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Bacillus Probiotics as Alternatives to In-feed Antibiotics and Its Influence on Growth, Serum Chemistry, Antioxidant Status, Intestinal Histomorphology, and Lesion Scores in Disease-Challenged Broiler Chickens

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In commercial poultry production, chickens are reared under intensive conditions, which may allow infections to spread quickly. Antibiotics are used at sub-therapeutic doses in livestock and poultry feed to prevent diseases and improve productivity. However, restrictions on the use of antibiotics at sub-therapeutic concentrations in livestock feed due to growing concerns of antimicrobial resistance (AMR), together with antibiotic residues in meat and eggs has prompted poultry researchers and feed producers to look for viable alternatives. Thus, there is increasing interest in developing natural alternatives to in-feed antibiotics to improve chicken productivity and health. Probiotics, specifically from the genus *Bacillus* have proven to be effective due to their spore-forming capabilities. Furthermore, their ability to withstand heat during feed processing and be stored for a long time without losing viability as well as their potential to function in the acidic medium of the chicken gut, provide them with several advantages over conventional probiotics. Several studies regarding the antimicrobial and antioxidant activities of *Bacillus* probiotics and their positive impact in chicken nutrition have been documented. Therefore, the present review shields light on the positive effect of *Bacillus* probiotics as alternatives to in-feed antibiotics on growth performance, serum chemistry, antioxidant status, intestinal histomorphology and lesion scores of disease-challenged broiler chickens and the mechanisms by which they exert their actions. It is concluded that *Bacillus* probiotics supplementation improve growth, health and productive indices of disease-challenged broiler chickens and can be a good alternative to in-feed antibiotics. However, more studies are required on the effect of *Bacillus* probiotics supplementation in broiler chickens to maximize productivity and achieve the ultimate goal of stopping the usage of antibiotics at sub-therapeutic doses in broiler chicken feed to enhance performance.

Keywords: broiler chickens, *Bacillus*, antibiotic alternatives, growth, health makers

INTRODUCTION

Demand for poultry source foods is increasing, particularly in developing countries (1), and this is driven primarily by population growth (2, 3). To keep pace with the growing request for poultry products, farmers now use antibiotics at minute doses to reduce the incidence of enteric pathogens, improve feed conversion ratio (FCR) and body weight gain (BWG) in broiler chickens (4). Despite the significant economic benefit of AGPs in large-scale broiler production, their continued use in animal feed has been heavily criticized because of growing concerns of AMR and the occurrence of multi-drug resistant bacteria. In view of this, several countries have banned the use of antibiotics at sub-therapeutic levels in feed for food animal production (5).

The ban on the use of antibiotics at sub-therapeutic doses in livestock feed resulted in economic losses because of reduced feed efficiency, decreased feed intake, BWG, higher morbidity and mortality of broiler chickens as a result of high prevalence of pathogens (6, 7). In addition, in-feed antibiotics increased the incidence of food-borne illness in humans (8). To maneuver the limitation linked to the usage of in-feed antibiotics in animal agriculture, poultry nutritionists and farmers have proposed the use of probiotics found to have no residual effect on animal products or result in antimicrobial resistance (9). A probiotic is viable or inactivated micro-organisms that exert beneficial effects to the host when administered in an adequate amount. *Lactobacillus spp.* is one of the common probiotics used in broiler chicken feed because they are considered safe, naturally present in the gut and with demonstrable health-promoting effects (10, 11). Other conventional probiotics with known beneficial impact in chickens include *Saccharomyces spp.*, *Aspergillus spp.*, Enterococcus *spp.*, and *Bifidobacteria* (12–15). The use of these probiotics is still limited as challenges have been noted during feed preparations, whereby these organisms are not able to withstand the high temperature of the feed pelletisation process. Furthermore, reduced shelf life and limited survival in the gut has to date, contributed to the poor adoption by the farmers into routine broiler production process (16) The limitations of conventional chicken probiotics during industrial production processes have been documented in the literature (17).

The use of *Bacillus* probiotics in broiler chicken feed is gaining attention since it has features that address the drawbacks related to Lactobacillus based probiotics. *Bacillus* organisms are gram-positive bacterium with the ability to form spores. The ability of *Bacillus* organisms to form spores ensures that they remain stable and viable during feed manufacturing processes, storage, and movement through the gastrointestinal tract (17–19) thus implying that *Bacillus* products are suitable for adoption in the poultry industry. Besides, evidence also exists that *Bacillus* strains were produced at high efficiency (20) and hence, one of the key advantages of using *Bacilli* as feed probiotics is their capability to resist the changing conditions in the gastrointestinal tract of chickens (17, 21). Importantly, as suggested by Ramlucken et al. (17) *Bacillus* spores can retain about 90% of their viability during the probiotic harvesting process and have a 5-year shelf life potential.

In another study, Aly et al. (22) prepared three probiotic supplemented diets, with diet 1 containing a mixture of 0.5 × 10^7 *L. acidophilus*/g feed and 0.5 × 10^7 *B. subtilis*/g feed, and diets 2 and 3 containing 1 × 10^7 L. acidophilus/g feed and 1 × 10^7 *B. subtilis*/g feed, respectively, and stored for 4 weeks at $4°$C and $25°$C. After, 4 weeks of storage at $4°$C and $25°$C, the authors discovered that diets 1 and 2 had significantly more viable cells than diet 3 after 4 weeks of storage at $4°$C and $25°$C. Broilers chickens challenged with *C. perfringens* fed a diet supplemented with *B. coagulans* experienced less intestinal damage and consumed more feed than those fed a diet supplemented with *L. fermentum* (23). In agreement with the present findings, Brzoska et al. (24) and Olnood et al. (25) found that dietary *Lactococcus lactis* and *Lactobacillus* spp. supplementation did not improve feed intake, FCR and BWG (24, 25), all of which are important parameters for probiotic acceptance in the broiler chicken industry. The poor performance of chickens on conventional probiotics supplementation (24, 25) compared to probiotic *Bacillus* could be partly attributed to their low survivability in the harsh conditions of the gastrointestinal tract (26, 27).

Bacillus probiotics used as probiotics in humans and animals include *B. coagulans, B. cereus, B. subtilis* and *B. licheniformis* (28, 29). Several studies have found that feeding *Bacillus* spp. to chickens enhances their growth and productivity (30–32). However, the impact of *Bacillus* probiotics supplementation on the performance indices of disease-challenged broiler chickens were inconclusive due to differences in strain of *Bacillus* probiotics used, dosage, the severity of infection, age of chickens, and rearing environment (7, 33).

Thus, the purpose of this review is to highlight the possible mechanisms of action of *Bacillus* probiotics and the influence of dietary *Bacillus* probiotics supplementation on growth parameters, blood metabolites, intestinal histomorphological indices and lesion scores in disease-challenged broiler chickens. In addition, the antioxidant and antimicrobial properties of *Bacillus* spp. in disease-challenged broiler chickens were discussed. We also discussed several conflicting research findings, reasons for these differences and offer recommendations on the potential of *Bacillus* spp. to replace to in-feed antibiotics in disease-challenged broiler chickens.

MECHANISMS OF ACTION

The probable mechanisms by which *Bacillus* spp. limit the proliferation of pathogens include competition for adhesion sites, production of organic acids leading to a reduction in gut pH, maintenance of normal gut microbiota *via* competitive exclusion (CE) and antagonisms, production of antimicrobial compounds, improvement in oxidative stability, modulation of immune system, improvement in digestive enzyme activity and competition for nutrients (21, 34). The inhibitory effect may be achieved through one or a combination of these actions. Probiotics improve the immune system by inducing the production antimicrobial compounds, and raising the concentrations of secretory immunoglobulin A (35). Upon

consumption, *Bacillus* spp. alters the intestinal environment and produces a variety of digestive enzymes which improves digestibility and nutrient absorption in poultry (36, 37). The mechanism of competition for binding sites on the intestinal mucosa is mediated by glycocalyx, a layer that protects the intestinal epithelial from mechanical damage and prevents pathogen colonization, thus protecting the host from infections (38). CE is one method for controlling enteric pathogens and zoonotic agents in chickens (8). Competition for adhesion sites on the intestinal mucosa is influenced by the pH of the digesta (21). Low pH promotes the growth of acidophilic bacteria such as *Bacillus* probiotics, which inhibit the proliferation of enteric pathogens (*Salmonella* spp., *Clostridium perfringens* and *Escherichia coli*) using the CE technique. *Bacillus* spp. has also been reported to produce bacteriocins which are toxic to enteric pathogens (39–41).

SOURCES OF BACILLUS PROBIOTICS AND CRITERIA FOR ITS DESIRABLE PROBIOTIC PROPERTIES

Currently, the *Bacillus* probiotics used in livestock production are isolated from the intestine, food, soil and pond. They are widely found in the dust, water, air, and soil (21). Several companies have successfully commercialized *Bacillus* probiotic products as detailed by Ramlucken et al. (17) in their recent review. The most common *Bacillus* probiotics used in livestock and poultry research is *B. subtilis* or its strains. *Bacillus* probiotics are identified based on standard morphological, biochemical, physiological tests, 16S rRNA gene sequencing or multilocus sequence analysis. To exert its positive influence on the host, probiotic bacteria should be able to survive, bind to the intestinal mucosa, maintain good viability, utilize the nutrients and substrates found in the diet, and remain non-pathogenic and non-toxic. *Bacillus* probiotic comes in two types: vegetative and spore. The vegetative type is destroyed by gastric acid and bile salts, but spores thrive in both conditions (42). Studies have shown that *Bacillus* probiotic produces different kinds of AMPs including bacteriocins, glycopeptides, lipopeptides, and cyclic peptides (39, 43). Bacteriocins are toxic to pathogens and are one the criteria for selecting a probiotic strain.

ANTIOXIDANT STATUS

Bacillus probiotics respond to oxidative stress by upregulation of catalases and thioredoxins (44). Abudabos et al. (30) reported that Salmonella-challenged broilers treated with *B. subtilis*-based probiotics had decreased serum concentrations of total antioxidant capacity (TAC), whereas those treated with avilamycin had increased serum concentrations of TAC. The significantly increased serum levels of TAC in Salmonella-challenged broilers fed antibiotic-based diet is consistent with Kabploy et al. (45), who linked the avilamycin's anti-oxidative activity to its scavenging effect on hydroxyl radicals. On the other hand, Rajput et al. (46) recorded higher serum TAC concentrations in *S. enterica* challenged birds fed diets containing

mixtures of *S. boulardii* and *B. subtilis* B10. Available information also revealed that probiotic bacteria can boost antioxidant status of broiler chickens (47). Free radicals are produced in chickens during an inflammatory process, and superoxide dismutase (SOD) is a zinc-based antioxidant enzyme that helps to degrade free radicals (48). Carillon et al. (49) found that chickens infected with 1×10^4 *S. enteritidis* cfu/bird had higher SOD concentration at 3 and 10 days of age than challenged broilers administered 10^4 spore/g *B. subtilis*. The authors hypothesized that the elevated SOD activity in *S. enteritidis*-challenged broilers could be attributed to the ability of chickens to counteract the oxidative damage caused by severe intestinal injury induced by *S. enteritidis* toxins, since SOD plays an important part in mitigating oxidative damage (49).

ANTIMICROBIAL PROPERTY

There are studies concerning the inhibitory influence of *Bacillus* probiotics on gram-positive bacteria (39, 50, 51). *B. subtilis* supplementation at 10^4 and 10^6 spores/g reduces the proliferation of Salmonella (gram-negative bacteria) in the crop, proventriculus and intestinal compartments of broiler chickens infected with *S. enteritidis* at 10^4 cfu (48). However, they did not detect Salmonella in the intestine when *B. subtilis* was given at a higher dose (10^6 spores/g), implying that *B. subtilis* has dose-related antibacterial effects in broiler chickens. Tactacan et al. (52) observed a similar pattern in disease-challenged broiler chickens that received 10^4 and 10^6 cfu/g *B. subtilis* (QST 713) for 28 days. Although the detailed mechanisms of antibacterial properties of *Bacillus* probiotics are unknown, however, they may have achieved this *via* maintenance of gut microbial ecology through CE and antagonism, production of AMPs, enhancement of digestive enzyme activity, stimulation of immune system and improvement in absorptive efficiency of the small intestine (33, 53, 54).

The addition of *B. subtilis* to chicken feed decreased *C. perfringens* (36, 55, 56), Enterobacteriaceae (36) and Campylobacter (57) count in the excreta. *Bacillus* spp. supplementation has been reported to reduce *C. perfringens* proliferation in the intestine (53). In a similar study, Deepak et al. (58) noticed that *C. perfringens* counts ($\log_{10}$ cfu/g of jejunal content) were significantly reduced in infected broilers administered *B. subtilis*. In agreement, Jayaraman et al. (56) and Lin et al. (59) discovered that dietary *B. subtilis* supplementation reduces *C. perfringens* proliferation in the gastrointestinal tracts of broiler chickens. Likewise, Liu et al. (60) observed a decline in microbial diversity of *C. perfringens* infected broilers fed *B. subtilis* PB6 supplemented diets. Arif et al. (61) observed that *Bacillus* probiotics supplementation increased the population of *Bacillus* spp. on day 21 and 35, while decreasing the proliferation of *C. perfringens*, *Salmonella* spp. and *E. coli* in the jejunum and ileum of broiler chickens reared under a low-level of biosecurity measures. The exact mechanisms that lead to the inhibitory activity of *B. subtilis* against enteric pathogens are not clear. However, according to Cladera-Olivera et al. (43) and Baindara

et al. (39), *Bacillus* probiotics produce bacteriocins that are active against other pathogens.

GROWTH PERFORMANCE

Influence of dietary *Bacillus* probiotics on feed intake, FCR and BWG of disease-challenged broiler chickens has been documented (**Table 1**). Abudabos et al. (30) found that administration of *B. subtilis* (2×10^7 cfu/g) and avilamycin (0.2 g/kg) to infected broiler chickens improved growth performance indices when compared with the infected control broilers, implying that *B. subtilis* could replace antibiotics in poultry nutrition. A similar finding has been reported by Roy et al. (62) in heat stressed broiler chickens fed *B. subtilis* (0.5 g/kg feed), 2.2% lincomycin (0.15 g/kg feed) and their blend (0.5 and 0.15 g/kg feed, respectively). The improvement in FCR could be linked to the capability of *B. subtilis* to increase production of digestive enzymes, which help in the breakdown of feed into smaller fractions that are easily absorbed by the chickens. Adhikari et al. (48) reported higher BWG and lower FCR in *S. typhimurium* and *C. perfringens* challenged broilers fed *Bacillus* probiotics in comparison with antibiotics. The beneficial effect of *Bacillus* probiotics on the gut microflora could lead to improved BWG and FCR (21). Furthermore, Abudabos et al. (63) reported higher BWG in Salmonella-challenged broilers fed 2×10^7 cfu/g *B. subtilis* in contrast to those fed diets supplemented with avilamycin at 0.2 g/kg and infected control, suggesting that probiotics may be a viable alternative to antibiotics. The authors also showed that infected broilers fed a basal diet without feed additives performed poorly in terms of BWG, FCR and villi morphology compared to non-challenged control broilers, confirming the negative impact of Salmonella infection in chicken growth and productivity (64). Abudabos et al. (63) recorded superior BWG in broilers fed *B. subtilis* compared to the broilers fed *B. licheniformis*, and the observed contradiction could be ascribed to differences in *Bacillus* strain used and dosage (33).

Low pH inhibits the growth and multiplication of pathogenic microbes (*S. typhimurium* and *C. perfringens*), while promoting the proliferation of beneficial microbes (33, 48, 74). It also facilitates the production and release of digestive enzymes such as pepsin, gastrin and cholecystokinin, all of which aid in digestion and nutrient utilization (33, 75). It is reported that *B. subtilis* PB6 improves feed intake, FCR and BWG in Necrotic enteritis (NE)-challenged broilers (72). Lee et al. (76) reported that *Bacillus* probiotics based DFMs ameliorated *Eimeria maxima* (EM)-induced reduction in BWG and intestinal lesions in broilers in comparison with EM infected control broilers. The growth-enhancing effect of *Bacillus* probiotics might be ascribed to it inhibitory effect against some enteric pathogens invasion in broilers (55, 77). This observation was consistent with Jayaraman et al. (56), who noticed an improvement in growth performance of *C. perfringens* infected broiler chickens fed *B. subtilis* PB6 additive. Addition of 1.0 kg *B. licheniformis*/metric ton (MT) of feed in broiler chickens reared for 35 days

under a low-level of biosecurity measures significantly enhanced BWG and FCR compared to those fed basal diet with no additives and basal diet plus *Bacillus subtilis* at 1.0 kg/metric ton (MT) feed and 4% flavomycin at 0.3 kg/MT feed (61). Dietary *Bacillus* probiotic has been found to positively influence growth and productivity of disease-challenged broilers (64, 66–69), and may serve as an alternative to in-feed antibiotics in broiler feed.

Recently, Ahmat et al. (73) assessed the impact of *B. amyloliquefaciens* LFB112 and *B. subtilis* CICC 20179 on performance of broiler chickens and observed that broilers fed *B. amyloliquefaciens* at 5×10^5 cfu/g had better BWG, FCR, carcass yield and cut-part weights (thigh and breast muscle) than broilers treated with *B. subtilis* (5×10^5 cfu/g) and antibiotic (150 mg of aureomycin/kg). Based on their findings, supplementation of *B. subtilis* and aureomycin to the level of 5 $\times 10^5$ cfu/g and 150 mg/kg feed, respectively in broiler chickens did not enhance growth performance, suggesting that *B. subtilis* and aureomycin may not have reached the threshold required to improve growth and productivity in broilers. Abdominal fat is the most common way animal accumulate fat, and is positively correlated with total fat. Increased abdominal fat content connotes inefficient use of energy in the feed. It is evident from the findings of Ahmat et al. (73) that abdominal fat weights were significantly lower in birds offered diets supplemented with *B. amyloliquefaciens* (5×10^5 cfu/g) and antibiotic (150 mg of aureomycin/kg) compared to control birds. This finding is in harmony with other authors (62, 78, 79), who found significantly lower abdominal fat weights in chickens fed *Bacillus* probiotics feed additives. The reduced abdominal fat pad weight could be due to a decrease in lipid content as probiotics have been reported to reduce the serum lipid content (63). The potential of *Bacillus* probiotics to limit the activity of fatty acid synthase in chicken liver could explain the significant reduction in abdominal fat pad.

INTESTINAL HISTOMORPHOLOGICAL INDICES AND LESION SCORES

Intestinal histomorphology is used as an important index for intestinal health and infection recovery. Studies have shown that broilers challenged with enteric pathogens had disorganized intestinal tight junctions, resulting in an increase in intestinal permeability (80, 81). A recent feeding study by Abudabos et al. (63) found deteriorated villi health in disease-challenged broiler chickens; however, treatment with *Bacillus* probiotics and antibiotics mitigated the negative effect. On the same hand, Hernandez-Patlan et al. (82) found that feeding *Bacillus* probiotic (1.0×10^6 spores/g) to necrotic enteritis (NE) infected birds alleviated its adverse consequences. Similarly, Aljumaah et al. (72) found that 0.5 g/kg *B. subtilis* PB6 lowers intestinal lesion scores in NE challenged-broilers suggesting enhanced recovery. This reduction in intestinal lesion scores may be attributed to *Bacillus* probiotics' ability to eliminate enteric pathogens through competitive exclusion and antagonisms (55, 70).

TABLE 1 | Influence of *Bacillus* probiotics on broiler performance.

Bacillus strain	Broiler strain	Dosage (cfu/g)	Duration of study (days)	Pathogen challenged	Response variables	Authors
Bacillus cereus var. toyoii	Ross	1×10^6 spores/g	47	*S. enteritidis*	*Bacillus cereus* var. toyoii significantly improved FCR and BWG in challenged groups compared to the infected control.	(65)
B. licheniformis (DSM17236)	Cobb	8×10^5 8×10^6 8×10^7	28	*C. perfringens*	Broilers fed diets having three inclusion levels of *B. licheniformis* and virginiamycin (15 g/ton) had significantly lower FCR, and higher BWG than the NE-challenged control.	(66)
B. subtilis (DSM17299)	Cobb	8×10^5	42	*Salmonella* Heidelberg	Broilers on *B. subtilis* had higher BWG than the challenged control	(67)
B. subtilis PB6 (ATCC-PTA 6737)	Cobb	5.0×10^7	35	*C. perfringens*	Challenged broiler chickens fed *B. subtilis* PB6 supplemented feed 5×10^7 cfu/g had significantly better body weight gain and feed efficiency than infected control and non-infected control birds.	(56)
B. subtilis (Avicorr^{TM})	Ross	1.5×10^5	28	Eimeria infected litter	Birds in *B. subtilis*-treated group had better WG than the controls	(68)
B. subtilis (ATCC PTA-6737)	Ross 308	2.0×10^7	14	*S. typhimurium*	Higher ($p > 0.05$) WG and lower FCR were observed in the *B. subtilis* and avilamycin (0.2 g/kg) groups when compared to the challenged control group.	(30)
B. licheniformis H2	-	1.0×10^6	22	*C. perfringens*	*B. licheniformis* improved BWG in *C. perfringens* challenged broiler chickens.	(69)
B. subtilis GalliPro-DSM 17299		1.2×10^6	42	*C. perfringens*	NE-challenged broilers had depressed BWG while, infected broilers fed *B. subtilis* had significantly higher BWG. However, feed intake and FCR were not significantly influenced.	(58)
Bacillus DFMs	Ross 708	0.80 cfu/g *Bacillus* A. and 0.82 cfu/g *Bacillus* L	42	*Eimeria species* and *C. perfringens*	*Bacillus* DFMs supplementation increased BWG and reduced FCR and mortality in subclinical challenged broilers.	(70)
B. licheniformis	Avian	1.2×10^6 cfu/g spore count	35	*C. perfringens*	Challenged broilers fed *B. licheniformis* and bacitracin methylene disalicylate (2 g/kg) had improved BWG and FCR than challenged control broilers.	(59)
B. subtilis B21 *B. licheniformis* B26	Arbor Acre	2×10^9	42	*C. perfringens*	Challenged broilers offered *Bacillus probiotics* and enramycin (5 mg/kg) recorded higher BWG and lower FCR than unchallenged control broilers.	(71)
B. subtilis (ATCC PTA-6737) *B. licheniformis*	Ross 308	2×10^7 2×10^6	nr	*Salmonella* spp.	*Bacillus* probiotics supplementation increased feed intake and BWG in diseased challenged broilers.	(63)

(Continued)

TABLE 1 | Continued

Bacillus strain	Broiler strain	Dosage (cfu/g)	Duration of study (days)	Pathogen challenged	Response variables	Authors
B. subtilis PB6	Ross 308	0.5 g/kg	35	*C. perfringens*	NE challenged broilers fed *B. subtilis* additive had better FCR post-NE period (28–35 d) than NE challenged broilers offered diet without *B. subtilis* additive. In addition, non-challenged birds fed *B. subtilis* had the highest BWG followed by non-challenged birds fed basal diet only, while the lowest BWG were recorded in NE challenged birds without *B. subtilis*.	(72)
B. amyloliquefaciens LFB112	Ross 308	5×10^5	39		Broilers fed *B. amyloliquefaciens* had heavier BWG and reduced FCR than birds fed *B. subtilis*, control (basal diet only) and antibiotics.	(73)
B. subtilis PB6	Arbor Acres	4×10^4 6×10^4	26	*C. perfringens*	Dietary *Bacillus* probiotics increased feed intake, FCR and BWG in challenged broiler chickens The challenged groups administered *B. subtilis* PB6 compete favorably with the group administered with 10 mg/kg enramycin.	(60)

Jayaraman et al. (56) investigated the impact of *B. subtilis* administration on the micro-anatomy of broiler chickens challenged with *C. perfringens*-induced NE and NE-challenged control birds had thickened mucosa, hemorrhages, intestinal lesions, and ballooning of the intestine. However, there were no signs of gross pathological changes in the intestines of NE-challenged broilers fed *B. subtilis* PB6 (5×10^7 cfu/g), indicating that inclusion of *B. subtilis* PB6 in chicken feed reduced intestinal *C. perfringens* counts and improved gut integrity. Jayaraman et al. (56) also reported distorted and damaged villi in *C. perfringens* challenged broiler chickens, implying loss of gut integrity. These findings also supported others who reported improved intestinal health in disease-challenged broilers (63, 83, 84) and healthy broiler chickens (31, 85, 86) fed *Bacillus* probiotics in their diets. The improved intestinal health in both healthy and infected broilers could be due to a decrease in the population of enteric pathogenic microbes, leading to increased digestion and utilization. These results corroborated Samanya and Yamauchi (87), who reported improved intestinal villus measurements in healthy chicken fed dried *B. subtilis*.

Deepak et al. (58) found severe histopathological changes in the jejunal villi of NE-challenged broiler chickens. However, the severity of the histopathological changes was reduced in infected broilers treated with *B. subtilis* compared to infected control birds. They also observed that the NE-challenged broilers administered *B. subtilis* had increased villi length compared to challenged control broiler chickens. In a similar study, Lee et al. (76) noticed significantly reduced intestinal lesion scores in EM infected broiler chickens fed diets treated with *B. subtilis* strains (15AP4 and Bs27) compared to infected

control chickens. These findings are consistent with previous research (88–90). A recent investigation by Konieczka et al. (91) showed improved gut morpho-structural indices and structure in *C. perfringens* infected broilers fed multistrain *Bacillus* probiotic in their feed when compared to those fed single strain *Bacillus* probiotic, implying that the multistrain *Bacillus* probiotic was more effective than the single strain *Bacillus* probiotic in improving gut histomorphological indices in diseased broiler chickens.

C. perfringens produces several harmful substances after colonization of the gut (92), and this affects the tight junction and their components by increasing gut permeability, resulting in compromised gut integrity and function (81). Administration of antibiotics (enramycin) and *B. subtilis* ($4.0–6.0 \times 10^4$ cfu/g feed) restored gut integrity, and reduced intestinal lesion score in Arbor Acre broilers challenged with 10^8 cfu/mL *C. perfringens* (60). Arif et al. (61) also found that inclusion of *B. licheniformis* at 1.0 kg/MT of feed in broiler chickens reared under a low-level of biosecurity measures had slightly better intestinal lesion score than groups fed diets with no additives, *Bacillus subtilis* PB6 (1.0 kg/MT feed) and 4% flavomycin (0.3 kg/MT feed). This agrees with Wu et al. (54), who found increased villus height (VH) and VH: crypt depth (CD) ratio in jejunum and reduced gut lesion scores in the small intestine in NE infected broilers fed a diet treated with *B. coagulans*. The damaged intestinal wall in NE-infected broilers could be one of the possible explanations for the poor growth performance of broilers infected with *C. perfringens*. Researchers have demonstrated that *B. subtilis* PB6 and enramycin can reduce lesion scores and enhance gut health in *C. perfringens* challenged broilers (71, 93). Enramycin kills enteric bacteria by inhibiting cell

wall formation, reducing the harm they can cause to the body (93).

SERUM BIOCHEMICAL CHARACTERISTICS

Blood indices are used in the nutritional assessment to determine the quality of test feedstuffs/additives and to reflect the physiological state of the animal (94). Abudabos et al. (63) discovered that dietary *B. licheniformis* (2×10^6 cfu/g) and *B. subtilis* (2×10^7 cfu/g) significantly increased serum cholesterol, glucose, total protein and globulin levels in Salmonella-challenged broiler chickens. This is supported by the earlier findings of Abudabos et al. (84), who reported an improvement in plasma total protein and glucose levels in Salmonella-infected broilers fed diets having supplemental levels of *B. subtilis, S. boulardii* and oregano. The improvement in serum protein level could be ascribed to the ability of *Bacillus* probiotics to improve dietary protein digestion and utilization by increasing the absorptive efficiency of the small intestine (51, 95, 96). Also, Abudabos et al. (30) demonstrated that the inclusion of *B. subtilis* and antibiotics in the chicken diet improved serum biochemical values in Salmonella-challenged broilers. These findings were in converse with Abudabos et al. (63), who evaluated the impact of *B. subtilis* and avilamycin/kg feed on serum biochemical characteristics of Salmonella-challenged broilers and noticed that supplementation of *B. subtilis* and avilamycin/kg feed in broiler feed had no adverse influence on plasma cholesterol, glucose, total protein and globulin values. Abudabos et al. (83) stated that the addition of *B. subtilis* in the diets of Salmonella infected broilers during the starter phase did not affect the content of serum glucose and protein. They also reported that *B. amyloliquefaciens* supplementation influenced blood metabolites in broiler chickens during the starter phase. The reasons for the discrepancy in serum biochemical values in broilers fed *Bacillus* probiotics is unknown; however, the difference may be related to differences in strains of *Bacillus* probiotics and concentrations, as well as the severity of infection.

Serum immunoglobulins (Ig), especially those generated from the b cells are used as indicators of humoral immunological state in poultry because of their role in immune regulation and resistance to several diseases (97, 98). Serum Ig levels in broilers were affected by *Bacillus* probiotics as Ahmat et al. (73) observed increased serum IgA and IgG in healthy broilers fed aureomycin at 150 mg/kg, and *Bacillus* probiotic (*B. amyloliquefaciens* and *B. subtilis*) and their metabolites at the level of 5.0×10^5 cfu/g in their diet. These results indicate that *Bacillus* probiotics and their metabolites that increased serum immunoglobulin concentrations will have more beneficial impacts on growth performance and broiler chickens' ability to combat diseases. This observation supports Luan et al. (99) and Wang et al. (100), who reported increased serum IgA, IgM and IgG in chickens fed *Bacillus* probiotics in their ration. This result supported Abudabos et al. (63) who found increased plasma globulin protein levels in Salmonella-challenged broiler chickens fed two

strains of *Bacillus* probiotics and their metabolites. Rajput et al. (101) discovered that adding 1.0×10^5 cfu/g *B. subtilis* B10 to chicken feed increased the amount of IgA-positive cells and concentration of cytokine secretory IgA in the small intestine.

DOSAGE, PRESENTATION FORMS AND ROUTE OF ADMINISTRATION

Different effective doses of *Bacillus* probiotics in broilers have been reported (60, 63, 66, 71). However, the efficacy may vary from one study to the other due to differences in *Bacillus* probiotics composition, dosage, duration of supplementation and strain used as well as chicken's age and health status (21, 33). The effective dose of *Bacillus* probiotics in broilers ranges from 1.0×10^6 to 8.0×10^7 cfu/g feed (33). The most common form and route for *Bacillus* probiotics is powder administered *via* feed (33, 71). The powder form is preferred by end users as it has advantages such as uniformity, stability, longer shelf life, ease of packaging and transportation, and easy incorporation into current feed manufacturing processes (102–104).

LIMITATIONS AND STRENGTHS

Some of the limitations of this study are: (1) In this review, we investigated the impact of *Bacillus* spp. on growth performance and health indices of disease-challenged broiler chickens and may not apply to other animal species; (ii) The variations in diet composition, degree of infection, species of *Bacillus* probiotics, dosage and duration of *Bacillus* probiotics supplementation and season may have effect on the reliability of the results. The differences in some of serum chemistry parameters in this review may depend on the age and sex of the chickens. In addition, differences in assay types and methods of analysis used by different studies may be a limitation. Despite constraints to their adoption, there is progressive increase in *Bacillus*-based probiotic studies used in poultry production. However, the results of this review have made an important contribution to knowledge on how *Bacillus* probiotics supplementation improves growth performance and health indices in disease-challenged broiler chickens.

CONCLUSION

This review indicated that *Bacillus* probiotics currently used in broiler chicken industry are isolated from the gut, food, soil and pond. This study also revealed that *B. subtilis, B. coagulans* and *B. licheniformis* were the most common *Bacillus* species used in broiler chicken nutrition. In addition, *Bacillus* probiotics had a positive effect on body weight gain and feed conversion ratio. It also improved blood characteristics and intestinal histomorphological indices of disease-challenged broiler chickens and may replace in-feed antibiotics in broiler chicken production. These findings also support the proposition that *Bacillus species* can assist chickens to fight enteric pathogens. *Bacillus* probiotics may have achieved these beneficial effects

through one or a blend of the following mechanisms of action: (1) decreasing pathogen proliferation in the gastrointestinal tracts *via* competitive exclusion and antagonism, (2) production of organic acids leading to a decline in gut pH, (3) production and release of antimicrobial compounds, (4) improvement in oxidative stability and immune systems, (5) modifications in gut metabolic processes to support the production and release of digestive enzymes, and (6) direct nutritional effects or enhancing feed consumption, digestibility and nutrient uptake. However, as with other probiotics, the potential of *Bacillus* probiotics in broiler chicken nutrition has not been fully realized, possibly because their effect is dependent on a variety of factors such as *Bacillus* strain, species, dose level, age of chicken among others. Thus, further research is required using meta-analysis and quadratic optimization model to determine the optimum dose level of *Bacillus* probiotics that enhance broiler chicken performance before *Bacillus* probiotics could be considered as a substitute for in-feed antibiotics. In addition, the use of transcriptomics technologies to understand the mechanisms by which of action of *Bacillus* probiotics improve health and productivity of disease-challenged broiler chickens is recommended as such information is lacking in the literature.

AUTHOR CONTRIBUTIONS

MM and IO: conceptualization. IO: writing—original draft preparation. NS, MM, and CM: review and editing. CM and MM: visualization. All authors have read and agreed to the published version of the manuscript.

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