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THE BENEFICIAL EFFECT OF *BACILLUS* SPP. AS PROBIOTICS IN POULTRY NUTRITION - A REVIEW

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Abstract

Over the last few decades, the use of probiotics as source of feed additives in animal nutrition has increased considerably. As you know, sub-therapeutic levels of antibiotics were used as growth promoters (AGP) in the animal field, with extensive use in poultry industries, but due to their multivarious side effects, it was necessary to find some alternatives in order to satisfy the consumer's demands. Probiotics are considered one of the options as a significant alternative to antibiotics for improving health, growth, and poultry production. In our day, among the extensive number of probiotic products in use are bacterial spore formers, mostly of the genus Bacillus. The current review presents the benefits of probiotic utilization based on Bacillus spp. in poultry feed highlighting their potential to form spores that can withstand harsh environmental stress and transition during poultry gastrointestinal tract. Furthermore, Bacillus spores involve more than 80% survivability during the probiotic in vitro tests, remaining stable in a fairly high concentration. Based on the information found from published articles, this review summarizes stronger information about the properties of Bacillus spp. obtained from in vitro and in vivo screening, which can provide researchers with a better understanding of the use of this species in poultry nutrition.

Key words*: Bacillus spp., poultry, probiotics.*

INTRODUCTION

The use of antibiotics as growth promoters (AGP) in animal diets has been of concern and has even been banned in many countries globally, due to the appearance of resistant bacteria to antibiotics, which was associated with human and animal illnesses (Cartman et al., 2004; Bajagai et al., 2017; Jiang et al., 2021). Excessive antibiotic utilization involves the appearance and transfer of gene resistance between bacteria, the disequilibrium of normal microflora, and the decline of beneficial intestinal bacteria (Sinol et al., 2012). Moreover, Cervantes (2015) affirmed that the elimination of antibiotics involves considerable consequences with negative effects on performance production, infections of the gut, and the possibility of a high mortality rate in the poultry industry.

Since 2006, many European Union (EU) countries prohibited using all commonly feed antibiotics added as growth promoters (EC Regulation No. 1831/2003). The EU has included this issue as the main point of ***"the farm to fork concept"*** and, shortly, the European Commission will act to decrease the total antimicrobials sales for livestock animals (European Commission, 2020). Therefore, probiotics are increasingly popular as considerable safe alternatives to replace and reduce antibiotics (Meng et al., 2010; Nguyen et al., 2018; Luise et al., 2022), as a viable solution to save the animal livestock sector (Hmani et al., 2017; Park et al., 2015), especially for young animals like broilers and piglets (Aar et al., 2016; Idriceanu et al., 2020).

According to the Food and Agriculture Organization (FAO, 2002) and World Health Organization (WHO, 2006) guidelines, probiotic bacteria are an important solution and have been proved the most favourited alternative to antibiotics as AGP and inhibitor of pathogens in the animal industry (Zhang & Kim, 2014).

Probiotics are defined as a preparation containing viable or inactivated known bacteria (Ramlucken et al., 2020a) and generally are recognized as safe (GRAS). Also, the use of probiotics or direct in-fed microbial (DFM) which, when administered in adequate amounts, confer “a health benefit to the host” (Fuller, 1989; Schrezenmeir & De Vrese, 2001) and seems to be one of the most promising strategies (Barba-Vidal et al., 2019).

Probiotics occur an important place due to their beneficial impact on body weight host, growth performance, improving the health profile (Abd El-Hack et al., 2020; Zhang & Kim, 2014), gut immunity by regulating the metabolism, and bacteria compositions from this area (Luise et al., 2022).

Generally, a probiotic strain is recommended to be isolated from the same source for which it was created. Based on the probiotic perspective, it is proclaimed that the candidate probiotic should be isolated from the source of the target population, which helps them to grow well inside the selected host (Elshaghabee et al., 2017).

Before a probiotic product can be included in poultry feed, it is essential to assay its stability (viability and growth) under simulation of the gastrointestinal tract (GIT) harsh conditions. As a level of inclusion, a probiotic must retain less than 1 x 10^6 CFU g^{-1} (Millette et al., 2013).

Probiotics improve digestion and nutrients absorption by inhibiting potentially pathogenic bacteria, regulating intestinal affection (Ding et al., 2021), and modulating the gut microbiota, which plays a critical role in sustaining beneficial health status (Patil et al., 2015). Also, probiotics addition as feed additives or supplements can re-establish the ecologic stability of gut microbiota by inhibiting pathogens and promoting the growth of representative bacteria (Bermúdez-Humarán et al., 2019; Del Toro-Barbosa et al., 2020).

An ideal probiotic is necessary to have the capacity to adhere to the intestinal mucosa, grow rapidly, and maintain its viability (Luise et al., 2022). Manufacturing is an additional trait of probiotics including transport and storage conditions, applied usually in the processes for obtaining animal feed, to keep as much possible the vital properties of these products (Banjagai et al., 2016), especially after feed pelleting, storage, and manipulation (Cutting, 2011).

The most commonly used probiotics are Gram-positive bacteria from the genus *Bacillus, Lactobacillus, Lactococcus, Streptococcus, Bifidobacterium, Enterococcus,* and non-bacteria (yeast or fungal) including *Aspergillus oryzae, Candida pintolopesii, Saccharomyces boulardii,* and *Saccharomyces cerevisiae,* which are widely used to prevent poultry diseases, pathogens multiplication and improve the growth performance (Mountzouris et al., 2007; Gaggia et al., 2010; Elshaghabee et al., 2017; Kerry et al., 2018; Dumitru et al., 2020; Yoha et al., 2021).

Currently, through the large number of probiotic products, bacterial spore formers are in use today (Hong et al., 2005) and have been most extensively studied. Species from the *Bacillus* genus present a distinct advantage over other probiotics due to the capacity of sporulation (Kim et al., 2019), germination, and proliferation within the GIT of animals (Dumitru et al., 2019; Ciurescu et al., 2020; Dumitru et al., 2021).

As Gram-positive or Gram-variable rods, catalase producing and efficient probiotic product, *Bacillus* spp. is necessary to survive during environmental stress, preparation conditions and application processes, tolerance to low pH (Lee et al., 2017; Penaloza-Vazquez et al., 2017), bile salts concentrations, and other severe conditions for the keep of their viability and properties within GIT (Barbosa et al., 2005; Shivaramaiah et al., 2011; Jiang et al., 2021; Dumitru et al., 2020). Morphologically, *Bacillus* species have rod-shaped cells with squared or rounded ends between 0.5 x 1.2 to 2.5 x 10 µm, occurring singly or in chains, and chains stability determines the colony form, which may differ from strain to strain (Logan & De Vos, 2009).

In comparison with other probiotic bacteria *Bacillus* spp. have notable advantages due to the capacity that are endospore-forming aerobic or facultative anaerobic bacteria. Sosa et al (2016) affirmed that *Bacillus* spp. under stressful environmental conditions can produce spores that remain in a dormant state for long periods (more than 2 years).

This trait makes them thermostable for storage and processing (i.e., extrusion and pelleting), with resistance to extreme temperatures up to 113°C for 8 min (Grant et al., 2018). This property makes it easier to control and enhances its probability of surviving during the animal feed production process. Further, Cartman et al. (2008) affirmed that *Bacillus* spp. can utilize nitrate or nitrite to facilitate anaerobic respiration, which enables them to survive in anoxic conditions.

Additionally, *Bacillus* spores were confirmed to survive at low pH in the stomach, bile salts, harsh conditions in the GIT environment of the host (Barbosa et al., 2005; Chaiyawan et al., 2010; Wang et al., 2010; Cutting, 2011; Bajagai et al., 2016; Dumitru et al., 2018; Dumitru et al., 2020), high pressures, and caustic chemicals, making them suitable for distribution and commercialization (Cartman et al., 2007).

Regarding the *Bacillus* group, the bacilli are easy to produce by conventional fermentation and do not involve expensive manufacture to ensure a stable commercial product (Cutting, 2011; Ramlucken et al., 2020b).

The addition of viable probiotics such as DFM, including bacteria from *Bacillus* group, involves beneficial health. Usually, *Bacillus* are examined as probiotic products in monogastric animals: *Bacillus subtilis, Bacillus licheniformis, Bacillus amyloliquefaciens*, *Bacillus coagulants, Bacillus cereus* and *Bacillus megaterium* (Cutting, 2011; Ciurescu et al., 2020; Dumitru et al., 2021; Uraisha et al., 2021; Mushtaq et al., 2022).

The anti-nutritional factor from feed materials raw could potentially be neutralized by using enzymes that occur a vital role in nutrient absorption by diminishing intestinal viscosity through catalyzing undigested starch polysaccharides (Popov et al., 2021).

Bacillus species possess several capacities such as secretion of beneficial enzymes (amylase, protease, cellulase, lipase, xylanase, phytase, and keratinase), antimicrobial molecules production (Ramlucken et al., 2020c; Sumi et al., 2015), and beneficial metabolites through modification of gut microflora (Grant et al., 2018; Dumitru et al., 2019; Shah & Bhatt, 2011; Jani et al., 2012). Further, the capacity of sporulation extends the percentage of survivability (heat tolerance, low pH of the gastric barrier, and longer viability during storage) in several environmental conditions compared to those containing non-spore-forming bacteria (Mingmongkolchai & Panbangred, 2018; Dumitru et al., 2021).

Bacillus improves the intestinal immune system by raising the levels of cytokines and chemokines as interleukin-1β (IL-1β) and interferon-γ (IFNγ) in the chicken gut (Lee et al., 2013).

In the last decade, the use of probiotics in animal feed has occupied significant attention, and the majority of the recent probiotics are represented by lactic acid bacteria, especially *Lactobacillus* spp. and *Enterococcus* spp. (Sorescu et al., 2019).

Based on published studies, this review will focus on the dietary supplementation with *Bacillus*-based probiotic in broiler chickens and the positive traits of this genus that has functional effects on the development of suitable commercial probiotics in poultry nutrition.

MATERIALS AND METHODS

To conduct this review, more than 160 references were necessary based on *in vitro* probiotic properties which establish the desirable *Bacillus* characteristics of several strains for survivability during GIT harvest conditions, and their effect as a probiotic product in poultry nutrition. The electronic search was carried out over the last 25 years from articles published in ISI Journals, Web of Science (WoS), and Scopus. For example, the systematic topic of research data was carried out from Google Scholar (https://scholar.google.com), ScienceDirect (https://www.sciencedirect.com), PubMed (https://pubmed.ncbi.nlm.nih.gov/), and NCBI-PCM (https://www.ncbi.nlm.nih.gov/pmc/). The topic of interest as a strategy for the search was based on probiotics effect on poultry nutrition. In addition, the keywords used for the search were: probiotics, alternative to antibiotics, probiotic properties, pH resistance, bile salts tolerance, immune response, spores viability, enzymatic activity, microflora, intestinal health, broiler performance, *Bacillus* spp., and poultry nutrition.

RESULTS AND DISCUSSIONS

Probiotics history, niche and mode of action

Elie Metchnikoff was the first investigator in the fermentation processes field and probiotic products. He reported that large soured milk

consumption increases human longevity. Furthermore, Metchnikoff affirmed that the lower gut can be affected by microbes, generally bacteria from *Lactobacillus* genus, for instance, *L. bulgaricus* (Ran et al., 2019).
The term probiotic is correlated with "life" being considered "microbial feed supplements that can affect positively the host". Over the years, the meaning of probiotics was changed. Later, in 1953, Werner Kollath gave other terminology as, "probiotika" and defined as "live microorganisms which are essential for the healthy development of the gut for life". The definition of probiotic was in continuous modification. Lilley and Stillwell (1965) defined probiotics as possible microorganisms with the capacity to help the proliferation of another beneficial microorganism. Our days, their definition is opposite to the antibiotic terms (Abd El-Hack et al., 2020). Morelli & Capurso (2012) defined probiotics as the consumption of enough live microorganisms with the capability to contribute health benefits to the host. Also, the authors affirmed that some strains ingested by the host may induce other reactions in the body. An example that can be given is related by *Bifidobacterium* spp. which can produce metabolic end products (acetate and lactate) with the capacity to diminish Gram-positive and Gram-negative pathogenic bacteria (Abd El-Hack et al., 2020).
Bacillus species have been isolated from a diversity of habitats as soil, vegetables, water, animals, and as a transient part of the human gut, contaminants of raw and prepared foods, aviation fuels (Kotb, 2014; Alou et al., 2015), feces from different animals as chickens, pigs, ruminants and aquatic animals (Mingmongkolchai & Panbangred, 2018). Hong et al. (2005) affirmed that species from *Bacillus* are normally allochthonous from GIT due to the ingestion of bacteria from soil and contaminated food. Table 1 is presented *Bacillus* probiotics isolated from different sources and their benefits in the poultry industry.

Table 1. *Bacillus* isolation from diverse sources with applicability in poultry

***Bacillus* designation**	**Sources**	**Benefits**	**Reference**
B. subtilis	Soil	Improve the growth performance, gut, excreta bacterial community, immune system and gut health, regulate intestinal microstructure and digestive enzymes.	Bar & Friedman (2018); Ciurescu et al. (2020); Liu et al. (2020); Oladokun et al. (2021).
B. subtilis CH16	Chicken GIT	Increase in daily weight gain (ADG), body weight (BW) and biofilm formation, reduce feed conversion ratio (FCR).	Nguyen et al. (2015)
B. subtilis (SC2362, 1781, 747, ATCC PTA-673, PB6)	Environment sources, soil	Capacity to germinate in GIT. Increase eggshell thickness, decrease excreta *Salmonella* counts without harmful effect on performance. Beneficial influence on selected performance parameters, egg quality, and the cholesterol content of yolk lipids. Greater resistance to the avian pathogenic *Escherichia coli* O78:K80 with a reduction in the colonization of the spleen, liver, and caeca.	Cartman et al. (2008); Sobczak & Kozłowski (2015); Park et al. (2020); La Ragione et al. (2001); Jayaraman et al. (2017)
B. subtilis fmbJ	Soil	Significantly decreased reactive oxygen species (ROS) contents in liver mitochondria of broilers.	Bai et al. (2017)
B. subtilis	Soil	Release antimicrobial and antibiotic compounds.	Jayaraman et al. (2017)
B. subtilis 1781 (PB1): *B. subtilis* 1104+*B. subtilis* 747	Environment sources	Modify intestinal activity and influence gut barrier integrity through increased tight junction gene expression.	Gadde et al., (2017b)
B. licheniformis	Unknown	Enhance meat, necrotic enteritis, and enhance growth performance.	Liu et al. (2012); Cheng et al. (2017)
B. subtilis	Soil	Improve feed conversion efficiency and diminish abdominal fat. Reduce the intestinal size and promote the growth of several digestive organs.	Samanya et al. (2002); Wang et al. (2018)
B. amyloliquefaciens	Soil	Increase serum immunoglobulin levels, decrease the number of *E. coli*, NH_3, and H_2S emissions. Enhances gut health and growth performance.	Ahmet et al. (2014); Tang et al. (2017); Li et al. (2015)

B. coagulans	Soil	Protective efficacy in *Salmonella enteritidis* infections.	Zhen et al. (2018)
Symbiotic: prebiotic (xylooligosaccharide and yeast) and probiotic (*B. licheniformis*, *B. subtilis* and C. *butyricum)*	Soil	Prevents necrotic enteritis and enhances growth performance.	Li et al. (2019)
B. subtilis KATMIRA1933 and *B. amyloliquefaciens* B-1895	Soil	Positively affected egg production, quality of sperm, quality and hatchery of eggs.	Mazanko et al. (2018)
BioPlus 2B (preparation of *B. subtilis* DSM 5749 and *B. licheniformis* DSM 5750)	Soybean mash and soils	Improve growth performance.	EFSA (2019)
B. subtilis and *B. licheniformis*		Performance improvement and control effects of *Salmonella* infection.	Abudabos et al. (2020)
B. pumilus and *B. subtilis*		Beneficial effects the intestinal and immune activities, specifically in day-14.	Bilal et al. (2021)

The presence of spores makes the *Bacillus* group to resist in extreme conditions (stomach acidity, bile salts concentrations etc.). Besides, during processing and storage, the bacilli spore-formers involve more stability, making them suitable as an ingredient for probiotic formulations (Elshaghabee et al., 2017).
Different supposable mechanisms for probiotic action have been investigated based on inhibition and stimulation of the host immunity (Guo et al., 2020). The main interest in animal nutrition is occurred to the relationship between nutrition and gut health, mainly in the small intestine (Luise et al., 2022). An important characteristic of this genus is its rapidity of growth and capacity for survival during chickens' GIT (Lattore et al., 2014).
The mechanisms of *Bacillus* spp. action in their vegetative state may function as probiotics are similar to those of other probiotic organisms (Ramlucken et al., 2020c). Figure 1 is illustrated the main actions of *Bacillus* strains in the organism host.

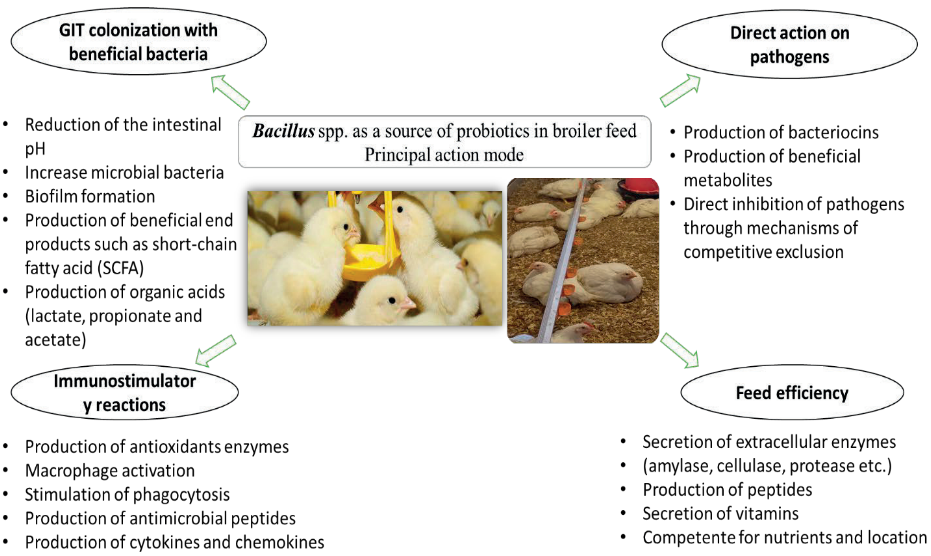

Figure 1. The effects of *Bacillus* as a probiotic source in poultry nutrition

It is known that the oral cavities of birds do not present teeth, compared to the mammals, so, feed going down to the esophagus into the crop (Scanes, 2014). Saliva secretion starts the process of feed humidification in the oral cavity involving a moist and good medium for the progress of bacteria in the crop (Scanes, 2014).
As a group of bacteria *Lactobacillus, Enterobacterium* and *Bifidobacterium* were most representative in the broiler chicken crop (Feye et al., 2020). Additionally, after a review

by Feye et al. (2020) large number of *Bacillus* (more than 70%) can be found in the poultry crop. In this region of the GIT, the feed can remain around 14 h, but many times stay between 1-3 h (Scanes, 2014).

It is very important when using a probiotic product based on *Bacillus* to be resistant to harvest environment conditions. Due to the low pH of the glandular stomach (2.3-4.8) of chickens, the probiotic of interest should not resist (Grist, 2006) which is why most of the microorganisms numbers are lower compared to the crop and intestine. Furthermore, *Bacillus* species have the capacity to produce extracellular enzymes; the first part of the enzymatic process of digestion of feed begins in the gizzard, the organ where feed is broken down and transported to the small intestine in small portions (Scanes, 2014).

The small intestines of poultry (duodenum, jejunum, and ileum) present a gradual pH from 5.0-6.0 (Ciurescu et al., 2020); however, in the small intestine, the feed remains 2 to 8 h, a process that is accompanied by secretion of enzymes and mucin (Scanes, 2014), digestion and absorption of nutrients.

A study by Dumitru et al. (2019) demonstrated the *B. licheniformis* capacity to produce extracellular enzymes by determining the carbohydrate fermentation profile through API 50CHB kits, resistance at low pH (2.0 and 3.0), and bile salts concentrations, properties that are necessary to be evaluated before administration of DFM probiotics in animal nutrition.

In the cecum, feed stays for 12-20 h (Oakley et al., 2014; Clavijo & Flórez, 2018). Gang et al. (2002) affirmed that the first function as a result of fermentation in the cecum is enzymatic activity and detoxification of damaging substances.

As mentioned above, the conditions during GIT are different, and not every bacteria can resist. In this case, the best option is to find significant probiotics through the commensal microorganisms that populate the interest intestine area (Popov et al., 2021).

Competition for adhesion to the intestinal epithelium

The selection of *Bacillus* as DFM candidate or probiotic product is based on adheration to the epithelial surface, colonization, and population of the GIT host, and afterward to form a strong barrier to prevent the adhesion of pathogens (Chauhan & Singh, 2019).

The results obtained by Nishiyama et al. (2020) suggest that *B. subtilis* C-3102 supplementation presented the potential to diminish *S. enterica* infection rates and accelerate the pathogen exclusion from the cecum, spleen, and chickens liver. Another study confirmed the potential of *B. amyloliquefaciens* US573 for exhibiting good adhesion efficacy to chicken enterocytes and the ability to create biofilms that may favour survivability in the animal tract. Moreover, the US573 strain neutralizes the antinutritional factor and maximizes nutrient absorption due to the enzymatic activity (xylanase, β-glucanase, and amylase). Therefore, the mode of action of poultry probiotics is not very clear, for this reason, further studies with *Bacillus* as DFM-probiotics will be in continuous research.

Secretion of inhibitory substances

Bacillus species are well-known producers of antimicrobial peptides such as bacteriocins, small ribosomal peptides with the capacity to inhibit the growth of pathogens. Khalique et al. (2020) showed that *B. subtilis* SP6 exhibits a wide antibacterial spectrum that has antagonist activity against *Clostridium perfringens*, a normal inhabitant in chicken intestinal microflora, usually found in low numbers in the posterior gut section (Arif et al., 2021).

Bacteriocins can attend as colonizing peptides by facilitating the population of probiotic strains into an already employed niche on the intestinal epithelium (Bahaddad et al., 2022). *B. subtilis* KATMIRA1933 and *B. amyloliquefaciens* B-1895 are probiotic strains that secret the bacteriocins subtilisin A and subtilin which have significant potential against *Salmonella* by inhibition of biofilm formation which may serve in decreased the pathogens microorganisms (Tazehabadi et al., 2021).

Modulation of the immune system

It is known that innate immunity is the first line of protection against pathogens. The probiotic spore-forming bacteria have been described for their aptitude to stimulate and/or control the poultry immune system by secreting cytokines and immune defence substances (Popov et al., 2021).

Probiotic supplementation, as an immunomodulatory answer, positively increased the level of serum immunoglobulin (Paturi et al., 20007). In addition, Mountzouris et al. (2007) affirmed that the inclusion of probiotics stimulates GIT immunity by decreasing the number of pathogens in microflora. Likewise, Xu et al. (2012) reported that *B. subtilis* stimulated the production of cytokines as Il-10 and Il-4. Furthermore, *Bacillus* spp. registered a vital role in cytokines regulation (Mushtaq et al., 2022), immune modulation, and activation of macrophages without cytotoxicity (Popov et al., 2021).

According to Bai et al. (2017), the inclusion of *Bacillus* spp. in broiler diets improves radically the IgA. Al-Khalaifa et al. (2019) reported that the administration of probiotics in broiler production improves significantly the immune globulin. Furthermore, Fathi et al. (2017) described those dietary probiotics in broiler chickens improved the level of immune-globulins (IgA, IgM, and IgG).

Several studies were demonstrated that the inclusion of *B. subtilis* in chickens' diets enhances the innate and acquired immune responses of broiler (Pagnini et al., 2010; Lee et al., 2015; Gadde et al., 2017a; Guo et al., 2020; Tarradas et al, 2020; Sikandar et al., 2020). Besides, *B. subtilis* was shown to modulate the responses of immune protective hosts against potential infections (Rajput et al., 2014; Zhang et al., 2017).

Spore formers as probiotic product

Due to physiological properties, *Bacillus* species have the capacity to produce a multitude of enzymes, metabolites, antibiotics, having thus a high spectrum of utilization in medical and pharmaceutical fields, agricultural and industrial processes, animal nutrition, etc. (Celandroni et al., 2019).

The most common bacteria from the *Bacillus* group used as a probiotic product in animal production including in the poultry industry is the *B. subtilis* strain (Joerger & Ganguly, 2017; Idriceanu et al., 2020).

Efficacy of probiotic inclusion can be ascribed to the species of bacteria and the formula of supplementation used, such as wet (liquid culture) or powdered (lyophilization) (FAO & WHO, 2001). Administration of *Bacillus* as a probiotic product in the poultry diet can be performed orally, directly in water drinker (as liquid inoculum culture), or homogenized along with the feed (Lei et al., 2015; Lattore et al., 2017; Ma et al., 2018; Ciurescu et al., 2021). During the manufacturing including fermentation, drying, freezing, thawing, and rehydration, *Bacillus* spores have the ability to resist passage through the GIT, proliferate and populate the host digestive tract (Elisashvili et al., 2019; Popov et al., 2021).

Bacillus is a group recognized as spore-forming bacteria, known for their capability to germinate, proliferate, and re-sporulate. Due to the production of endospores, Bacilli involve long viability making them more stable and resistant to harvest environmental conditions. Keller et al. (2020) showed that when used *in vitro* human model, the *B. coagulans* GBI-30 can proliferate up to 97% in the GIT with active metabolically cells. Lattore et al. (2014), also, noted that 90% of *Bacillus* spores germinate in the small intestine of chickens within one hour. The spores can adhere to the intestinal walls, germinate and sporulate under anaerobic conditions as commensal to the animal intestines (Hong et al., 2009; Auger et al., 2009).

Probiotic benefits in poultry diets

The administration of probiotics in the poultry field specifically in broiler chickens has positive impacts on growth performance, feed efficiency, gut histomorphology improvement, immunity status, increase diseases resistance, and a beneficial microbiota increment (Simon et al., 2001; Mountzouris et al., 2010; Grant et al., 2018; Zhen et al., 2018).

As a direct effect, *Bacillus* probiotics can act on pathogenic bacteria such as *E. coli, Salmonella, Clostridium,* and *Campylobacter*, ensuing inhibition of their growth and population of the animal gut (Luise et al., 2022), thus preventing or reducing the incidence of infections (Zhang et al., 2014; Ding et al., 2017; Castaneda et al., 2021). For example, necrotic enteritis (NE) in the broiler industry, is produced by *Clostridium perfringens,* which is a digestive tract infection with negative effects on host profitability (Abd El-Hack et al., 2022) and has conducted over the years to economic losses (Salem and Attia, 2021). As an enteric disorder, *C. perfringens* can be found in air, wastewater, healthy human, and animal GIT (Khelfa et al., 2012). Through 2 and 6 weeks, NE can occur in the broiler chickens tract due to the strong characteristics caused by *C. perfringens* pathogen (anaerobic, Gram-positive, endospore-forming, without motility), which could survive and stay life in extreme environmental conditions such as disintegration of organic matter and soil, due to the capacity to form endospores (Khelfa et al., 2015).

Studies have presented that inclusion of *Bacillus* spp. improves overall intestinal health and performance growth in broiler chickens (Grant et al., 2018). Teo & Tan (2007) showed that two types of *B. subtilis* strains, isolated from the chicken gut, involved antagonistic action against *C. perfirngens* ATCC 13124. Later, in 2010, Knap et al. observed a reduction of *C. perfringens* in chickens at the addition of three levels of *B. licheniformis* s (8×10^5 CFU/g feed, 8×10^6 CFU/g feed, and 8×10^7 CFU/g feed). All three concentrations maintained similar body weight (BW) and feed conversion ratio (FCR) in the chicken's trial. Further, intestinal *Salmonella typhimurium* was significantly decreased in the presence of *B. subtilis* B2A (1×10^4 CFU/g, 1×10^5 CFU/g, and 1×10^6 CFU/g). Park and Kim (2014) reported a better feed conversion ratio (FCR) and a less feed intake (FI) in the experimental chicken group feed with *B. subtilis* B2A. As mentioned, *Bacillus* is in a vegetative state it is possible to not persist in the chicken's intestinal epithelium for a long time (Latorre et al., 2014). However, *Bacillus* once inside, the chicken's GIT germinates rapidly and vegetative cells can outnumber spores within 20 h of oral administration as mentioned by Cartman et al. (2008). The presence of spores could be detected over GIT. So, in the gut wall, bacteria from *Bacillus* group start to colonize these host section which competes with and block the pathogenic bacterial sites (Mushtaq et al., 2022).

The pathogens prevention could be due to the secretion of antimicrobial peptides by *Bacillus* spp. such as amylase and protease enzymes (Dumitru et al., 2018) and metabolites (lipopeptides, surfactins, bacteriocins, inhibitory substances) which involve antagonistic results for microorganisms (Baruzzi et al., 2015; Sumi et al., 2015). It is known that, when an enzymatic bacterium is added to animal feed, the absorption and nutrient availability will improve (Amerah et al., 2017).

During competition with pathogens from the gut, the host can recover a part of the energy lost by captivating nutrients and metabolites such as lactic acid and volatile fatty acids resulting from fermenting bacteria (Grant et al., 2018).

Utilization of nutrients

An effective probiotic for growth and proliferation within the host is necessary to use nutrients and energy (Jha et al., 2020). The effects of the inclusion level of five probiotic bacterial strains (*L. reuteri* DSM 16350, *L. salivarius* DSM 16351, *Enterococcus faecium* DSM 16211, *Bifidobacterium animalis* DSM 16284, and *Pediococcus acidilactici* DSM 16210) were investigated by Mountzouris et al. (2010) as sources of probiotics in broilers feed (hybrid Cobb, male). The author's study found that the higher inclusion level ($> 10^9$ CFU/kg feed) modulates the cecal microflora composition and enhanced the growth performance and nutrient utilization in a corn-soybean diet.

Higher digestive absorption of nutrients in animal nutrition in the presence of probiotic supplementation is followed by an improvement of the intestinal structure and environment gut modulation (Choi et al., 2011). For example, the place of proliferation and differentiation of intestinal epithelial cells that stimulate villus growth is occurred by crypts.

The addition of *B. subtilis* DSM 29784 improved interior eggs quality with a significant increase in nutrient retentions and dietary apparent metabolizable energy (AME) in laying hens during the production cycle (Neijat et al., 2019).

Among of four inclusion levels (100, 150, 200, and 250 mg/kg, time 42 days) of *B. subtilis* improved the apparent metabolism of crude protein, crude fat, dry matter, and organic matter. Overall, Gao et al. (2017)

recommended that the inclusion of *B. subtilis* at 200 mg/kg could improve the broiler's performance.

He et al. (2019) investigated the effect of *B. subtilis, B. licheniformis,* and *S. cerevisiae* as an antibiotic substitute on growth performance and intestinal health status in broilers. The results obtained showed that the inclusion of probiotic complex as an alternative to chlortetracycline could improve performance growth, nutrient digestibility, serum antioxidant capacity, jejunal mucosal barrier function, and intestinal broilers morphology.

Even in unsuitable farming conditions, such as spore-forming bacteria, *B. subtilis* is implied in improving nutrient digestibility (Jha et al., 2020). After intramuscular inoculation with *E. coli,* the control + 0.1% *B. subtilis* increased the digestibility of nutrients ($P < 0.01$) followed by a reduction of *E. coli* respectively broiler colibacillosis disease (Manafi et al., 2017).

A large number of microorganisms are used as probiotics in poultry; the inclusion of *B. subtilis* DSM 17299 in broilers was correlated well with high nutrient digestibility of dry matter (DM), crude protein (CP), and AME (Reis et al., 2017). Due to their capacity to synthesize enzymes, *Bacillus* species occur as an essential solution in animal nutrition. Several studies reported that dietary probiotic supplementation enhanced the ileal digestibility of some nutrients like CP and most amino acids (Apata, 2008; Oso et al., 2019).

Due to their intense activities, digestive enzymes can affect nutrient digestibility (Zaghari et al., 2016). The improvement of digestibility, nutrients absorption, and digesta viscosity may be associated with the production of extracellular enzymes by the vegetative form of *B. subtilis* which secrete protease, amylase, and lipase (Chen et al., 2009) improving, in the end, the productive animal profile (Ravindran, 2013). The addition of *B. subtilis* enhances the feed efficiency, highlighting the production level through oxygen utilization in host GIT and secretion of various enzymes like subtilisin and catalase (Bajagai et al., 2016).

Guo et al. (2020) proved that *B. subtilis* used in their study had the capacity to produce protease. Another study affirmed that *B. licheniformis* ATCC 21424 was found as an effective enzyme producer (amylase and protease) through the submerged fermentation process (Dumitru & Habeanu, 2021). Enzymes supplementation aims to reduce the presence of indigestible components and can subsidize better digestion and therefore intensify the nutritional value of feed and energy in animal nutrition (Hmani et al., 2017).

Health status

According to the literature data, the administration of *Bacillus* diminishes the broiler chickens mortality (Teo and Tan, 2007; Knap et al., 2010; Abdel Baset et al., 2020; Qiu et al., 2021). In addition, *B. subtilis* used as probiotic (8×10^5 CFU/g) in poultry feed decreased mortality by 2.51% compared with non-supplemented groups (Harrington et al., 2016). Indeed, due to the possibility to germinate, with rapid multiplication, *Bacillus* strains have more advantages providing a wide range of health benefits to the host. Sen et al. (2010) reported that the addition of *B. subtilis* LS 1-2 improved the intestinal microbial balance and gut health of broiler with a decrease in cecal *Clostridium* and Coliforms counts. Further, *B. amyloliquefaciens*-based DFM as replace of antibiotics decreased the cecal population of *E. coli* followed by an increment of *Lactobacillus* counts comparatively with the control broiler group (Lei et al., 2014).

Probiotics affect the host (Fuller, 1989) and develop beneficial gut microflora that suppresses the growth of pathogens and modulate intestinal health in broiler (Shim et al., 2010; Reddy et al., 2010; Li et al., 2011; Rajput et al., 2012; Ciurescu et al., 2020).

Growth Performance

Growth performance characteristics (BW, ADG, ADFI, and FCR) are some of the most important aspects used to assess the economic benefits of broiler production (Zhang et al., 2021).

In poultry, the spore-forming probiotics affect positively FCR and are able to improve the growth and productivity of broilers in a variety of ways (Hooge et al., 2004; Jeon et al., 2014; Park et al., 2014; Ciurescu et al., 2020).

Studies from literature data have shown that the administration of *Bacillus* as probiotic product in the poultry industry can significantly promote the growth performance of broilers (Knarreborg et al., 2008; Zhou et al., 2010; Jeong & Kim, 2014; Park & Kim, 2014; Rhayat et al., 2017). Zhang also reported that ADG was enhanced by the inclusion of *Bacillus*-based probiotics in a dose of 10^5 and 10^8 CFU/kg feed (Zhang et al., 2012, 2013).

Rhayat et al. (2017) study presented that the addition of *B. subtilis* DSM 29784 in broilers significantly improved the FCR ($P < 0.05$). Reis et al. (2017) observed an improvement of FCR ($P = 0.07$) at the addition of *B. subtilis* DSM 17299 (1.6×10^9 CFU/g), results which are in agreement with other data (Jeong & Kim, 2014; Zhang et al., 2021). Bai et al. (2017) investigated the effect of *B. subtilis* fmbJ (2×10^{10} CFU/kg feed) and observed significantly improved ADG, (ADFI), and FCR of broilers ($P < 0.05$) from 21 to 42 d, respectively on the entire period. In addition, the authors noted an increase of BW in experimental groups (**BS-1**: 2519.47 ± 87.59 g; **BS-2**: 2528.10 ± 71.30 g) compared with the control (2287.34 ± 60.88 g).

B. licheniformis DSM 28710 improved the broiler performance including BW and FCR (Trela et al., 2020). Also, *B. licheniformis* has a beneficial role on performance parameters in poultry nutrition (Lei et al., 2013; Hanuszewska et al.,2018; Musa et al., 2019). The feed supplementation with two strains of *B. subtilis* (DSM 32324 and DSM 32325) involved significantly higher BW with a lower FCR than the control group during the starter phase (Sandvang et al., 2021).

Further, in the last years, spores-forming bacteria have become a topic of great interest. Single or in combination with other types of bacteria with/without the addition of minerals in the diets, probiotic-based on *Bacillus* spp. involve positive results regarding the broiler's growth promoters.

CONCLUSIONS

In summary, the present review founded on literature data shows the *Bacillus* efficacy as a probiotic and potential supplementation product for reducing antibiotics administration in the poultry industry. Based on the references from the literature, this review is focused on the influence of *Bacillus* spp. used as probiotic source in broiler feed. To point out that due to the capacity of sporulation, the *Bacillus* group has an important advantage founded on their stability through host GIT. As an end-product, in terms of inclusion in the diets or drinking water, the use of *Bacillus* strains is easily done. Future knowledge is necessary and more investigations of the probiotic administrations in poultry should focus on *in vitro* tests for determining the action mechanism of *Bacillus*-probiotic and, as well, for clarifying the correlation between bacterial properties, level of inclusion, optimal concentration, and the host profile (age, health and production conditions, hybrid type) which can influence the purpose pursued.

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