Use of *Bacillus* Species as Probiotic in Broiler Production

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Abstract

The rising demand for antibiotic-free chicken farming has generated interest in probiotics as viable alternatives. *Bacillus* species stand out due to their spore-forming ability, robustness, and multiple benefits. This chapter covers the significant significance of Bacillus probiotics in improving broiler performance, emphasizing their modes of action, which encompass competition exclusion, immunomodulation, enzyme synthesis, and enhanced gut morphology. *Bacillus* spp. help in maintaining gut microbiota and increase the metabolism of nutrients; this enhances protection and results in improved feed conversion ratios (FCR), body weight gain (BWG), and resistance to disease—mainly necrotic intestinal diseases and coccidiosis. In terms of adaption to changing ecological and gut circumstances, *Bacillus* spp. outcompete other probiotics such as *Saccharomyces* and *LactoBacillus*. Moreover, this chapter explores the potential of *Bacillus* probiotics to reduce antibiotic usage while improving the sustainability of chicken production. In view of such benefits, strain specificity, ambient conditions, and formulation issues must be addressed in order to maximize their use. Future research directions include the determination of strain-specific benefits, studying synergistic mixtures of probiotics, and the development of cost-effective manufacturing methods. The use of *Bacillus* probiotics in chicken production can increase productivity, animal welfare, and consumer safety while meeting global expectations for an eco-friendly, antibiotic-free food chain.

Keywords: *Bacillus* probiotics; Gut health; Feed conversion ratio; Antibiotic alternatives; Poultry production

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Introduction

One of the biggest industries in the world for food production is classified as farming of poultry birds (Sharopatova et al., 2020). The extra usage of antibiotics has been practised in chicken farming in developed countries and continues in developing countries for fruitful outcomes, which results in the development of resistance in them and faced a lot of criticism for their usage in human food. Alternative to these antibiotics have been introduced as a novel concept to overcome this criticism; probiotics belong to them. Probiotics can lead to improved productivity and better feed conversion in contemporary broiler chicken farming. These are defined as living microbial substances which have the potential to improve the gut microbiome for better outcomes when consumed in therapeutical dosage (Chen et al., 2013). Direct inclusion of them results in a positive impact on the microflora of the gut, which has given them characteristics of an antibiotic substitute (Bahaddad et al., 2023). They can disrupt the growth of bacteria that are the culprit of illness and death of farm animals, including *Salmonella*, *Escherichia coli*, and *Clostridium* species (Remus et al., 2014). The probiotic industry, which is expected to expand by 14% between 2023 and 2030 and reach USD 77 billion in 2022, has benefited from the greater knowledge of its health advantages (Payne et al., 2024). Four essential requirements must be met for an organism to be classified as a probiotic (Fenster et al., 2019): it must be a microbe, be alive when consumed, be given in enough amounts (minimum 106cfu/g to 109cfu/g), and have positive impacts on the host's health.

It is crucial to establish and preserve a healthy microbiota in the bird's digestive system because it aids in the removal of harmful bacteria (Yousaf et al., 2022). Day-old hatched chicks have sterile stomachs. At the age of about seven to ten weeks, these chicks start picking up pathogens from the environment. This period is most critical because chicks can easily get infection from outer environment harmful bacteria. With time, a balance between helpful and harmful microorganisms is established. Consequently, when internal or external factors such as stress or viral pressure disturb the balance between the two, probiotic supplementation becomes an integral option (Rashid et al., 2023).

In the current era, the consumption of alternatives to antibiotics especially *bacillus* spp. has taken a swift change in the poultry feed industry along with its usage in human consumption as probiotics. This change is living proof of the well-established advantages of *Bacillus*, such as its usage in the procurement of gastrointestinal problems, urinary tract-related infections, the production of digestive enzymes, antimicrobials, and immunological modulation (Elshaghabee et al., 2017). A huge number approximately, 700 to 795 antibiotics have been produced from *Bacillus* bacteria (Sorokulova, 2013). This genus has the credit for many productive mechanisms like aid in the overall better performance of the immune system, and GI system (Dodoo et al., 2017). *Bacillus* projects "good" microflora, which has advantages including optimizing gut function, reducing food sensitivities and allergies, increasing nutritional impact, eliminating pathogens competitively, and having anti-inflammatory and immune-modulating properties (Chugh & Kamal-Eldin, 2020). *Bacillus*'s proactive health benefits and resilience to harsh conditions make it a more wanted functional food ingredient.

1. Probiotics and Poultry Production

Poultry's production performance is maximised and their gut flora is balanced when probiotics—live, non-pathogenic microorganisms—are included in their diet (Khan et al., 2020). The World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO) define probiotics as "live non-pathogenic micro-organisms, which when used in adequate quantity confer health benefits on the host" (Jha et al., 2020).

Competitive exclusion along with immune system modulation are probiotics' two major mechanisms of action a) Probiotics utilise their power to competitively exit the pathogens by producing inhibitory compounds such as bacteriocins, mucins, defensins, and others, (c) promoting the competition for the available resources, (d) reducing the bioavailability of the poisonous compounds, and (e) altering the host immune system by increasing both innate and adaptive immunity (Hernandez-Patlan et al., 2020).

Probiotics are a very essential dietary supplement for providing a stable bacterial population in the poultry intestine that is necessary for proper immune function and feed assimilation. In poultry farming, probiotics become beneficial for numerous reasons: to maintain a healthy gut microbiota, to increase nutrient intake, and to reduce ammonia output (Dhama & Singh, 2010). Chicks and poultry have been demonstrated to gain advantages from the usage of efficient probiotics. Probiotic spray therapies can prevent and cure *Salmonella* infections in birds, including chicks. Several worldwide studies have proven that the benefits of these additives for poultry nutrition are constant and universal. Several studies have indicated that particular probiotic strains and species improve broiler development efficiency (Fesseha et al., 2021). Incorporating probiotics to layers' and turkeys' diets enhance the number of eggs produced, weight/size, and food absorption ratios (Yousaf et al., 2022). This type of synchronization suggests that local chicken raising procedures might be altered to make better use of these products.

At present, more than 30 probiotic brands are registered with the EU, and mixtures of many varieties of bacteria are permitted under regulation. As per Krysiak et al. (2021) commonly found probiotics include *Bacillus*, *Enterococcus*, *Bifidobacterium*, *Lactococcus*, *Saccharomyces*, *LactoBacillus*, *Streptococcus*, *Candida* and *Aspergillus spp*. Probiotic are basically bacterial isolates which generate chemicals that activate phytases, cellulose, xylanases and proteases. Probiotics are mostly delivered through diet on chicken farms, although additional ways include aerosols, pellets, capsules, pills, powder pouches, and vaccines/drops. The approach stays the same: target the infectious agent. *Saccharomyces cerevisiae*, or baker yeast, is utilized as a probiotic in broiler feed. This yeast contains β-glucan and mannan oligosaccharides, which promote consumption of feed and gain in weight in commercial flocks. *Bacillus*, a spore-forming bacteria, has a greater chance for use as a probiotic in poultry. Its strain may affect the decomposition of non-starch polysaccharides and phytates in feed, as well as the intake of nutrients through secreted enzymes (Popov et al., 2021). *Bifidobacterium spp*. are used as probiotics, either alone or in combination with other bacterial species. It affected the ileal ecology by boosting lactic acid-producing bacteria and decreasing overall coliform numbers (Gul & Alsayeqh, 2022).

2. Characteristics of *Bacillus* Species

Bacillus species are frequently utilized in commercial probiotics for chicken production. Different types of *Bacillus* species includes *B. Subtilis* (*Bacillus natto*), *Bacillus clausii*, *B. cereus*, *B. pumilus*, *B. coagulants*, *B. licheniformis*, and *B. polyfermentans* have been thoroughly studied, because they are suitable as probiotics due to their spore-forming nature and ability to live in extreme temperatures and pH levels. These qualities improve the survival of good bacteria throughout feed preparation and preservation, as well as their transit through the digestive tract. *Bacillus* spp. probiotics have demonstrated antagonism towards selected bacteria *in vitro*, *in vivo* and are viable alternatives to antibiotics for the management of *necrotic enteritis* (Fernández et al., 2019).

Bacillus Subtilis

Bacillus Subtilis produces spore that can withstand harsh environmental conditions such as alkali, acid, and heat. *Bacillus Subtilis* spores can quickly germinate in the digestive system, and for this reason, it is a very good alternative to antibiotics. *Bacillus Subtilis* is an aerobic microorganism that requires high free oxygen in the gut. This can inhibit the growth of pathogenic aerobic bacteria and stimulate the proliferation of anaerobic bacteria such as yeast, *LactoBacillus*, and *Bifidobacterium* (Ramlucken, Lalloo, et al., 2020). The addition of *Bacillus Subtilis* to the diet of broiler chickens enhances the productivity and quality of their carcasses (Ciurescu et al., 2020). *Bacillus Subtilis* may synthesize exogenous digesting enzymes (Abd El-Moneim & Sabic, 2019). In chickens, administering *B. Subtilis* strains solely or in mixture with other bacteria may enhance feed conversion and body weight, minimize *Clostridium perfringens* scarring, expand intestinal villi in *necrotic enteritis*, and regulate the microbiota to boost *LactoBacillus* amounts, while lowering pathogens like *C. perfringens*, *E coli*, and *Salmonella enteritidis*. Probiotic activities of *B. Subtilis* strains towards *C. jejuni* in chicken have been well reported. However, their anti-Campylobacter actions are varied and strain specific *B. Subtilis* is arguably the best-characterized bacterium for the inhibition of *C. perfringens*, and it has been demonstrated to be an efficient probiotic in *in vitro* and *in vivo* investigations (Jayaraman et al., 2013). Chen and Yu, (2022) studied the *B. Subtilis*-fermented products (SFPs) generated using solid-state fermentation and observed enhanced broiler development and decreased gut inflammation during inflammatory trials. The later authors stated that SFPs alter the cecal microbiota in broilers, favoring short-chain fatty acid-producing bacteria and decreasing mucin-degrading bacteria in the digestive tract. *B. Subtilis* treatments resulted in better duodenal performance (Ogbuewu & Mbajiorgu, 2022). The later authors observed improvements in duodenal histomorphological parameters, enhanced villus histomorphometry, and improved ileal VH and VH:CD.

Bacillus licheniformis

Bacillus licheniformis K-508, a type of aerobic probiotic, has the ability to increase the breakdown, intake, and consumption of nutrients. Fermented goods containing *B. licheniformis* can inhibit pathogen development and increase gut health. *Bacillus licheniformis*, a gram-positive and endospore-forming probiotic bacterium, produces surfactin, an antibacterial cyclic lipopeptide, which effectively inhibits *C. perfringens in vitro* (Horng et al., 2019). *B. licheniformis* produces antibacterial cyclic lipopeptide, indicating antimicrobial action against microorganisms

(Lin et al., 2019). *B. licheniformis* can reduce *C. perfringens*-induced necrotic intestines and improve broiler performance during growth. Fermented *B. Licheniformis* fermented compounds have been shown to increase broiler production and reduce gastroenteritis incidence (Chen & Yu, 2020). Probiotics have been shown to change the intestinal microbiota while avoiding pathogen infections in broilers. Adding to broiler diets with *B. licheniformis* HJDY01 improves development, enhances short-chain fatty acid synthesis, and regulates cecal microbiome (Xu et al., 2021). The *B. licheniformis* H2 can restore the ileal microbiota in hens affected with *necrotic enteritis*. Dietary *B. licheniformis* PY79 and *B. licheniformis* CGMCC 1.3448 can enhance egg yield and quality, reduce heat-induced egg loss, and control laying hen reproduction hormone production (Wang et al., 2017).

Bacillus amyloliquefaciens

B. amyloliquefaciens, a very similar microbe to *Bacillus Subtilis*, has been identified as a potential bio-control microorganism capable of producing enzymes including α-amylase and proteases (Hassanein & Soliman, 2010). *B. amyloliquefaciens* has been shown to boost broiler productivity, reduce immunological stress, and improve gut microbiota for disease resistance (Tsukahara et al., 2018). The *B. amyloliquefaciens* US573 strain, collected from volcanic soil in Southern Tunisia, has previously been shown to generate extraordinarily salt-tolerant and highly thermally stable extracellular phytase, with an excellent prospect for use as a supplement in feed industry (Boukhris et al., 2015). Chistyakov et al. (2015) studied that *B. amyloliquefaciens* B-1895 combination improved both broiler body mass and intake of feed.

3. Mode of Action

Ahmad (2006) proposed that probiotics can boost chicken immunity by two mechanisms broadly: (a) bacteria from probiotics moves across the intestinal wall and multiplies to a limited amount, or (b) antigens produced by dead bacteria are taken in and promote immunity. As per the (Vasquez, 2016) *Bacillus* spores have a distinct method of working in the GI-track per se that allows them to soak up toxins and elicit the innate immune system reaction by contact with the host cells' Toll-like receptors (R2 and R4). It leads to activation of the NF-bk pathway. Boosting the numbers of NK cells, activation of cytokines (IFN-g), increase the health of the gut and maintain the microbiota, promote the daily weight gain of the poultry and the FCR. Detailed and well explained mode of action has been given in Figure 1 (Khalid et al., 2022).

Production of Antimicrobial Compounds

Bacilli's release of antimicrobials (*amicoumacin*, *coagulin*, and *Subtilisin*) might limit the expansion of competing bacteria and intestinal pathogens, perhaps providing a probiotic benefit. *Bacilli's* antimicrobial properties are linked to their capacity to create peptides, tiny extrinsic effector molecules, and communicate with hosts through adhesion and attaching characteristics (Khochamit et al., 2015). *Bacilli* release antimicrobials such coagulin, amicoumacin, and *Subtilis* which restrict the spread of competing microorganisms and intestinal pathogens, creating a probiotic effect. Bacteriocin-producing probiotic *Bacillus* spp. might be used to preserve food and treat illnesses in humans and animals. The scientists found that *B. Subtilis* MA139 releases antimicrobial compounds, allowing probiotic microorganisms to limit enterobacteria development while offering a cost-effective solution for producing solid-state fermentation feed. Abdhul et al. (2015) stated that *B. coagulans*, a probiotic bacteria that produces bacteriocin, was found in conventionally fermented seafood from Manipur, India. The pure bacteriocin with reduced molecular weight demonstrated wide antibacterial efficacy against food-borne and clinically relevant infections, with lesser cytotoxicity. The pure bacteriocin with reduced molecular weight demonstrated wide antibacterial efficacy towards food-borne and clinically relevant infections, with lesser cytotoxicity. The later authors described a *B. Subtilis* strain that produces bacteriocin. The partly purified bacteriocin was found to have strong antibacterial action towards ulcerated feet bacterial pathogens, particularly *Klebsiella spp*. Bacteriocin-producing *Bacillus* strains have the ability to be used as a food biopreservative and antibacterial in human and animal illnesses.

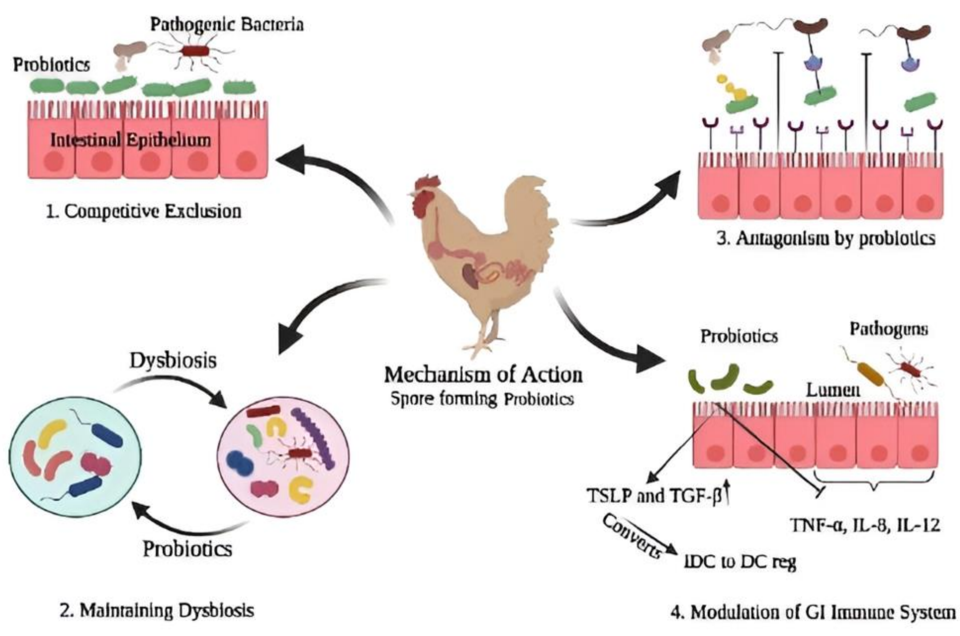

Fig. 1: (Khalid et al., 2022): Probiotics' mechanism of action. (1) Probiotics' competitive exclusion. (2) Probiotics can repair dysbiosis induced by any source. (3) Probiotics create bactericidal chemicals that lyse bacteria, while receptors on their surface deactivate them. (4) Pathogen-exposed gastrointestinal cells create pro-inflammatory chemicals (TNF-α, IL-8, and IL-12). Probiotics reduce pro-inflammatory chemicals and boost anti-inflammatory mediators including TSLP and TGF-β, transforming immature dendritic cells into regulatory dendritic cells. TSLP stands for thymic stromal lymphopoietin, and TGF-β for transforming growth factor-β.

Competitive Exclusion of Pathogens

Competitive exclusion (CE) occurs when probiotic organisms eliminate undesired bacterial infections (Callaway et al., 2008). The CE is also defined as antagonistic actions of probiotics include the formation of organic acids that reduce pH to compete with pathogenic bacteria in gut. *Bacillus* probiotics utilize a variety of strategies to inhibit the expansion of pathogenic species, such as battle for physical attachment points and area, both direct and indirect rivalry for vital nutrients, antimicrobial compound generation, gut microbiome control, and combined effects of the mechanisms (Mingmongkolchai & Panbangred, 2018). Probiotics colonize the epithelium cells of gut, eliminating opportunistic infections through spatial domination. This technique has been utilized to manage infections in poultry, particularly in day-old chicks that are biologically weak and susceptible to colonization by foreign organisms.

A different form of CE is the competing absorption of critical nutrients required for pathogen development. The probiotic's rapid absorption of nutrients including glucose, carbon, and iron allows it to prevent pathogen development. Heterotrophic *Bacillus* spp. are picky and have a greater rate of using organic carbon and protein, giving them an advantage over pathogens. *Bacillus* spp. synthesize siderophores, low-molecular-weight chelating chemicals that promote competing iron absorption and play a role in pathogen elimination.

CE also produces antibacterial chemicals. *Bacillus* spp. may produce a wide range of antimicrobial peptides (AMP), including surfactins, lipopeptides, bacteriocin and bacteriocins-like suppressive compounds. Bacteriocin-mediated death involves destroying pathogenic cells by forming pores, inhibiting cell wall construction, and disrupting DNA, RNA, and protein metabolism. Bacteriocins' antimicrobial efficacy in poultry farming is hard to evaluate *in vivo*, but has been demonstrated *in vitro* through pathogen inhibition experiments. According to Grant et al. (2018), *Bacillus* spp. create several AMPs that damage bacterial membranes. *Bacillus* spp. produced cytotoxic AMP against Eimeria spp., lowering avian coccidiosis and *Clostridium perfringens* colonization.

Another process that results in CE is the synthesis of tight junction proteins in chickens, which produces an ongoing barrier that protects against infections. Gadde et al. (2017) found a substantial rise in tight junction genes in stressed broiler hens fed feeds supplemented with *B. Subtilis*. Probiotics help regulate and maintain the gastrointestinal tract through several methods that boost their effectiveness.

Enzyme Secretion for Nutrient Digestion

Probiotics can boost nutrition intake through two mechanisms: producing digestive enzymes and altering intestinal villi shape. Optimizing feed component costs has led to the use of non-soluble and soluble non-starch polysaccharides (NSP) in carbs. These diets frequently include maize replacements such oats, wheat, rye, and barley. NSP-rich feeds limit intake of nutrients, resulting in reduced development performance. Poultry lack the enzymes needed to break down NSP, leading to poor feed efficiency. *Bacillus* probiotics make desired enzymes, providing a substitute to free enzymes. Probiotics make enzymes only in the presence of a substrate, resulting in a system that is more advanced. Bacilli make enzymes such *β-glucanase*, *α-amylase*, *protease*, *xylanase*, *lipase*, *phytase*, and *cellulase* that aid in carbohydrate, protein, and fat digestion in the poultry industry (Latorre et al., 2015). They make glycosyl hydrolase enzymes which tear down compound NSP into edible monosaccharides, lowering intestinal stiffness and boosting absorption. Enzymes can improve the amount of metabolizable energy (AME) in poor-quality food items by breaking down fibrous materials. Probiotic enzymes improve nutrition bioavailability for microbial flora in the gastrointestinal tract. Feeding broiler chickens with *B. coagulans* NJ0516 boosted amylase and protease activity, leading to improved growth rates (Wang & Gu, 2010). Using *B. Subtilis* spores as an additive to feed lowered the need for amino acid and protein supplements, resulting in lower feed costs. Using a multiple modes *Bacillus* probiotic, the probiotic impact was more noticeable on a low-energy diet compared to a regular diet, possibly due to enzyme activity. Farhat-Khemakhem et al. (2018) found that *B. amyloliquefaciens* US573 strain secretes *β-glucanase*, *xylanase*, and *amylase*, leading to a 48% increase in wheat digestion. Gut wellness as well digestion are heavily influenced by the intestinal epithelium's morphology. Increased villus height and crypt depth ratio enhance nutrition uptake in the small intestine. Feeding *Bacillus Subtilis* to broiler chicks improves villus height, cell area, and mitotic rates. Study (Ramlucken, Ramchuran, et al., 2020) on *Bacillus* spp. in broiler chickens have shown better villi height and crypt depth-to-height ratio. *Bacillus*-based probiotics increase digestibility through enzyme production and gut shape, leading to better broiler chicken yield, as measured by FCR. *Bacillus* spp. help increase intestinal villi, leading to better digestion and assimilation. This might be due to enzyme activity, which is one of the species' primary mechanisms of action.

4. Benefits of *Bacillus* Probiotics for Poultry Production

Bacillus probiotics have several benefits on poultry production including: improved growth performance, better egg production, enhanced immunity, increased enzymatic secretion, production of AMP, healthy gut along with microbiota, and reduced production of ammonia. Figure 2 summarises benefits of probiotics usage in poultry production.

Growth Performance

Stabilizing intestinal microbiota improves feed conversion ratio, metabolism, and uptake of nutrients (Pan & Yu, 2014). Adding *B. Subtilis* C-1302 to the diets of broilers at 300 and 600 mg/kg resulted in enhanced daily average growth and decreased FCR (Jeong & Kim, 2014). Fathi et al. (2018) found that supplementing laying hens with *B. Subtilis* did not significantly affect their FCR levels. Lei et al. (2013) found that addition *Bacillus licheniformis* to the diet had no major impact on laying hens' feed intake or FCR. Probiotics have shown conflicting outcomes for average daily feed intake (ADFI), with some indicating no impact. Supplementing Hy-Line Brown laying hens' feed with *Bacillus velezensis* resulted in a considerable rise in ADFI levels. Many studies, including those with Broiler Cobb 500 fed a *B. Subtilis*-supplemented feed, found no significant increase in ADFI (Oladokun et al., 2021). Zhu et al. (2009) pointed out that the efficacy of probiotics is influenced by various factors, such as species, strain, method of administration, age of the flock, farm sanitation and housing conditions, and external environmental factors.

Abudabos et al. (2017) demonstrated that feeding *B. Subtilis* and avilamycin to infested broiler chickens improved growth parameters compared to infected control broilers. This indicates that *B. Subtilis* may potentially replace antibiotics in poultry diet. Roy et al. (2015) obtained similar findings in heat-stressed broiler hens fed *B. Subtilis*, 2.2% lincomycin, or a combination of both. *B. Subtilis* may enhance FCR by producing digestive enzymes that break down diet into smaller fractions that hens can absorb. *Bacillus* probiotics increase gut microbiota, which might lead to improved BWG and FCR. A low pH value inhibits pathogenic microorganisms (*S. typhimurium* and *C. perfringens*) while promoting the development of beneficial microbes (Ogbuewu et al., 2019). It enhances digestion and the use of nutrients by encouraging the production and release of digestive enzymes such cholecystokinin, gastrin, and pepsin. *B. Subtilis* PB6 has been shown to enhance consumption of feed, FCR, and BWG in broilers with *necrotic enteritis*. Lee et al. (2010) found that *Bacillus* probiotic-based DFMs improved Eimeria maxima (EM)-induced decrease in BWG and gastrointestinal abnormalities in broilers compared to control broilers (Bodinga et al., 2020). Adding 1.0kg *B. licheniformis*/metric ton of feed to broiler raised for 35 days within low biosecurity standards significantly improved BWG and FCR over those fed a basal diet with no additives, and 4% flavomycin at 0.3 kg/MT (Arif et al., 2021). *Bacillus* probiotics have been shown to improve disease-challenged broiler performance and development, suggesting a possible substitute to antibiotics in broiler diet. Animals often acquire abdominal fat, which is positively connected with overall fat. Abdominal fat indicates poor energy usage in the meal. Birds fed diets enriched with *B. amyloliquefaciens* and antibiotic had considerably lower fat in their abdomens weights than control birds. Previous research (Khajeh Bami et al., 2020) has shown that hens given *Bacillus* probiotics had considerably reduced belly fat weights. Probiotics have been shown to lower blood lipid levels, which might explain the decreased weight of the abdominal fat deposit. *Bacillus* probiotics may reduce fatty acid synthesis expression in chicken liver, resulting in an important decrease in abdominal fat pad.

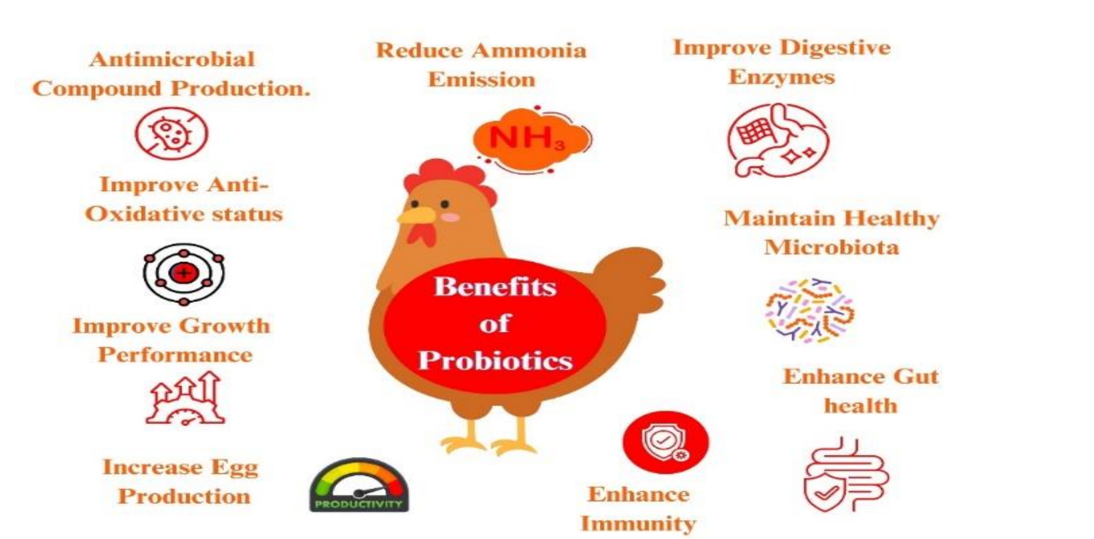

Fig. 2: Benefits of Probiotics usage in Poultry Production

Improved Gut Health

Supplementing with *Bacillus* had a beneficial and substantial influence on components of gastrointestinal villus histomorphometry (villus height [VH] and VH: crypt depth (CD)) in the duodenum and ileum area of the small intestine of broiler chickens (Ogbuewu & Mbajiorgu, 2022). These studies (Abd El-Moneim & Sabic, 2019; El-Moneim et al., 2020; Olnood et al., 2015) indicated that dietary probiotic supplementation affects VH and CD in small intestine segments. Probiotics are thought to promote gut epithelial cell proliferation and mitotic cell division, leading to increased villus length (Bai et al., 2013). Greater VH leads to a rise in villi uptake surface area, which improves intake of nutrients and consequent development performance. *LactoBacillus*-based probiotic supplements can generate a wave-like configuration of villi in broiler jejunum (Pelicano et al., 2005). The wavy structure of villi improves nutrient absorption by reducing the pace of transit through the gastrointestinal system.

Enhanced Immunity

The immune-modifying impacts in chickens occur when living microorganisms travel along the lining of the gut and proliferate to a limited extent, while dead ones are absorbed and activate the immune system. The immunoglobulin commonly known as antibodies, is essential to the chicken immune system because it binds to certain antigens like as germs or viruses, facilitating in their elimination. IgA plays an important part in the immunological function of mucosal membranes, whereas IgM acts as the initial line of security against illness (Bian et al., 2016). Chickens make IgY antibodies, which operate similarly to mammalian IgG antibodies. Adding *Bacillus* to the diets of broilers significantly enhanced IgA, IgM, and IgG levels in sera, suggesting that *Bacillus* therapy enhances broiler chicken immunity (Ogbuewu & Mbajiorgu, 2022). B. Zhang et al. (2021) found that feeding *B. coagulans* to 6-week-old broiler chicks increased immunoglobulin levels in their serum. Dong et al. (2020) observed 32 and 31% spikes in IgG and IgM concentrations in broiler sera given *B. Subtilis* for 5 weeks. Probiotics improve gut barrier function by modulating the cytoskeleton and epithelium tight connections in the intestinal mucosa, which is one of their strategies for preventing infections. Probiotics can help birds fight pathogenic microbes and reduce the amount of bacteria in the intestine, reducing the risk of fecal contamination in the housing (Edea et al., 2022). Probiotics can boost antibody levels against viral infections such as Newcastle disease and Infectious Bursal Disease. Improving immunological function can prevent intestinal infections and minimize secondary infections in birds, which are often caused by viral illnesses or immunosuppressive circumstances. Probiotics taken orally stimulate the formation of natural antibodies in the stomach and circulation against a variety of antigens.

Better Feed Efficiency

Stability of gut flora as result of probiotics can aid to increase the feed conversion ratio and hence boost digestion and nutrient absorption (Pan & Yu, 2014). *Bacillus spp.* probiotic strains can reduce antibiotic use while boosting chicken development and well-being (Luise et al., 2022). Several studies (Jeong & Kim, 2014; Panda et al., 2003) showed beneficial impacts on the development rate (increased average daily gain) and decreased the FCR in broilers. *B. licheniformis* DSM 28710 also enhanced body weight of birds by 20g and improved feed conversion ratio by 0.7% from day 11 to day 22 when compared with salnomycin (Trela et al., 2020).

Whereas some studies (Fathi et al., 2018; Oladokun et al., 2021; Zhou et al., 2020) showed no influence on the intake of food and FCR of the poultry. Zhu et al. (2009) highlighted that the effects of probiotics are determined by a variety of parameters, including probiotic species, strain, treatment technique, flocks maturity, overall cleanliness and living conditions on the premises, and outside environmental variables.

Ammonia Reduction through Feces

At present, surplus ammonia emissions are a serious environmental issue in the chicken business. The Probiotics and the environment of the digestive system can help address this critical issue. The chicken liver produces uric acid, which gets driven into the colon and converted into ammonia by bacterial urease. Activity for Urease is present in many intestinal bacteria, including *Bacteroides*, *Clostridium*, and *Proteus*. This enzyme hydrolyzes uric acid into ammonia. Gram-positive probiotics have been shown to limit urease-producing bacteria by suppressing harmful microbes, generating antimicrobial chemicals, or reducing pH, resulting in reduced ammonia synthesis in ceca. *Bacillus Subtilis* can inhibit pathogenic microorganisms in broiler chickens, which enhances growth rate and reduces ammonia emission (Chen et al., 2013). The stable gut microbiota contributes to maintaining gut wellness in chickens. S. Zhang et al. (2021) indicated that *B. Subtilis* supplementation improves gut microbiota function and balance, hence reducing ammonia generation. The usage of probiotics in feed enhances its utilization and balances the ecology of bacteria in the GI tract, thus minimising the levels of ammonia. Jeong and Kim (2014) concluded that the inclusion of *Bacillus* strains in chicken feed leads to a significant decrease in ammonia emissions. Zhou et al. (2020) stated that *Bacillus amyloliquefaciens* can reduce ammonia generation and improve the GI tract environment.

Conclusion

The application of different types of *Bacillus* as probiotics has a magical positive impact on broiler productivity. Their ability to balance the gut microbiota, enhance the absorption of nutrients, better protection, and reduce the chance of diseases such as necrotic enteritis has categorised them quite suitable as alternatives to antibiotics in chicken production. *Bacillus spp.* deliberate benefits rather than better growth achievement, including more feed utilization and lower environmental pollution through the decrease in ammonia emission. These characteristics make *Bacillus*-based probiotics a reliable and economical alternative for modern-age poultry farming systems.

Future Directions

Different strains of *Bacillus* give a wide variety of benefits. Although *Bacillus spp*. offer a bundle of benefits, the influence of individual strains differs as per their impact. More speculated research is required to recognise those strains that perform well under special conditions, like changing ambient temperatures, feed utilization, and chicken strains. The clear mechanisms of probiotic activity by *Bacillus* species, in particular as immunomodulation and competitive exclusion, require more authentic studies. Further research experiments on multi-strain or multi-component formulations can result in synergistic benefits, further improving poultry health and production. Internationally emphasized legal terms for probiotic use in chicken farming are required to facilitate safety, consistency, and user confidence.

By addressing these points, the *Bacillus* species could be well integrated into the poultry production farming systems to give practically used alternatives to fulfil the increasing world need for poultry and associated products while maintaining the welfare of animals and the natural environment for them.

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