undissociated form of short-chain fatty acids (Ali et al., 2022; Szabó et al., 2023). The supplementation of probiotics helps in the manipulation of gut microbiota. For example, broilers supplemented with a *C. butyricum* diet enhanced the gut flora and immune response (Li et al., 2021).

Gut health and immunity in poultry

Gut health

Prebiotics act indirectly as a substrate for the membrane of the poultry gastrointestinal tract microbial population, which in turn responds by increasing in numbers and generating metabolites and other mechanisms that can be considered antagonistic to foodborne pathogens in poultry (Ricke, 2018). The impact of dietary-supplemented prebiotics on the gastrointestinal tract of poultry is likely a function of the chemical composition of the respective prebiotic and the metabolic capabilities of the gastrointestinal microbiota present (Ricke et al., 2020). In the avian gut, it's presumed that prebiotics are hydrolyzed and utilized by gastrointestinal tract microorganisms present in the various compartments because the prebiotics are characterized as being indigestible by the host. The dietary fibers, which are known as the undigested dietary materials, generally transit through the upper part of the gastrointestinal tract and reach the ceca, where they are available as substrates for the resident cecal microbial population (Jha & Mishra, 2021). Prebiotics that get to the ceca would likely be utilized by the cecal population would resulting in a variety of fermentations. A study showed that chickens supplemented with *S. boulardii* had a beneficial impact on intestinal health and also had good results on chickens infected with *S. enteritidis* (Halder et al., 2024). The enhanced intestinal bacteria with probiotics mainly promote intestinal fermentation by promoting short-chain fatty acids, which are one of the most important critical positive factors in the expansion of probiotics (Markowiak-Kopeć & Śliżewska, 2020). The administration of probiotics prevented the establishment and spread of pathogenic bacteria in the gastrointestinal tract of broiler chickens. The administration of commercial probiotics formulated from Lactobacillus and *S. cerevisiae* reduced the stress on *E. coli K88*-infected Hubbard broiler chicks and further reduced *E. co*li proliferation in the gastrointestinal tract (Tsega et al., 2019).

In the past few years, non-specific immunomodulators such as probiotics, prebiotics, synbiotics, and many others have emerged as an alternative to commercial antibiotics and are also used to enhance gut microbiota (Halder et al., 2024). A variety of research has established the positive effects of both probiotics and prebiotics and their link with intestinal disorders. However, there is still information that could delve into diseases apart from gastrointestinal disorders, which can be concentrated directly or indirectly. The gut microbiota is regulated by several factors that have also been related to disease prevention and treatment. The dysbiosis of intestinal microbiota has been linked to the development of disorders. A homeostatic gut microbiota population is required for the host and the microbiome to coexist in a symbiotic association (Talapko et al., 2022).

Poultry immunity

The word "*immunity*", derived from the intestine, has become more important in the poultry industry because probiotics have proven helpful in the fight against diseases of bacterial origin, against zoonoses. Immunity plays an essential role in maintaining animal health and thereby achieving an adequate level of performance (Niu et al., 2022). Immunity can be stimulated by various methods of preparation; this could facilitate the effectiveness of the immune system of birds. Chicken has a strong and built-in defense against diseases that are caused by the overrunning of the body by microorganisms and toxins. There are a variety of mechanisms employed to achieve immunity, including inactivation of biological agents, agglutination or precipitation of molecules or cells, or phagocytosis of foreign agents (Paludan et al., 2021). Poultry health management practices are crucial for the industry's success in productivity and reproduction. The boosting of poultry farming productivity, disease resistance, and reproductive success through health management practices. Avian has made valuable contributions to understanding immunology. For instance, the bursa Fabricius, which is an immune organ in chickens, has played an essential role in immunity. The major blood antibody class in chickens is immunoglobulin Y(IgY), whereas immunoglobulin E (IgE) antibodies are absent in chickens (Ferreira Júnior et al., 2018). The immunomodulatory activities of probiotic microorganisms have long been known. The epithelial and dendritic cells, as well as the monocytes, macrophages, and lymphocytes, may all converse with these bacteria. The immune system is innate and adaptive, dependent on the B and T lymphocytes. The immune systems react with the pathogen-associated molecular patterns (PAMPs), which are observed in a large spectrum of disorders (Mogensen, 2009).

Probiotics and prebiotics act as a novel feed supplement that improves the health and immunity of poultry. A study revealed that the supplementation with *B. licheniformis* Dahb1 could improve innate immune function by reducing the oxidative stress linked to ammonia accumulation in tissues and blood (Ramirez-Olea et al., 2022). The study of specific probiotic strains that give the most effective prevention and mitigation of oxidative stress must be continued to produce novel products with the potential to prevent oxidative stress. Another study proves that lactic acid bacteria affected the proinflammatory cytokines expressions (IL-6, IL-10, IL-1β, INF-γ, and TNF-α), and helped in reducing the inflammation in broiler chicken (Basiouni et al., 2023). A study by Bai et al. (2013) showed that a mixture of *L. fermentum* and *S. cerevisiae* increased the T-cell generations in broiler chickens.

Conclusion

In conclusion, this comprehensive review discussed the characteristics of *B. licheniformis and S. cerevisiae*, the mechanisms by which these probiotics inhibit pathogens, and their synergistic effects in poultry production. Our review highlighted the benefits of using a dietary combination of both probiotics and prebiotics in poultry, which has improved health, performance traits, and the immune system's ability to combat diseases. The synergy of these feed additives has been identified as a potential tool for reducing pathogenic load in poultry and positively influencing intestinal microbiota by promoting epithelial barrier integrity and other mechanisms. Additionally, the review provided a better understanding of *B. licheniformis and S. cerevisiae* as antibiotic substitutes and their impact on poultry health. Future researches should focus on the digestive enzymes produced by the synergy of *B. licheniformis* and *S. cerevisiae* the molecular mechanisms of these enzymes.

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