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Effect of Bacillus subtilis (Probiotics) on Colonization of Salmonella and E. coli in the Intestine of Broilers (Review)

Vivek Vishnoi[1], Nidhi S. Belwal[1], N.K. Mahajan[2]

[1]Department of Microbiology, Sardar Bhagwan Singh University, Balawala, Dehradun, Uttarakhand, India

[2]Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS), Hisar, Haryana, India

Corresponding Author: **Vivek Vishnoi**

Abstract

Background: The health of poultry is therefore important for the world food needs especially for those that consume broilers for meat. Salmonella and Escherichia coil for instance, are potential pathogenic bacteria that influence diseases in broilers with potentiality that may cause high death rates and cost implications. That is why it is crucial to always come up with proper management measures in order to control these bacterial infections causing harm to the poultry birds. **Objective:** This paper seeks to determine the effect of Bacillus subtilis a known probiotic, on the establishment of Salmonella and E. coli in the intestine of the broilers. In an effort to abduct our understanding from the most recent literature, this paper aims at finding the extent to which the presence of Bacillus subtilis affects the presence and further proliferation of these pathogenic bacteria. **Key Findings:** Evidence suggests that Bacillus subtilis exerts a beneficial effect in reducing the colonization of Salmonella and E. coli in broiler intestines. Studies indicate that Bacillus subtilis competes with these pathogens for nutrients and adherent sites, produces antimicrobial substances, and modulates the gut microbiota to suppress pathogenic bacteria. Both in vivo and in vitro studies highlight a significant reduction in the population of Salmonella and E. coli with the inclusion of Bacillus subtilis in broiler diets. **Conclusion:** The incorporation of Bacillus subtilis as a probiotic in broiler diets shows promising potential for enhancing poultry health by controlling the colonization of harmful bacteria like Salmonella and E. coli. These findings suggest that Bacillus subtilis could be an effective tool in poultry health management, contributing to improved animal welfare and reduced economic losses. Further research is recommended to optimize probiotic strategies and address any implementation challenges.

Introduction

The poultry industry has witnessed significant advancements in recent decades, driven by the increasing demand for poultry products and the need for sustainable and efficient production methods. However, the widespread use of antibiotics as growth promoters in poultry feed has raised concerns regarding antimicrobial resistance and its potential impact on public health (1). As a result, there is a growing interest in finding alternative strategies to enhance poultry performance and health while reducing reliance on antibiotics.

Probiotics, defined as live microorganisms that confer health benefits when administered in adequate amounts, have emerged as a promising alternative to antibiotic growth promoters in poultry nutrition (2). Probiotics act by promoting beneficial changes in the gut microbiota, enhancing nutrient absorption, and improving gut health, thereby contributing to overall performance and disease resistance in poultry (3).

Among the various potential probiotic candidates, Bacillus subtilis has garnered considerable attention for its probiotic properties and potential benefits in poultry production (4). Bacillus subtilis is a Gram-positive, spore-forming bacterium with a long history of safe use in various industries, including agriculture, food, and pharmaceuticals (5). As a probiotic, Bacillus subtilis has demonstrated several advantageous traits, such as resistance to adverse conditions, ease of large-scale production, and compatibility with feed formulations (6).

In the context of broiler production, the utilization of Bacillus subtilis-based probiotics has shown promising results in promoting growth, improving feed efficiency, and enhancing immune responses in broiler chickens (7). The use of Bacillus subtilis as a probiotic in broiler diets not only contributes to better bird performance but also supports sustainable poultry production practices by reducing the reliance on antibiotics and mitigating potential environmental impacts (8).

This review aims to provide a comprehensive overview of the current state of research on Bacillus subtilis as a probiotic for broilers. It will delve into the mechanisms of action by which Bacillus subtilis exerts its probiotic effects in the avian gastrointestinal tract, the impact on broiler performance, and the immunomodulatory properties that contribute to enhanced disease resistance. Additionally, the review will discuss practical considerations for incorporating Bacillus subtilis-based probiotics into commercial broiler production, addressing challenges, and highlighting areas for further research and development (9).

In conclusion, Bacillus subtilis represents a promising probiotic candidate for broiler production, offering potential benefits for both producers and consumers alike. Understanding its role as a probiotic and its practical applications in poultry nutrition can pave the way towards sustainable and efficient broiler production systems while ensuring the health and welfare of the birds and minimizing the use of antibiotics (10).

1.1. Importance of Broiler Health

Broiler production is a cornerstone of global food systems, providing a major source of meat for billions of people worldwide (11). The industry's efficiency in

delivering high-quality poultry meat at competitive prices is pivotal to food security and economic stability. However, maintaining the health and productivity of broilers is a complex challenge fraught with numerous issues, one of the most pressing being the management of pathogenic bacteria in poultry (12).
Pathogenic bacteria such as Salmonella and Escherichia coli are significant threats to broiler health. These pathogens can lead to a range of serious diseases, including salmonellosis and colibacillosis, which not only compromise the well-being of the birds but also pose risks to human health through the contamination of meat products (13). Infected broilers often exhibit symptoms such as diarrhea, reduced growth rates, and increased mortality, resulting in substantial economic losses for producers (14). Moreover, the need to control these pathogens frequently leads to the use of antibiotics, which can contribute to antibiotic resistance and further complicate disease management (15).
Effective strategies to mitigate bacterial infections are essential for ensuring the health and productivity of broiler flocks. Advances in poultry management practices, including the use of probiotics, offer promising alternatives to traditional antibiotic treatments (16). Probiotics, particularly those containing beneficial microorganisms like Bacillus subtilis, have gained attention for their potential to enhance gut health and prevent the colonization of harmful bacteria (17). By leveraging the natural mechanisms of probiotics to outcompete pathogens and strengthen the gut microbiota, poultry producers can improve the overall health and efficiency of their flocks while reducing reliance on antibiotics (18).

1.2. Problem of Pathogenic Bacteria

Pathogenic bacteria, particularly Salmonella and Escherichia coli, are major concerns in broiler production due to their impact on both poultry health and food safety (19).
Salmonella is a genus of bacteria known for causing salmonellosis, a serious infection that can affect both poultry and humans. In broilers, Salmonella can establish itself in the intestinal tract, leading to symptoms such as diarrhea, fever, and dehydration. Infected birds often suffer from decreased growth rates and increased mortality. The presence of Salmonella in poultry also poses a significant risk of contamination in meat products, which can lead to foodborne illness outbreaks in humans (20). This pathogen is particularly problematic due to its ability to survive in various environmental conditions and its potential for rapid spread within flocks (21).
Escherichia coli (E. coli), while commonly found as a normal inhabitant of the avian gut microbiota, can become pathogenic under certain conditions. Pathogenic strains of E. coli, such as those producing enterotoxins, can cause colibacillosis, which manifests as severe enteritis or septicemia in broilers (22). This condition can result in high morbidity and mortality, as well as economic losses due to reduced feed efficiency and increased treatment costs. Pathogenic

E. coli strains are often associated with poor sanitation, overcrowding, and stress, all of which can disrupt the balance of the intestinal microbiota and promote the proliferation of harmful bacteria (23).

The persistence and proliferation of these pathogens in the broiler intestines are influenced by various factors, including environmental conditions, diet, and management practices (24). The ability of Salmonella and pathogenic E. coli to colonize and persist in the gut highlights the need for effective intervention strategies to manage these infections.

Control measures traditionally involve the use of antibiotics, but this approach can lead to the development of antibiotic-resistant strains, posing additional challenges to poultry health management (25). As a result, there is a growing interest in alternative strategies, such as the use of probiotics, to mitigate the impact of these pathogens and enhance gut health in broilers (26). Probiotics like Bacillus subtilis are being explored for their potential to out compete harmful bacteria, modulate the gut microbiome, and improve overall poultry health (27).

1.3. Role of Probiotics:

Probiotics are live microorganisms that, when administered in adequate amounts, confer health benefits to the host by enhancing the balance of the gut microbiota. In poultry, probiotics are increasingly recognized for their potential to improve gut health, boost immune function, and control pathogenic bacteria. They function through several mechanisms, including competition for nutrients and adhesion sites, production of antimicrobial substances, and modulation of the host's immune response [28].

One prominent probiotic in poultry nutrition is Bacillus subtilis. This bacterium is a Gram-positive, spore-forming microorganism known for its ability to survive harsh conditions and colonize the gut effectively. Bacillus subtilis produces various antimicrobial compounds, such as bacteriocins and enzymes, which can inhibit the growth of pathogenic bacteria like Salmonella and E. coli [29,30]. Additionally, it competes with harmful pathogens for resources and attachment sites in the intestinal tract, further reducing their ability to colonize [31].

The potential benefits of Bacillus subtilis in poultry include:

- Enhancement of Gut Health: By promoting a balanced microbiota, Bacillus subtilis supports optimal digestion and nutrient absorption [32].
- Reduction of Pathogenic Bacteria: The antimicrobial activity of Bacillus subtilis helps inhibit the growth of harmful pathogens [33].
- Immune System Modulation: Bacillus subtilis may enhance the host's immune response, improving resistance to infections [34].
- Improved Performance: Healthier broilers with reduced pathogen load often exhibit better growth rates and feed conversion ratios [35].

The use of Bacillus subtilis as a probiotic offers a promising alternative to traditional antibiotics, aligning with the growing emphasis on sustainable and health-conscious poultry management practices [36].

1.4. Objective of the Review

The objective of this review is to systematically summarize and evaluate current research on the effects of Bacillus subtilis on the colonization of Salmonella and E. coli in the intestines of broilers. By reviewing in vivo and in vitro studies, we aim to elucidate the mechanisms through which Bacillus subtilis influences these pathogenic bacteria, assess its efficacy in reducing their populations, and discuss the implications for poultry health management. This comprehensive overview seeks to provide insights into the potential role of Bacillus subtilis in enhancing gut health and improving the overall productivity and welfare of broiler chickens.

2. Bacillus subtilis as a Probiotic

2.1. Characteristics of Bacillus subtilis

Bacillus subtilis is a Gram-positive, rod-shaped bacterium belonging to the family Bacillaceae. It is widely known for its ability to form resilient spores, which allow it to survive harsh environmental conditions, such as high temperatures and desiccation. These spores make Bacillus subtilis particularly well-suited for use as a probiotic in poultry, as they can remain viable through feed processing and delivery [37].

- **Microbial Properties:** Spore Formation: The ability to form spores enhances its stability and longevity in various environments, including the gastrointestinal tract of poultry [38]. Growth Characteristics: Bacillus subtilis exhibits robust growth in a wide range of temperatures and pH levels, making it adaptable to the variable conditions of the poultry gut [39]. Metabolic Activity: It produces various enzymes, such as proteases, amylases, and cellulases, which aid in the breakdown of complex nutrients and improve feed digestibility [40].
- **Biochemical Properties:** Antimicrobial Production: Bacillus subtilis synthesizes a range of antimicrobial compounds, including bacteriocins, antibiotics, and lipopeptides (such as surfactin), which inhibit the growth of pathogenic microorganisms [41]. Biofilm Formation: It can form biofilms on gut mucosa, creating a protective barrier that prevents the colonization of harmful pathogens [42].

2.2. Mechanism of Action Bacillus subtilis exerts its probiotic effects through several key mechanisms:

1. **Competition for Nutrients and Adhesion Sites:** Bacillus subtilis competes with pathogenic bacteria for essential nutrients and adhesion sites in the intestinal lining. By occupying these sites and consuming available

nutrients, it reduces the resources available to harmful pathogens like Salmonella and E. coli, thereby limiting their growth and colonization [43].

2. **Production of Antimicrobial Substances:** The bacterium produces a variety of antimicrobial compounds that directly inhibit the growth of pathogenic bacteria. These substances disrupt the cell membranes of pathogens, interfere with their metabolism, and inhibit their ability to adhere to the gut mucosa [44].
3. **Modulation of Gut Microbiota:** Bacillus subtilis promotes the growth of beneficial gut microbiota while suppressing harmful microorganisms. This balance helps maintain a healthy intestinal environment and enhances overall gut health [45].
4. **Immune System Stimulation:** It stimulates the host's immune system, enhancing the production of immune cells and cytokines that help fight off infections. This immune modulation contributes to the overall resistance of broilers to pathogenic challenges [46].

2.3. Benefits in Poultry

The use of Bacillus subtilis as a probiotic in poultry offers several advantages:

1. **Improved Growth Performance**: Bacillus subtilis enhances nutrient digestibility and absorption, leading to better feed conversion ratios and improved growth rates in broilers [47].
2. **Reduction in Pathogen Load:** By inhibiting the growth and colonization of pathogenic bacteria such as Salmonella and E. coli, Bacillus subtilis helps reduce the incidence of gastrointestinal diseases and related symptoms [48].
3. **Enhanced Immune Response:** It boosts the immune system's ability to respond to infections, reducing the need for antibiotics and improving overall flock health [49].
4. **Stress Mitigation:** Bacillus subtilis helps in alleviating stress-related conditions in poultry, contributing to a more resilient and robust flock [50].
5. **Better Feed Utilization:** The enzymatic activity of Bacillus subtilis improves the breakdown of feed components, leading to more efficient use of nutrients and reducing feed wastage [51].

3. Salmonella and E. coli in Broilers

3.1. Pathogenicity of Salmonella

Salmonella is a major pathogen in poultry that causes significant health and economic issues. The pathogenicity of Salmonella in broilers can be detailed as follows:

1. **Clinical Symptoms:** Infected broilers often exhibit symptoms such as diarrhea, fever, dehydration, and general malaise. In severe cases, Salmonella infection can lead to systemic disease, characterized by septicemia and high mortality rates [52].

2. **Impact on Growth and Production:** Salmonella infections can impair growth rates and feed conversion efficiency. The stress and energy expenditure associated with the infection reduce the birds' overall performance, leading to decreased meat production and increased feed costs [53].
3. **Economic Consequences:** The presence of Salmonella in broilers poses serious economic challenges due to increased veterinary costs, potential losses from reduced growth performance, and the risk of contamination in meat products. This contamination can lead to foodborne illnesses in humans, resulting in regulatory penalties and loss of market access [54].
4. **Public Health Risk:** Salmonella is a major concern for public health as it can be transmitted to humans through the consumption of contaminated poultry products. This can lead to salmonellosis, which presents symptoms such as gastroenteritis, abdominal pain, and fever in humans [55].

3.2. Pathogenicity of E. coli

Pathogenic strains of Escherichia coli (E. coli) also pose significant health risks to broilers. The impact of E. coli on poultry includes:

1. **Enteric Diseases:** Pathogenic E. coli strains, such as those producing enterotoxins or adhering to the intestinal mucosa, cause enteric diseases in broilers. These infections can lead to symptoms such as diarrhea, dehydration, and severe enteritis, which can impair growth and increase mortality [56].
2. **Systemic Infections:** Certain pathogenic E. coli strains can lead to systemic infections, including septicemia and pericarditis. These conditions are associated with high mortality and significant economic losses due to reduced bird performance and increased treatment costs [57].
3. **Economic Impact:** The health issues caused by E. coli infections result in decreased feed efficiency, lower growth rates, and higher veterinary expenses. Additionally, E. coli contamination can compromise meat quality and safety, affecting marketability and consumer confidence [58].
4. **Food Safety Concerns**: Pathogenic E. coli strains can pose a risk to human health through contamination of poultry products. Infections in humans can cause symptoms ranging from mild gastrointestinal distress to severe conditions such as hemolytic uremic syndrome (HUS), especially with certain virulent strains [59].

3.3. Mechanisms of Colonization

Both Salmonella and E. coli employ specific mechanisms to establish and maintain infection in the intestinal tract of broilers:

1. **Adherence to Mucosa:** Salmonella and pathogenic E. coli have specialized structures such as fimbriae (pili) and adhesins that facilitate attachment to

the intestinal mucosa. This adherence is critical for colonization and persistence in the gut environment [60].

2. **Invasion of Intestinal Epithelium**: Some strains of Salmonella and E. coli are capable of invading and penetrating the intestinal epithelial cells. This invasion disrupts the integrity of the gut lining, leading to inflammation and further facilitating bacterial spread [61].
3. **Nutrient Acquisition:** Both pathogens can compete with host and commensal bacteria for essential nutrients in the gut. Their ability to utilize available resources more effectively contributes to their persistence and proliferation [62].
4. **Immune Evasion:** Salmonella and E. coli have evolved various mechanisms to evade the host's immune system. For example, Salmonella can alter its surface antigens and inhibit the host's immune response, while pathogenic E. coli may produce toxins that interfere with immune functions and promote disease [63].
5. **Biofilm Formation:** Some strains of these bacteria can form biofilms on the gut epithelium. Biofilms provide a protective environment that enhances bacterial survival and resistance to both host defences and antimicrobial treatments [64].

Your reference list is mostly consistent with Vancouver style, but to fully align with standard formatting, here's how it should be presented, including author formatting, journal name abbreviation, volume(issue), page numbers, and publication year, as per NLM (National Library of Medicine) guidelines:

4. Effect of Bacillus subtilis on Salmonella Colonization

- **4.1. In Vivo Studies:** Review studies that have investigated the effect of Bacillus subtilis on Salmonella colonization in broilers.

1. **(Knap I et al; 2011):** This study evaluated the impact of Bacillus subtilis DSM17299 supplementation on Salmonella colonization and shedding in broiler chickens under production-like conditions. The results showed that supplementation significantly reduced Salmonella shedding by 58% and decreased Salmonella load in the cecum by 3 log units, indicating a substantial reduction in gut colonization. While the study observed potential improvements in feed conversion rate and body weight gain, these were not statistically significant. The findings suggest that Bacillus subtilis DSM17299 can enhance food safety by reducing Salmonella risks in poultry production [65].
2. **(Shanmugasundaram R et al; 2020):** This study assessed the impact of Bacillus subtilis and Bacillus licheniformis probiotic supplementation on broilers challenged with Salmonella enterica serovar Enteritidis. The results showed that probiotic supplementation significantly reduced

Salmonella counts in the cecum and lessened the negative effects of the Salmonella challenge on growth performance, feed consumption, and gut health. Probiotics also enhanced the immune response and protected gut integrity. The study concluded that these probiotics are effective in reducing Salmonella loads and can help improve food safety by reducing contamination in broiler carcasses [66].

3. Xing JH et al; 2021: This study investigated the protective effects of a probiotic strain (BSH) in chicks infected with Salmonella. The results demonstrated that BSH supplementation reduced pathological changes in the liver, decreased Salmonella invasion in the liver and spleen, and improved the survival rate of infected chicks from 26% to 60%. Additionally, BSH helped alleviate disruptions in the intestinal flora caused by Salmonella infection and promoted the proliferation of beneficial bacteria, such as Lactobacillus salivarius, in the cecum. The study highlights BSH as a promising candidate for combating Salmonella infections in poultry [67].
4. **Oh JK et al; 2017:** This study explored the effects of Bacillus subtilis CSL2 supplementation on the gut microbiota of broiler chickens, both challenged and unchallenged with Salmonella Gallinarum. Using 16S rRNA gene sequencing, the researchers analyzed microbial shifts over 24 hours. The dominant bacterial phyla were Firmicutes, Bacteroidetes, and Proteobacteria. Salmonella-infected chickens had a higher abundance of Bacteroidetes, while the control and Bacillus-treated groups showed a high presence of Lactobacillus. The Bacillus supplementation also enhanced gut-associated energy mechanisms, helping to maintain microbiota stability and gut integrity. The study concluded that S. Gallinarum infection and B. subtilis CSL2 supplementation significantly influenced the diversity and functionality of the gut microbiota, providing insights into how probiotics can protect against infections in broiler chickens [68].
5. **Hayashi RM et al; 2018:** This study investigated the effects of feeding a blend of three Bacillus subtilis strains (PRO) on broilers challenged with Salmonella enterica serovar Heidelberg. Supplementation with PRO at 250 or 500 g/ton significantly reduced Salmonella counts in the liver and cecum of challenged birds. PRO also enhanced macrophage mobilization in SH-challenged birds but decreased it in non-challenged birds. While PRO at 250 g/ton improved broiler performance without altering gut histology, PRO at 500 g/ton did not enhance performance but caused immune-related histological changes in the ileum. Additionally, the 500 g/ton PRO group exhibited greater cecal microbiota diversity, and the 250 g/ton PRO group showed increased ileal microbiota richness compared to controls. Overall, PRO effectively reduced Salmonellacolonization and modulated immune responses and gut microbiota, with varying effects based on dosage [69].

6. **Sikandar A et al; 2020:** This study compared the effects of Bacillus subtilis probiotic and the antibiotic enrofloxacin on broilers challenged with Salmonella gallinarum. Four groups of chicks were tested: uninfected and untreated (NN), infected and untreated (SN), infected and treated with enrofloxacin (SE), and infected and treated with B. subtilis (SP). While infected groups initially showed reduced growth, by week four, both SE and SP groups had recovered to or exceeded the weight of the NN group. The B. subtilis group exhibited better cellular immunity and increased bursa and thymus weights compared to the other groups. B. subtilis also showed superior efficacy in reducing intestinal pathology and Salmonella numbers in the ceca compared to enrofloxacin. Overall, B. subtilis proved to be as effective as antibiotics in improving growth performance, immune function, and gut health in broilers [70].
7. **Park JH et al; 2018:** This study evaluated the effects of Bacillus subtilis RX7 and C14 on broiler chickens' growth, blood profiles, nutrient retention, and caecal microflora. Four groups of 288 Ross 308 male broilers were fed either a basal diet, the basal diet with 40 ppm avilamycin (positive control), or the basal diet supplemented with 0.1% B. subtilis RX7 or C14. Results showed that both B. subtilis strains significantly improved weight gain and energy retention compared to the control groups. Supplementation also reduced serum haptoglobin levels, indicating lower inflammation. Additionally, the B. subtilis groups had higher caecal Lactobacillus counts and lower Salmonella numbers. Overall, B. subtilis RX7 and C14 enhanced growth performance, nutrient utilization, and gut health in broiler chickens [71].
8. **Nishiyama T et al; 2021:** This study investigated the protective effects of Bacillus subtilis C-3102 against Salmonella enterica infection in specific pathogen-free (SPF) chicks. In the first experiment, chicks fed a basal diet with 1×10^6 CFU/g of B. subtilis C-3102 showed significantly lower S. enterica detection rates in the cecum and liver compared to the control group, although body weight remained unchanged. The second experiment evaluated different dosages of B. subtilis C-3102 and found that higher dosages (1×10^6 CFU/g) were more effective in reducing S. enterica infection rates and promoting its exclusion from the liver and spleen compared to lower dosages. Overall, B. subtilis C-3102 supplementation was effective in decreasing S. enterica infection rates and accelerating its clearance from the chicks [72].
9. **Park JH et al; 2015:** This study evaluated the effects of Bacillus subtilis RX7 and B2A on broilers challenged with Salmonella typhimurium. Four groups of Ross 308 chicks received different diets: a negative control (NC), a positive control with virginiamycin, and diets supplemented with B. subtilis RX7 or B2A. Results showed no differences in growth performance among treatments. However, both Bacillus supplements lowered serum

haptoglobin levels, reduced intestinal and excreta Salmonella populations, and decreased excreta ammonia emissions compared to the NC group. Additionally, while breast meat quality parameters like pH, color, and water-holding capacity were unaffected, supplementation improved drip loss and increased relative gizzard weights. Overall, B. subtilis RX7 and B2A demonstrated effectiveness in mitigating Salmonella and ammonia issues, and enhancing meat quality under challenge conditions [73].

Maruta K at al: 1996: In experiments with chickens, Bacillus subtilis C-3102 was found to effectively reduce intestinal pathogens such as Campylobacter and Salmonella. Laboratory trials showed a significant decrease in the presence of Campylobacter in the experimental group, with only one positive case out of ten by day 49, compared to six in the control group. Similarly, Salmonella colonization was lower in the experimental group by day 10 and remained reduced compared to controls. Field trials confirmed these findings with significant decreases in Campylobacter and Salmonella detection rates and numbers, as well as reductions in Clostridium perfringens and Enterobacteriaceae. Additionally, a significant increase in lactobacilli was observed, highlighting B. subtilis C-3102's efficacy as a probiotic additive in improving intestinal health [74].

5. Effect of Bacillus subtilis on E. coli Colonization

1. Jeong JS et al; 2014: In a study evaluating Bacillus subtilis C-3102, the probiotic was found to positively impact broiler performance, nutrient digestibility, and intestinal health. Supplementation with B. subtilis at 300 and 600 mg/kg of feed significantly increased Lactobacillus counts and reduced Escherichia coli, Clostridium perfringens, and Salmonella levels in the cecum, ileum, large intestine, and excreta compared to the control group. Broilers fed B. subtilis showed improved average daily gain (ADG), feed conversion ratio, and digestibility of dry matter and gross energy. Additionally, ammonia emissions were reduced. While there were no significant differences in meat quality, organ weights, or blood profiles, the study confirmed that B. subtilis C-3102 enhances growth performance and feed efficiency with minimal side effects [75].

2. Ciurescu G et al; 2020: This study assessed the effects of Bacillus subtilis (BS) ATCC 6051a as a probiotic in broiler diets with two protein sources: soybean meal (SBM) and cowpea seeds (CWP). Broilers fed BS, regardless of the protein source, showed significantly improved body weight gain (BWG) and feed efficiency, especially during the grower and finisher periods. BS supplementation also decreased abdominal fat, cecum weight, and cecal pH, while increasing tibia bone phosphorus concentration. Additionally, BS reduced populations of Escherichia coli and Staphylococcus spp. in the cecum and excreta. Carcass traits, bone mineralization, and overall growth performance were enhanced with BS, indicating its beneficial impact on broiler diets with either SBM or CWP [76].

3. **Zhang S et al 2021:** In a study investigating the effects of Bacillus subtilis (B. subtilis) on chickens, feeding a diet containing this probiotic for 21 days led to significant improvements in various health and performance metrics. Chickens on the B. subtilis diet showed increased body weight, elevated levels of serum immunoglobulins IgA and IgM, and enhanced intestinal health, characterized by greater villus height, shallower crypt depth, and a higher VH/CD ratio in the jejunum. Additionally, B. subtilis improved intestinal microbiota balance, fostering beneficial bacteria while inhibiting pathogenic ones. Following an E. coli challenge, chickens fed with B. subtilis had a higher survival rate (66.67%) and lower E. coli levels in their spleens and lungs compared to controls. The probiotic also stimulated toll-like receptor 4, leading to increased production of proinflammatory cytokines and enhanced innate immune responses. Overall, B. subtilis in the diet positively affected growth performance, immune system function, intestinal morphology, and disease resistance in chickens [77].

4. Wu BQ et al; 2011: A new strain of Bacillus subtilis, identified as KD1, was isolated from healthy broilers and its potential as a probiotic was assessed. The strain demonstrated excellent growth characteristics and high tolerance to gastric acid and bile salts. Notably, B. subtilis KD1 produced a substantial amount of neutral protease (1,369.3 U/mL) during its logarithmic growth phase. In an animal trial, broilers were fed B. subtilis KD1 in varying concentrations (10^9, 5 × 10^9, and 10^10 bacilli/kg of feed), which led to a significant improvement in intestinal flora. Specifically, the strain increased lactobacilli and reduced Escherichia coli levels compared to controls. These results suggest that B. subtilis KD1 is a promising probiotic for enhancing gut health in broilers [78].

5. La Ragioneet al; 2001: In an experiment with newly hatched specific pathogen-free chicks, a single oral dose of 2.5×10^8 Bacillus subtilis spores effectively suppressed Escherichia coli O78 infection, a strain known for causing avian colibacillosis. The B. subtilis spores reduced E. coli colonization in deep organs by over 90% and in the intestine by more than 70%. E. coli shedding was significantly lower throughout the 35-day study period. Although B. subtilis persisted in the chicks' intestines, its numbers declined over time. When the same dose of E. coli was administered 5 days after the initial B. subtilis treatment, the protective effect of the spores was lost [79].

6. Manafi M et al; 2017: In a study with 360 Ross 308 chicks, the impacts of Bacillus subtilis (BS) and bacitracin methylene disalicylate (BMD) on broiler performance were evaluated following an E. coli challenge. The chicks were divided into six treatment groups: control, E. coli alone, control + 0.1% BS, control + 0.05% BMD, E. coli + BS, and E. coli + BMD. Both BS and BMD significantly improved body weight and feed conversion ratio (FCR) compared to the control. However, they did not fully mitigate the growth reduction caused by E. coli. E. coli-infected chicks had reduced vaccine titers and increased levels of albumin, globulin, cholesterol, triglycerides, LDL, ALT, and ALP, which were partially addressed by BS and BMD. Both probiotics also improved intestinal health metrics and reduced harmful bacterial populations in the ceca [80].

7. Molnár AK et al; 2011: An experiment evaluated the effects of various Bacillus subtilis concentrations on broiler chickens' productivity, carcass quality, immune response, and gut microflora. Five groups were studied: a control with no B. subtilis and four groups with different B. subtilis concentrations. Supplementation with B. subtilis significantly increased weight gain and improved feed conversion rates across all supplemented groups. Chickens receiving B. subtilis had larger breast muscles but smaller carcasses and thighs compared to the control. Enhanced immunological responses were observed in these chickens, including increased lymphohistiocytic infiltration and solitary lymphoid follicles in the mucosa, and a stronger response to Newcastle disease virus (NDV) vaccination. Although higher B. subtilis concentrations did not boost Lactobacillus levels in the ileum or caecum, they significantly reduced E. coli populations. Overall, B. subtilis supplementation improved growth performance and immune response while reducing harmful bacteria [81].

8. Teo AL et al; 2006: An experimental trial tested Bacillus subtilis PB6 (CloSTAT), isolated from healthy chicken gut, for its impact on broiler performance in both infected and uninfected conditions. Broilers supplemented with B. subtilis PB6 showed significant improvements in feed conversion ratio (FCR), with a 10- and 8-point enhancement in uninfected birds compared to negative and antibiotic controls, respectively. Infected birds treated with B. subtilis PB6 had a notable 15-point FCR improvement over those in the negative control group. Additionally, body weights increased by 97 g in uninfected and 152 g in infected birds receiving B. subtilis PB6 compared to the control. Mortality rates in infected birds decreased from 14% to 6% with antibiotics and 8% with B. subtilis PB6. B. subtilis PB6 also maintained higher levels of lactobacilli in both uninfected and infected birds, demonstrating its potential as a growth promoter and alternative to traditional antibiotics [82].

6. Bacillus subtilis vs. Other Probiotics: Comparative Efficacy in Controlling Salmonella and E. coli

Bacillus subtilis is a well-studied probiotic known for its potential in controlling harmful gut bacteria like Salmonella and E. coli. Here's how it compares with other probiotics:

Sr. No.	Probiotics	Effectiveness	Comparison
1	**Lactobacillus species**	Lactobacillus probiotics, such as Lactobacillus acidophilus and Lactobacillus rhamnosus, are widely used for their	While Lactobacillus is effective against E. coli, Bacillus subtilis has shown a strong ability to reduce both Salmonella and E. coli

		ability to inhibit pathogenic bacteria through competition for nutrients and production of antimicrobial substances like lactic acid. [83]	populations. Studies often indicate that Bacillus subtilis can be more resilient under harsh gut conditions and offer prolonged benefits due to its spore-forming capability. [84]
2	**Saccharomyces boulardii**	This yeast probiotic is effective in controlling E. coli and has shown some success against Salmonella. It works by enhancing intestinal barrier function and producing antimicrobial peptides.[85]	Saccharomyces boulardii is less effective against Salmonella compared to Bacillus subtilis, which may provide a more comprehensive control over both Salmonella and E. coli due to its broader spectrum of antimicrobial activity.[86]
3	**Enterococcus faecium**	This probiotic can help control E. coli and has been shown to compete with Salmonella in the gut. [87]	While Enterococcus faecium is beneficial, Bacillus subtilis often demonstrates superior efficacy in reducing both Salmonella and E. coli due to its ability to produce a range of antimicrobial compounds and spores that enhance its survival in the gastrointestinal tract. [88]

Overall, Bacillus subtilis tends to be highly effective against both Salmonella and E. coli, sometimes outperforming other probiotics due to its spore-forming nature and broad-spectrum antimicrobial activity.

6.2. Synergistic Effects: Interactions between Bacillus subtilis and Other Probiotics or Prebiotics

Synergistic interactions between Bacillus subtilis and other probiotics or prebiotics can enhance their overall efficacy:

Sr.No.	Probiotic	Synergy	Effectiveness
1	**With Lactobacillus species**	Combining Bacillus subtilis with Lactobacillus species can result in improved	Studies have shown that such combinations can lead to enhanced inhibition of Salmonella

		gut health, as they may complement each other's mechanisms. For instance, Lactobacillus can acidify the gut environment, which may help Bacillus subtilis in maintaining a stable presence and promoting a more balanced microbiota. [85]	and E. coli due to the additive effects of both probiotics on gut health and pathogen suppression.[85]
2	**With Prebiotics (e.g., inulin, oligosaccharides):**	Prebiotics provide substrates that support the growth and activity of beneficial bacteria, including Bacillus subtilis. The presence of prebiotics can enhance the survival and colonization of Bacillus subtilis in the gut, boosting its effectiveness against pathogens. [88]	This combination often results in improved gut microbiota balance, reduced pathogen load, and enhanced overall health. For instance, prebiotics can stimulate the growth of beneficial bacteria that complement the actions of Bacillus subtilis.[88]
3	**With Other Probiotics (e.g., Saccharomyces boulardii)**	Bacillus subtilis and Saccharomyces boulardii can work synergistically to enhance gut health. Saccharomyces boulardii may aid in managing diarrhea and intestinal infections, while Bacillus subtilis contributes through its broad-spectrum antimicrobial properties.[87]	Such combinations may offer a more comprehensive approach to gut health, reducing pathogenic bacteria and promoting a healthier microbiome through complementary mechanisms.[87]

In summary, Bacillus subtilis demonstrates significant efficacy in controlling harmful bacteria like Salmonella and E. coli, often outperforming or complementing other probiotics. Its synergistic effects with other probiotics and prebiotics can further enhance gut health and pathogen control.

7. Practical Implications and Recommendations

7.1. Application in Broiler Production

Incorporating Bacillus subtilis into Poultry Feed

Incorporating Bacillus subtilis into poultry feed involves several practical considerations to maximize its benefits:

- **Formulation**: Bacillus subtilis can be included in broiler feed as a direct-fed microbial (DFM) additive. It is typically blended with other feed ingredients to ensure even distribution. Using pelleted or mash feed allows effective integration of the probiotic [84, 86].
- **Integration**: Introducing Bacillus subtilis from the early stages of broiler growth helps establish a beneficial microbial community in the gut. This can enhance growth performance, improve feed conversion, and boost immune responses [88].
- **Feed Management**: Proper storage of feed is essential to maintain the viability of Bacillus subtilis. Probiotics are sensitive to environmental factors such as moisture, heat, and oxygen. Appropriate storage facilities help preserve the feed's freshness and effectiveness [84].

7.2. Dosage and Administration

Effective dosages and methods of administration for Bacillus subtilis are critical to achieving the desired outcomes:

- **Dosage**: Studies and field practices suggest Bacillus subtilis is effective at dosages ranging from 1×10^6 to 1×10^9 CFU per kg of feed. The exact dosage may vary depending on the strain, production system, and broiler health status [86, 89].
- **Administration Methods**: Bacillus subtilis may be added to feed either as a dry powder or in liquid form. Uniform mixing is essential to ensure consistent probiotic levels across the feed. Manufacturer guidelines should be followed for optimaldosage and mixing techniques [84].
- **Monitoring**: Routine monitoring of broiler performance and health helps evaluate the effectiveness of probiotic supplementation. Improvements in growth rates, feed conversion ratio, and overall health status can indicate successful administration [88].

7.3. Potential Challenges

Several issues may impact the efficacy of Bacillus subtilis as a probiotic in poultry production:

- **Survival in Feed**: The viability of Bacillus subtilis may be compromised by feed processing and storage conditions. However, spore-forming strains of Bacillus subtilis are more resistant to environmental stressors, including high heat and humidity [86,90].

- **Strain Variability**: The effectiveness of Bacillus subtilis depends on the specific strain used. Different strains vary in their ability to adhere to the gut lining, produce antimicrobial substances, and inhibit pathogens [89].
- **Interactions with Other Additives**: Bacillus subtilis may interact with other feed components such as antibiotics, vitamins, or minerals. Ensuring compatibility is necessary to avoid reducing probiotic efficacy [87].
- **Regulatory and Cost Considerations**: The use of probiotics in animal feed requires regulatory approval, which varies by region. Additionally, cost-effectiveness should be evaluated to balance input cost with improvements in performance and health [87].
- **Monitoring and Adjustment**: Continuous monitoring of the flock's performance allows for timely adjustments in probiotic dosage or formulation based on observed outcomes [88].

Conclusion

Incorporating Bacillus subtilis into broiler feed can significantly enhance growth, immune response, and feed efficiency. Careful attention to formulation, dosage, administration, and potential challenges is necessary to maximize its probiotic benefits.

8. Future Research Directions

8.1. Gaps in Knowledge

Despite growing evidence supporting the use of Bacillus subtilis in poultry production, several research gaps remain. **Strain-specific efficacy** needs further investigation to understand how different Bacillus subtilis strains perform under various conditions and their precise mechanisms of action [89,5]. Long-term studies are required to evaluate the **sustained impact** of supplementation on broiler health, performance, and **microbiota stability**, including potential adverse effects or resistance development [91, 87].

Research into **interactions with feed components** is essential to identify synergistic or antagonistic effects with prebiotics, antibiotics, and vitamins, thereby optimizing feed formulations [92,87]. At a deeper level, exploring the **molecular mechanisms** through which Bacillus subtilis contributes to gut barrier function, immune modulation, and competitive exclusion of pathogens will enhance the precision of probiotic application [89,94]. Finally, understanding the **variability in response among different broiler breeds** or genetic lines will support tailored strategies that maximize probiotic effectiveness across diverse poultry populations [93].

Sr.No.	Area	Research Needed	Focus
1	**Strain-Specific Efficacy**	To understand how different strains of Bacillus subtilis perform under various conditions and their specific mechanisms of action. While some strains show promising results, the efficacy can vary significantly based on the strain.	Investigate strain-specific benefits, including optimal conditions for activity and interactions with other gut microbiota.
2	**Long-Term Effects**	To assess the prolonged impact of Bacillus subtilis supplementation on broiler health, performance, and overall microbiota stability.	Examine the long-term sustainability of health benefits and potential for resistance development or adverse effects over extended periods.
3	**Interaction with Feed Components**	To understanding how Bacillus subtilis interacts with other feed additives, including prebiotics, antibiotics, and vitamins, is crucial.	Identify any synergistic or antagonistic effects with common feed ingredients and additives to optimize feed formulations.
4	**Mechanisms of Action**	For further exploration into the specific mechanisms by which Bacillus subtilis exerts its beneficial effects on gut health and pathogen control.	Study the probiotic's impact on gut barrier function, immune modulation, and competition with pathogens at a molecular level.
5	**Host-Specific Responses**	To investigate how different broiler breeds or genetic backgrounds respond to Bacillus subtilis supplementation.	Tailor probiotic applications to breed-specific needs and performance characteristics.

8.2. Emerging Trends

Advancements in probiotic research are shaping several emerging trends aimed at enhancing poultry health, performance, and sustainability. One key development is the rise of **precision probiotics**, which are designed based on the host's genetic makeup and gut microbiota composition to achieve targeted and consistent outcomes [95]. These customized formulations may improve colonization efficiency and functional compatibility within specific poultry breeds or production systems.

The use of **combination therapies**, particularly **symbiotic**—integrating probiotics with prebiotics or bioactive compounds—is gaining attention for their ability to deliver synergistic benefits. These combinations can support more robust gut health, enhance immune modulation, and improve resistance to enteric pathogens such as Salmonella and Clostridium perfringens [92].

Innovations in **microbiome engineering** are advancing strategies for fine-tuned manipulation of the intestinal microbiota. Through selective enrichment or exclusion of microbial populations, these techniques offer opportunities to promote beneficial communities and suppress disease-associated microbes [96]. Simultaneously, progress in **spore technology** is improving the resilience and shelf-life of spore-forming probiotics like Bacillus subtilis, allowing them to withstand feed processing stressors such as heat and moisture without losing viability [84].

Furthermore, the integration of **artificial intelligence (AI)** and **data analytics** is enabling more precise and adaptive probiotic applications. Real-time monitoring of flock health, performance metrics, and environmental data can inform dynamic adjustments in probiotic dosing and combinations, supporting more responsive and data-driven management strategies [97].

Lastly, there is increasing emphasis on **environmental sustainability**. Probiotic use has been shown to reduce the reliance on antibiotics, improve feed conversion efficiency, and lower nitrogen and phosphorus excretion, thereby contributing to improved waste management and reduced environmental impact in poultry operations [86].

Collectively, these innovations hold the potential to bridge current knowledge gaps, refine probiotic deployment strategies, and support the transition to more **efficient, sustainable, and antibiotic-free poultry production systems**.

Sr. No.	Area of Emerging Trends	Development	Trend
1	**Precision Probiotics**	Advances in genomics and microbiome research are leading to the development of precision	Customizing probiotic strains based on genetic and microbial profiles of the poultry to enhance

		probiotics tailored to the specific needs of individual animals or flocks.	efficacy and performance.
2	**Combination Therapies**	Combining probiotics with prebiotics (synbiotics) or other bioactive compounds to enhance overall gut health and pathogen resistance.	Research into synergistic effects of multi-strain or multi-ingredient formulations to provide broader health benefits.
3	**Microbiome Engineering**	Using advanced techniques to engineer the gut microbiome of poultry, including targeted delivery systems for probiotics and prebiotics.	Developing tools and technologies for more precise modulation of the gut microbiome to improve health and productivity.
4	**Spore Technology**	Innovations in spore technology are improving the stability and viability of probiotics during feed processing and storage.	Enhanced formulations of spore-forming probiotics to ensure better survival and efficacy in challenging feed environments.
5	**Integration of AI and Data Analytics**	Utilizing artificial intelligence and data analytics to optimize probiotic use and predict health outcomes based on real-time data.	Implementing smart farming technologies to monitor and adjust probiotic supplementation dynamically based on performance data and environmental conditions.
6	**Environmental and Sustainability Focus**	Research into how probiotics like Bacillus subtilis can contribute to more sustainable poultry production systems.	Exploring the role of probiotics in reducing the environmental impact of poultry farming, such as lowering antibiotic use and improving waste management.

Future research in these areas will help bridge current knowledge gaps, refine probiotic applications, and leverage emerging technologies to enhance poultry health and productivity.

9. Conclusion

This review examines the role of Bacillus subtilis in broiler production, focusing on its efficacy in controlling pathogens such as Salmonella and Escherichia coli, and its broader impact on poultry health and productivity.

- **Effectiveness against Pathogens**: Bacillus subtilis has shown strong efficacy in reducing gut colonization by Salmonella and E. coli in broilers. Its antagonistic activity helps lower pathogen load, reduce disease incidence, and support a healthier gut microbiota [86,83].
- **Performance Improvement**: Supplementation with Bacillus subtilis enhances broiler growth performance, leading to improved body weight gain and feed conversion ratio (FCR), even under stress or pathogen challenge [88,84].
- **Immune Response Enhancement**: Bacillus subtilis promotes immune system function, indicated by elevated immunoglobulin levels and improved vaccine response. It stimulates gut-associated lymphoid tissue (GALT), supporting a more effective defense system [86,92].
- **Intestinal Health**: Morphological improvements such as increased villus height and improved villus-to-crypt ratio have been reported in broilers receiving Bacillus subtilis, facilitating nutrient absorption and gut function [88].
- **Comparative Efficacy**: Compared with other probiotics, Bacillus subtilis often exhibits equal or superior antimicrobial effects and gut health benefits. Its spore-forming nature grants it greater stability and survivability during feed processing [83,84].
- **Practical Applications**: The effectiveness of Bacillus subtilis is dose-dependent. Proper dosage, timing, and storage conditions are critical to maintaining probiotic viability and ensuring benefits [86,84].
- **Future Research**: Research gaps include the need for strain-specific evaluations, long-term efficacy data, and insights into interactions with dietary components. Emerging trends such as precision probiotics, combination therapies, and microbiome engineering are expected to enhance probiotic applications [92,88].

Bacillus subtilis is a potent probiotic for broiler production, particularly in mitigating Salmonella and E. coli colonization. Its positive effects on growth performance, gut morphology, and immune function support its role as a valuable alternative to antibiotics. With continued research and formulation optimization, Bacillus subtilis holds promise for sustainable and effective poultry health management.

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