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Invited Review Article

Assessment of *Bacillus subtilis* based probiotics on health and productive performance of poultry: A review

S. Soren[1*], G. P. Mandal[1], B. Roy[1], I. Samanta[2] and R. N. Hansda[3]

[1]Department of Animal Nutrition, West Bengal University of Animal and Fishery Sciences, Kolkata- 700 037, West Bengal, India; [2]Department of Veterinary Microbiology, West Bengal University of Animal and Fishery Sciences, Kolkata- 700 037, West Bengal, India; [3]Department of Veterinary Pathology, West Bengal University of Animal and Fishery Sciences, Kolkata- 700 037, West Bengal, India

Abstract

Probiotics are live microorganisms that can offer health benefits to humans and animals when taken in sufficient amounts and have become a popular choice for poultry producers as an alternative to antibiotics. This review examines the effects of *Bacillus subtilis* as probiotics on various aspects of poultry production, such as growth performance, carcass traits, gut histomorphology, immunity, and gut microbiota. Findings suggest that supplementing with *Bacillus subtilis* probiotics may improve growth performance, modify the gut microbiome and stimulate immune system; however, no impact on carcass trait was found. The success of probiotics in poultry production depends on the intestinal health condition of birds, the probiotic inclusion rate, feedstuff provided, and water quality offered. This systematic review summarizes all current research on the use of *Bacillus subtilis* probiotics in poultry production and their possible contribution to overall performance parameters.

Keywords: *Bacillus subtilis*, Chicken, Gut microbiota, Gut-morphology, Nutrition

Highlights

- Supplementation with *Bacillus subtilis* has resulted in improved growth performance in broiler chickens.
- *Bacillus subtilis* has been shown to improve the intestinal morphology of broiler chickens.
- *Bacillus subtilis* may have a positive influence on the gut microbiota of broiler chickens.
- Broiler chickens experience increased antibody titres when given *Bacillus subtilis*.
- Broiler chickens do not exhibit changes in carcass traits when supplemented with *Bacillus subtilis*.

INTRODUCTION

The poultry industry has experienced immense development in its production system over the last half-century due to advances in genetics, management manoeuvres, and nutritional science (Gadde *et al.*, 2017). Antibiotics have been used for decades to increase production and protect birds from pathogens (Hakimulue *et al.*, 2020). However, their improper use can lead to increased antimicrobial resistance (AMR) bacteria as a threat to public health, residues in animal products, and environmental pollution (Gadde *et al.*, 2017). The increasing concern over the use of antibiotics in animal feed has resulted AMR in numerous countries imposing restrictions and bans on their use, driving nutritionists to engage in search for alternatives that ensure efficient poultry production while providing safe poultry meat and eggs. Various animal antibiotic substitutes, such as prebiotics, probiotics, synbiotics, and postbiotics, have been thoroughly researched in order to develop more secure biological control products for the industry. These substitutes may help reduce antibiotic resistance issues while supplying animals and humans with safe and efficient food sources. This review critically examines the latest research on *Bacillus subtilis* probiotics use in broiler production. The results from various studies are highly diverse; thus, the primary purpose of this review is to analyze and interpret the critical conclusions about probiotics in broilers and gain insight into their potential effects. This review is expected to reveal a better understanding of specific probiotic applications in broilers and its implications for poultry health.

Concept of probiotics and common varieties

Probiotics, as defined by Fuller (1992), are live microbial feed supplements that promote gut microbiota balance when ingested. They can be a single strain or a

[*]Corresponding Author, E-mail: drsoren1507@gmail.com

combination of strains with potential beneficial effects. Essential components of probiotics include bile/acid resistance, strain specificity, no side effects, reduction in pathogenic microorganisms numbers, ability to withstand feed processing, and viability during storage (Stęczny and Kokoszyński, 2021). Common bacterial species used as probiotics in poultry production include *Bifidobacterium*, *Bacillus*, *Streptococcus*, *Enterococcus*, and *Lactobacillus* (Gadde *et al*., 2017), while fungi such as *Aspergillus oryzae* and yeast like *Saccharomyces cerevisiae* are becoming increasingly popular options (Ghosh *et al.*, 2012).

Mechanisms of action of probiotics

Probiotics have been found to provide various benefits for overall health through multiple mechanisms such as competitive exclusion, production of antibacterial substances and organic acids, immune-modulatory activities (Salzman *et al.*, 2003), enhancement in the absorption of proteins and minerals from the gut (Raghuwanshi *et al.*, 2018), and regulation of gut microbiota homeostasis (Salminen *et al.*, 2021). Moreover, probiotic consumption is associated with a reduced risk of cardiovascular disease due to its ability to decrease blood cholesterol levels in hosts (Jones *et al.*, 2013). Therefore, probiotics are recommended for maintaining good health.

Effect of probiotic on growth performance

With the phasing out of Antibiotic Growth Promoters (AGPs) in poultry feed, there has been a renewed focus on evaluating probiotics for their potential to improve growth performance in commercial poultry production. As stated by Niewold (2007), AGPs restrict the release and elimination of catabolic mediators by intestinal inflammatory cells, ultimately leading to a reduction in intestinal microflora population. In contrast, probiotics exert their effects by modulating the gut environment and enhancing gut barrier function. This is accomplished through the reinforcement of beneficial intestinal microflora, competition against pathogenic bacteria, and stimulation of the immune system. Following supplementation with probiotics, non-pathogenic bacteria compete with pathogenic bacteria in terms of access to nutrients; they also colonize and limit space for harmful bacteria to occupy or establish within the intestine. Additionally, probiotics secrete digestive enzymes such as β-galactosidase and α-amylase which aid in increased nutrient absorption and consequently improved growth performance in animals (Jadhav *et al.*, 2015). Nevertheless, both could result in an improved growth performance. An increase in body weight gain (BWG) and feed conversion ratio (FCR), which thereby improves production performance, is commonly observed with the use of probiotics (Cervera and Carmona, 2020). However, feed consumption will not always be improved (Angelakis, 2017). Nevertheless, when there is a lack of a significant improvement in FCR with probiotic supplementation, average daily gain (ADG) can still increase. The BWG, average daily feed intake (ADFI), FCR, and production performance of poultry birds may be affected by several elements such as strain selection, application mode, time, concentration and absorption of dietary supplementation with probiotics (Askelson *et al.*, 2018). Yadav *et al.* (2018), Pereira *et al.* (2019), Kakar *et al.* (2021), Ortega *et al.* (2021) and Zhang *et al.* (2021) demonstrated that supplementation of *Bacillus subtilis* at various dosages was beneficial in increasing ADG, ADFI and FCR, when compared to the control groups. In contrast, studies conducted by de Oliveira *et al.* (2019), Qiu *et al.* (2021) and Mohamed *et al.* (2022) demonstrated that dietary supplementation with *Bacillus subtilis* had no significant effect on ADFI, ADG, or FCR among chicken flocks. Other research has similarly shown that there is no variation in growth performance indicators when *Bacillus subtilis* is added to feed at varying levels, such as 750 g/t (Zhang *et al.*, 2021), 100 g and 50 g/tonne (Sarangi *et al.*, 2016), 1.5 × 105 cfu/g (Gadde *et al.*, 2017), 0.4, 0.6, and 0.8 g/kg (Islam and Nishibori, 2023). In general, it is apparent that extensive research has been conducted on the impact of supplementing *Bacillus subtilis* on growth performance measures in poultry flocks. While some studies had found positive, beneficial effects on ADG, ADFI and FCR when *Bacillus subtilis* was added to feed at varying levels, other studies showed that there was no significant effect (Qiu *et al.*, 2021; Zhang *et al.*, 2021; Islam and Nishibori, 2023). This suggests that the effects of dietary supplementation of *Bacillus subtilis* may be highly variable in poultry birds, and hence, future research should aim to uncover more precise details.

Effect of probiotic on intestinal morphology

The critical factors for maximizing nutritional absorption are the parameters linked to intestinal morphology, namely increased villus height (VH), reduced crypt depth (CD), and a higher villus height-to-crypt depth ratio (Jha *et al.*, 2020). An increase in VH leads to an increased surface area of the villi, which boosts nutrient absorption and improves growth performance. Conversely, expanded CD can decrease enzyme secretion, reduce nutrient absorption, and negatively affect growth (Singh *et al.*, 2011). Probiotics are often utilized to improve gastrointestinal histomorphology; however, the effects vary depending on the strain used (Barkhidarian *et al.*, 2021). A recent study by Oladokun and Adewole (2023) sought to investigate the effect of probiotics (*Bacillus subtilis*) on gut morphology in broiler chickens, with the conclusion that *B. subtilis* increased jejunal and ileal villus height to crypt depth ratios (P<0.001). This was corroborated by Qiu *et al.* (2021), whose results indicated that *B. subtilis* supplementation at a rate of 500 mg/kg caused increases in villus height (VH), VH/CD, and intestinal wall thickness (P<0.05), and decreases in crypt depth (CD; p<0.05). Moreover, Zhang *et al.* (2021) reported an increase in the ileum villus height to crypt depth ratio when compared to control groups (P<0.05). Similarly beneficial effects on gut morphology have also been recorded upon supplementation of *Bacillus subtilis* at doses of $1x10^9$ cfu/kg (Aziz e*t al.*, 2019), 1, 3 and 5 X 10^8 (Mohamed *et al.*, 2022), 1.25 and 5 g/kg (Šimunović *et al.*, 2022) or 0.1% (Manafi *et al.*, 2017). The evidence from the studies mentioned above indicates that probiotics can improve broiler chickens' growth performance by increasing villus height (VH) and higher villus height-to-crypt depth ratio (VH/CD), while crypt depth (CD) was slightly decreased. Maximizing nutritional absorption in the gut is closely related to intestinal morphology, so by enhancing this key factor, it can be concluded that probiotic supplementation can be an effective way to improve the overall gut morphology of broiler chickens as well.

Effect of probiotic on gut microbiota

Studies have demonstrated that probiotics can impact the composition and activity of the gut microbiome by outcompeting other microorganisms for nutrients, ability to attach themselves to specific sites on the intestinal mucosa, and generating antimicrobial compounds that restrict pathogenic microbial growth (Abd El-Hack *et al.*, 2020). Possible mechanisms behind probiotic antagonism include the reduction of gastrointestinal pH, modulation of the immune system and production of organic acids (Rajput *et al.*, 2020). Furthermore, probiotics may also heavily contribute towards improved intestinal barrier integrity, strengthened immunogenicity and adjustments to microbial signalling pathways in intestinal epithelial cells (Arif *et al.*, 2021). Various tools are utilized to examine the impacts of probiotics on gut microbiota function, variation, and composition, such as culture-dependent approaches, metagenomic sequencing, and *in vivo* assays. Nevertheless, *in vivo* administration of probiotics is the most accurate and efficient method for obtaining precise outcomes (Foligne *et al.*, 2007). Several studies have revealed that probiotics, especially lactic acid bacteria, can successfully inhibit *Salmonella*, *Enteritidis* and *Escherichia coli* infections in poultry (Jiratitipat *et al.*, 2019). A study conducted by Rivera-Pérez *et al.* (2021) evaluates *Bacillus subtillis* as a substitute for an antibiotic growth promoter. The results demonstrated that supplementation with *B. subtilis* probiotic led to significantly lower counts of *E. coli* and *Enterococcus* (p<0.05), while also significantly increasing *Lactobacillus* counts (p<0.05) compared to both the control group and the group receiving antibiotic growth promoters. Results obtained by Qiu *et al.* (2021) indicated that dietary supplementation of *B. subtilis* (500 mg/kg diet) significantly increased the number of *Lactobacillus* and *Bifidobacteria* in the ileum and cecum and decreased the *Coliforms* and *Clostridium perfringens* in the cecum. Another study conducted by Jeong and Kim (2014) evaluate the efficacy of *B. subtilis* at the rate of 300 mg and 600 mg/kg feed on gut microbiota of broilers. The findings suggest that *B. subtilis* supplementation had a significant positive effect on *Lactobacillus* populations in the cecum, ileal, and excreta. It also showed a decrease in *Escherichia coli* counts in the cecum and excreta when compared to the control group. Furthermore, there was a tendency for *B. subtilis* to reduce *Clostridium perfringens* populations in the large intestine and excreta. Additionally, there was a linear reduction in *Salmonella* counts observed in the cecum, ileal, large intestine, and excreta with *B. subtilis* supplementation when compared to the control group. Another study conducted by Gao *et al.* (2017) reported that *B. subtilis* at the rate of 100, 150, 200 and 250 mg/kg decreased the *Escherichia coli* and *Salmonella* populations in the cecum. *Bacillus subtilis* supplementation has been found to be a successful method for improving beneficial bacteria such as *Lactobacillus* and *Bifidobacteria*, while decreasing pathogenic bacteria including *Escherichia coli*, *Coliform*, *Enterobacteriaceae* and *Salmonella*. This improvement was observed at various levels of *B. subtilis* supplementation- 0.1% (Manafi *et al.*, 2017); $1x10^7$ CFU/g of feed (Mardanova *et al.*, 2020); 1.0×10^9 spores/kg of diet (Ma *et al.*, 2018); 100, 150, 200 and 250 mg/kg (Gao *et al.*, 2017); $1x10^8$, 3 X 10^8 and 5 X 10^8 (Mohammed *et al.*, 2022). Based on various studies, it is evident that probiotics can be effective in reducing *Salmonella* and *Escherichia coli* counts while increasing the counts of *Lactobacillus* and *Bifidobacteria* in the cecum of broiler chickens. Thus, incorporating probiotics into their diet can be beneficial for them in terms of reducing diseases. This could help farmers to benefit financially as they would have fewer losses due to reduced mortality and improved performance.

Effect of probiotic on immune response

Studies on the effect of probiotics on Newcastle disease vaccine (NDV) and Infectious bursal disease vaccine (IBDV) antibody titre have shown that probiotic supplementation can increase the level of specific antibodies to both viruses (Sikandar *et al.*, 2022). This indicates that probiotics may help prevent or reduce the severity of NDV and IBDV infection, likely by enhancing the immune system's response to potential pathogens. Additionally, research has found that adding certain types of bacteria (*Bacillus subtilis*, *B. animalis and B. bifidum*) to poultry feed and drinking water for an extended period prior to vaccination can improve immunogenic responses in birds for both NDV and IBDV, suggesting that probiotics might be a viable method for improving protection against these diseases (Sikandar *et al.*, 2022). A study conducted by Hatab *et al.* (2016) observed that Newcastle antibody titres were significantly higher for broiler chicken given probiotics *Bacillus subtilis* and *Enterococcus faecium* as a supplement in their diet plan compared to the control group. Another study reported that broiler chickens fed a diet supplemented with probiotics showed higher Newcastle antibody titers than the control group (Rowghani *et al.*, 2007). In addition, King and Seal (1998) found that birds given diets enriched with probiotics containing *B. subtilis* displayed elevated antibody titers against Newcastle disease (ND) when compared to the control group of birds. Comparably, Manafi *et al.* (2017) found that broiler chickens supplemented with *B. subtilis* displayed higher concentrations of antibodies targeting Newcastle disease. The significant increase in antibody titre production against Newcastle disease, compared to control group may be due to immune-stimulatory and immune-modulatory effect of using biological supplementation. Some probiotics could stimulate a protective immune response sufficiently to enhance resistance to microbial pathogens (Noverr and Huffnagle, 2004). Another study conducted by Manafi *et al.* (2017) found that broiler chickens supplemented with *B. subtilis* displayed higher concentrations of antibodies targeting the bursal disease. Similar results in terms of improved antibody titre were reported when *Bacillus subtilis* was supplemented at 0.1 g/kg (Sikandar *et al.*, 2022). These findings suggest that probiotic supplementation in chicken diets can enhance immunological response in poultry, which could help improve resistance to various diseases. In contrast, antibody titre against ND did not vary significantly (P>0.05) due to probiotic inclusion in the diet of broilers (Rehman *et al.*, 2020). Similarly, no influence of titer against ND was observed (P>0.05) against the inclusion of probiotics at the dose rate of 0.9 g/kg in the basal diet of the broiler (Salehimanesh *et al.*, 2016). Again, Kalandakanond-Thongsong *et al.* (2008) found no significant difference in the antibody titre responses to ND between treated and untreated groups. Another study conducted by Toghyani *et al.* (2015) concluded that antibody titre against ND was not statistically (P>0.05) influenced due to a probiotic-supplemented diet. Studies by Sikandar *et al.* (2022) have demonstrated that probiotic supplementation can significantly enhance the immune response of broilers by increasing antibody production following vaccination and improving gut integrity, reducing inflammation and limiting bacterial translocation. These effects result in improved defence against pathogenic bacteria and other infections, helping to regulate the immune system and protect against disease. Thus, probiotic supplementation has been shown to be a viable dietary intervention for enhancing the humoral immunity of broilers compared to un-supplemented diets.

Effect of probiotic on carcass traits

The evaluation of carcass traits is critical for profitable broiler production. Thus, numerous researchers have devoted their attention to this topic. Since the early days of probiotic use as a growth promoter, its effectiveness in evaluating carcass traits in broilers has been studied. In a study conducted by Tang *et al.* (2021), the effects of dietary supplementation with *Bacillus subtilis* at 500 mg/kg feed on carcass qualities of broiler chickens were evaluated. It was found that there were no significant differences in carcass weight, dressing percentage, semi-eviscerated percentage, eviscerated percentage, and leg muscle percentage between treatments (P>0.10). Furthermore, similar results were observed in another study by Yadav *et al.* (2018), which investigated the efficacy of probiotic (*Bacillus subtilis*) on carcass traits of broiler birds after analysis of the weight of liver, heart, and intestine as well as the importance of different cuts (thigh, wing, and back) relative to live weight; no significant variations were detected among other groups. Similarly, Islam and Nishibori (2023) reported that there was no significant effect on carcass traits when *Bacillus subtilis* was supplemented at different levels (0.4, 0.6, and 0.8 g/kg diet). However, carcass traits were found to be significantly increased in probiotic (*Bacillus subtilis* @ 5×10^{10} cfu/g) supplemented group compared with control group (Abdel-Hafeez *et al.*, 2017). Studies have revealed that probiotic supplementation does not have a meaningful impact on the carcass traits of broiler chickens, such as meat yield, muscle weight, and fat content (Yadav *et al.*, 2018). This conclusion was reported after extensive research analyses which measured the effects of probiotics in terms of growth performance in birds. Results showed that there were no differences between groups supplemented with different levels of probiotic and control (Tang *et al.*, 2021; Islam and Nishibori, 2023). Therefore, these findings suggest that adding probiotic to poultry diets has little or no effect on the final carcass characteristics of broiler chicken production.

Conclusion

The potential of probiotics as feed additives may be explored for providing an array of benefits such as improved gut health, enhanced immune response, and better growth performance. Probiotics may be able to substitute for some sub-therapeutic antibiotics use in poultry production, thus helping to reduce the risks associated with antimicrobial resistance. Despite numerous studies striving to compare results at different inclusion levels, there has been no conclusive recommendation on optimal dosing due to discrepancies between methodologies. As researchers continue to study the modes of action of these beneficial microorganisms, there is still much more to be discovered. Therefore, further research is necessary to comprehend probiotics' effects on poultry health.

Conflict of interest: The authors of this study have no financial or personal interests that could be seen to influence the results of the study, therefore there is no conflict of interest.

Author's contributions: SS: Conceived and designed, data collection and drafted the manuscript; GPM: Conceived and designed and interpreted the results; BR: Conceived and designed; RNH: Data collection; IS: Edited the manuscript. All authors reviewed the results to approve the final version of the manuscript.

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