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Chapter 11

BACILLUS DIRECT-FED MICROBIALS: SPORE GERMINATION, DISTRIBUTION IN THE GASTROINTESTINAL TRACT, AND EFFECT ON HEALTH AND PERFORMANCE PARAMETERS IN POULTRY

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ABSTRACT

Indiscriminate and inappropriate use of antibiotics has led to the emergence of multidrug resistant pathogens, resulting in the ban of the use of antibiotics in animal diets in several countries. As an alternative, probiotics have been under investigation as feed additives to establish an adequate intestinal microflora that improve productive responses in animals. Bacteria from the genus *Bacillus* are receiving important attention, because of their properties to control enteropathogens and their remarkable capacity to produce endospores. In addition, some *Bacillus* species have the capacity to synthesize different exogenous enzymes, including protease, phytase, xylanase, keratinase, lipase, and cellulase, that have been reported to improve absorption of nutrients and diminish intestinal viscosity in non-starch polysaccharide rich diets. These benefits make supplementation of *Bacillus* spores an available and applicable alternative for the use of antibiotic growth promoters, reducing the incidence of various GIT diseases and improving production performance in poultry under commercial conditions. In this review, we summarize the fate, dissemination, and efficacy of *Bacillus* direct-fed microbial candidates in the gastrointestinal tract of broiler chickens and their effect on health and performance parameters.

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1. INTRODUCTION

Due to current intensive management practices in poultry production, animals are susceptible to enteric microflora imbalances leading to diminishment on performance parameters. To mitigate the effect of dysbiosis in the gastrointestinal tract, diets have been commonly supplemented with antibiotics as growth promoters showing effective decrease in the presentation of digestive disorders and increase in performance [1]. However, the indiscriminate and inappropriate use of antibiotics has led to the emergence of multidrug resistant pathogens, leading to the prohibition of antibiotics in animal diets in the European Union in 2006 [2]. The worst scenario is the possible contribution of these resistant bacteria from animal production resulting in serious medical implications in humans [3].

As an alternative to the use of antibiotic growth promoters (AGP) in poultry diets, probiotics have been under investigation as feed additives to establish an adequate intestinal microflora, promoting adequate productive responses in animals [4, 5]. Among the species of microorganisms used as probiotics, some facultative anaerobic gram positive bacteria from the genus *Bacillus* are receiving important attention through enhancing digestion and absorption of nutrients, and control of enteropathogens such as *Salmonella spp., Clostridium perfringens, Campylobacter spp., and Escherichia coli* in the gastrointestinal tract (GIT) of different animal species [2, 6-8]. Additionally, the genus *Bacillus* has the extraordinary capacity to produce endospores under stressful environmental conditions; some of these spores have the ability to resist high temperatures used during feed preparation (pelletization), extreme pH, dehydration, high pressures and contact with caustic chemical substances [9]. These admirable features make selected *Bacillus* spores a direct-fed microbial (DFM) suitable for commercialization and distribution due to a long-shelf life and stability [10, 11].

On the other hand, it is really important to understand the factors that affect germination and distribution of *Bacillus* spores throughout the gastrointestinal tract. *Bacillus* spores germinate into vegetative cells depending on nutritional and non-nutritional factors known as germinants (such as L-alanine, asparagine, glucose, fructose, potassium chloride) and the effect of a non-lethal heat treatment under different pressures (100 – 600 Megapascals) [12]. There is evidence supporting the idea that some *Bacillus* spores germinate in the GIT of chickens, mice, pigs, dogs and humans, therefore, being metabolically active and having responses such as, production of antimicrobial substances, immunomodulatory effects on the intestinal mucosa, and function as competitive exclusion agents interacting with host cells [13-15]. Furthermore, some *Bacillus* species have the capacity to produce different exogenous enzymes, including protease, phytase, xylanase, keratinase, lipase, and cellulase [16-21]. These enzymes help to degrade complex feed molecules, improve absorption of nutrients, reduce intestinal viscosity in non-starch polysaccharide rich diets (NSP), and decrease the amount of substrates available for growth of pathogenic bacteria. Additionally, it has been shown that the presence of *Bacillus* species such as *Bacillus subtilis*, enhance growth of other beneficial microorganisms such as *Lactobacillus* by production of subtilisin, catalase, and also decreasing intestinal pH [22].

All the benefits related to the utilization of *Bacillus*-DFM in the diet, make supplementation of *Bacillus* spores an available and applicable alternative instead of the use of antibiotic growth promoters, avoiding an increment in the presentation of different

gastrointestinal diseases and maintaining or improving performance parameters in poultry production under commercial conditions.

2. SPORE GERMINATION AND GERMINANTS

Although *Bacillus* spores are in a dormancy state and are considered one of the most stable and resistant forms of life. Spores have an active sensor system that allows them to undergo the process of germination when environmental and nutritional conditions are favorable. Spores respond to the presence of nutritional or non-nutritional factors known as germinants, which trigger the start of a series of interconnected reactions that will finally have as a result the generation of a metabolically active vegetative cell. Among the nutritional germinants involved in germination of *B. subtilis*, L-alanine is the most common, but also the mixture of asparagine, fructose, glucose and potassium chloride have been demonstrated to stimulate the initiation of germination [12]. In the case of *B. megaterium*, L-proline has been recognized as an important germinant [23].

The first step implicated in germination is the interaction between a nutritional germinant like L-alanine and the receptor of the spore located in the inner membrane. These receptors are composed by different proteins (GerA, GerB and GerK), that in the case of *B. subtilis* are encoded by *gerA*, *gerB* and *gerK* operons [24]. Following the binding of the germinant with the cell receptor, there is an increase in the spore core permeability, due to the movement of Ca^{++} cations and dipicolinic acid (DPA) from the core accompanied by uptake of water into the core. These steps are considered the first phase of the germination process, however, some researchers mention that a non-lethal heat treatment is important to activate the spore receptors before binding the germinant [25]. The second phase of the germination process includes the activation of cortex-lytic enzymes that finally allow the complete rehydration of the core and activation of the metabolic enzyme activity of the future vegetative cell [12].

The mechanism by which the cortex-lytic enzymes are activated is not completely known. *B. subtilis* enzymes CwlJ and SleB are involved in the degradation of the cortex peptidoglycan after previous activation of the cell receptor by a specific germinant [26, 27]. One hypothesis is that CwlJ activity may be induced by the presence of Ca^{++} and DPA released from the inner membrane of the spore, starting in this way the hydrolytic disruption of the cortex and permitting the movement of water inside the spore core. Moreover, the increment of stress in the structure of the dissolving cortex could also induce SleB activity, having as result the expansion of the germ cell wall, which is going to be the cell membrane of the vegetative cell. The substrate of the cortex-lytic enzymes is the muramic-δ-lactam present in the peptidoglycans layer, being the target of enzyme activity, however, other enzymes and other compound of the cortex may be involved in this process in different *Bacillus* species [28].

In addition to nutrient germinants, there are also non-nutrient factors that stimuli germination, such as; heat shock, salts, presence of Ca^{++} cations and DPA from other germinating spores, and different amounts of pressure (100 – 600 MPa). Nevertheless, there is contradictory evidence related to low and high pressures and their effect on germination. Wuytack and co-workers observed that low pressures (100 – 200 MPa) may influence the activation of inner membrane receptors promoting germination; however, at high pressures

(600 MPa) germination was not completed due to interruption of the final phase steps of the process [29]. In contrast, Paidhungat et al. [24], reported that higher pressures (500 – 600 MPa) incentive germination even without presence of nutritional germinants, indicating that pressure may affect the release of Ca^{++} and DPA, therefore avoiding the first phase of activation of spore receptors, and acting directly over degradation of cortex peptidoglycans.

Additionally, different aspects such as osmoregulation of the core by the cortex layer, degradation of small acid-soluble proteins (SASPs) as source of amino acids for cell growth, and permeability of the coat and cortex to different nutrient germinants to reach the inner membrane of the core are fundamental to complete germination, together with the chain of reactions mentioned before. Spore germination is still a process that must be investigated in detail, because there are still unknown facts about how the binding of the germinant and the receptor is integrated in a response that finish with the activation of cortex-lytic enzymes, and also how proteins of the inner membrane receptor interact between each other and with different germinants in variable *Bacillus* species [12, 25]. Have knowledge about the aspects that affect germination and sporulation of the genus *Bacillus* is crucial to understand how these bacteria may act in the GIT of animals when supplemented as direct-fed microbials.

3. Distribution and Germination of *Bacillus* Spores throughout the GIT in Different Animal Models Including Poultry

Due to *Bacillus* spores are recognized to be in a dormant state in comparison to other probiotic bacteria such as *Lactobacillus*, determination of germination of *Bacillus* spores in the GIT is of vital importance. The capacity of certain spores to germinate under gastrointestinal conditions is directly related with the possible mechanism of action through which these bacteria will benefit the host [30]. Metabolically active cells are required to secrete antimicrobial substances, stimulate beneficial microbiota, and act as competitive exclusion agents [31]. Additionally, vegetative cells of some, but not all, *Bacillus* isolates have shown to produce exogenous enzymes that may promote an increase in digestibility of different nutrients from the diet [32]. On the contrary, according to Tam and co-workers, other beneficial effects of *Bacillus* spores such as competition for attachment sites and immunomodulation do not require germinated spores to have positive effects on the host, therefore both stages (vegetative cells and spores) of the *Bacillus* life cycle could provide a different set of advantages supporing their utilization as functional feed additives [14].

Equally essential is to know the distribution of the spores throughout the GIT to realize where the major advantages of the direct-fed microbial supplementation would be expected to occur. Furthermore, it is also crucial to recognize if these bacteria have the capacity to accomplish some full life cycle in the GIT or if they are transient occurring, requiring constant supplementation in the diet to persist in the digestive tract [33].

In the case of poultry, Cartman et al. [34] demonstrate that *B. subtilis* spores, when provided orally, germinate in the GIT. Identification and quantification of spores and vegetative cells were done using RT-PCR, qRT-PCR and a strain of *B. subtilis* (SC2362) that harbored a fusion gene (*rrnO-lacZ*) and a chloramphenicol acetyltransferase gene (*cat*). After 20 hours of spores administration, the number of vegetative cells was higher in comparison to

the number of spores present in different segments of the GIT. This finding suggests that even when *Bacillus spp.*, are considered aerobic bacteria, spores have the ability to germinate into vegetative cells and survive in the anoxic environment of the digestive tract. This could be the result of the use of nitrite or nitrate molecules as terminal electron acceptors in the electron transport chain by some *Bacillus* strains [35]. Additionally, Studies conducted in our laboratory have shown that approximately 90% of *B. subtilis* spores germinate within 60 min in the presence of starter broiler feed *in vitro*, this was observed after a heat-shock treatment (75ºC for 10 minutes) that allowed counting of spores only (Table 1). Extended *in vivo* studies confirmed that viable *B. subtilis* spores were recovered during 120 h, from different sections of the GIT of broiler chickens after constantly receiving feed supplemented with spores (10^6 spores/g) or a single oral-gavage dose (10^6 spores/0.25 mL). Approximately a 1 $\log_{10}$ colony forming units (CFU) reduction of spore numbers was observed after 24 hours of administration, which may suggest a germination rate of around 90% in the GIT (Figure 1) [36]. Similarly, Jadamus and co-workers [6] observed that spores of *B. cereus* var. *toyoi* germinated fast in the crop of chickens. Moreover, when vegetative cells were orally administered, spores were collected from different segments of the GIT, meaning that both processes were occurring, either germination of spores or sporulation of vegetative cells. Furthermore Cartman et al. [34], found that *B. subtilis* spores can be detected after six weeks of a single oral administration (10^9 spores/0.1mL), meaning that compared to the passage rate of the digesta in chickens (6 -7 hours), spores tend to persist over time in the GIT [37].

Table 1. Evaluation of germination and growth of *Bacillus* PHL-NP122 ($\log_{10}$ cfu/g) spores in an *in vitro* crop assay using a corn and soybean feed with or without heat shock†. Adapted from Latorre et al. [36]

Time (min)	No heat shock ($\log_{10}$ cfu /g)	Heat shock ($\log_{10}$ cfu /g)
0	6.98 ± 0.1^{a}	6.78 ± 0.1^{a}
10	6.58 ± 0.2^{a}	6.52 ± 0.2^{a}
15	6.78 ± 0.2^{a}	6.56 ± 0.2^{a}
30	7.06 ± 0.1^{a}	6.66 ± 0.1^{b}
40	7.12 ± 0.1^{a}	6.58 ± 0.1^{b}
60	7.16 ± 0.1^{a}	6.33 ± 0.2^{b}

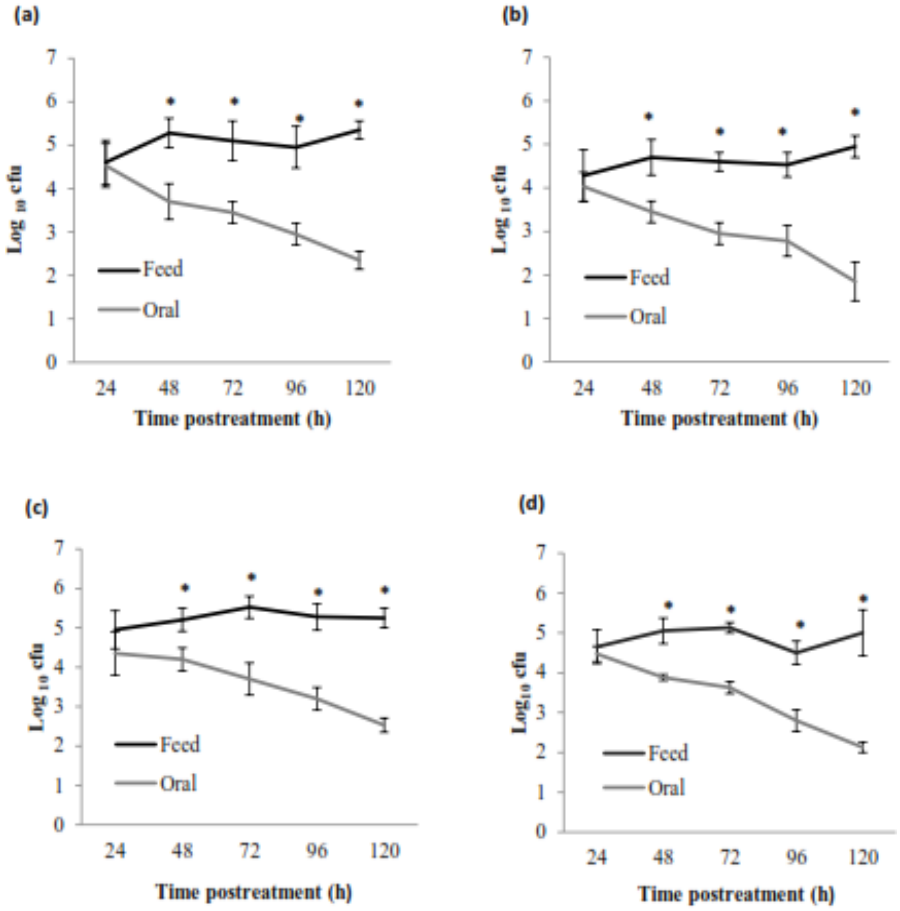
[a–b] Means within a row with different superscripts differ ($P < 0.05$).

†Data is expressed as mean ± SE of five replicates per treatment in each timepoint.

This behavior was also supported by examination of the fate of *Bacillus* spores in the GIT of different animal models such as mice and pigs. For instance, Hoa and co-workers evaluate the amount of spores and vegetative cells present in different parts of the GIT of pathogen-free mice, showing that occasionally the amount of spores excreted was higher than the single oral original inoculum administer to the mice [38]. This result may imply that spores can germinate, growth and sporulate, completing a full-life cycle under digestive tract of conditons.

Furthermore, Leser et al. [32], evaluate the germination and outgrowth of *B. subtilis* and *B. licheniformis* spores in the GIT of pigs using a flow cytometry technique (FCM) and also

plate counting spores after heat-treatment. In the quantitation of spores and vegetative cells using FCM, cells were stained with a dye (Syto-13), and differentiate by the low concentration of the dye present in the spore cell wall, in contrast to the high concentration observed in the cell wall of vegetative cells. It was revealed that, similar to the GIT of poultry, the number of spores diminished with time, meanwhile the number of vegetative cells tend to increase. Additionally, in this research was also possible to appreciate that the number of vegetative cells slightly increased in comparison with the original inoculum administered, suggesting little outgrowth of *Bacillus* cells in the GIT [32].



*Data is expressed as mean and SE of 5 replicates in each time point ($P < 0.05$). Comparisons of spore counting were performed between constant supplementation of spores in the feed or a single oral dose. Adapted from Latorre et al. [36].

Figure 1. *B. subtilis* (Log_{10} cfu/g) in crop (a), ileum (b), ceca (c) and feces (d) of broiler chickens given a single oral dose or constant administration of spores in the feed.

Even when conditions of the GIT are not completely suitable for germination of *Bacillus* spores, it has been shown that in different animal species some *Bacillus* isolates are capable to develop a transformation from metabolically dormant spores to metabolically active vegetative cells. Spores are tolerant to the acidic pH of the stomach or proventriculus,

additionally it has been suggested that the change of pH can trigger germination in the fore and hind gut [6, 32].

However, vegetative cells are highly susceptible to the presence of bile salts, probably in this way influencing the beginning of the sporulation process inside the GIT of the host, and promoting competition of some full life cycle development of the *Bacillus* bacteria before being excreted [39].

4. EFFECT OF *BACILLUS* DIRECT-FED MICROBIALS ON HEALTH

In day-old hatch birds, the digestive tract has a reduced bacterial population, making this almost unpopulated environment susceptible for colonization by pathogenic bacteria, therefore, affecting from the beginning the future performance of affected animals. This is one of the reasons for the utilization of probiotics or direct-fed microbials starting from early phases of life in livestock animals. Additionally, the prohibition of inclusion of antibiotics in animal diets since 2006 (Europe Community), increase the necessity to find alternatives to prevent presentation of diseases without diminishing production standards [33]. The most common bacteria used as probiotics are from the genus *Lactobacillus* (LAB), however, they must be administered for example in the drinking water, and maintained under optimal conditions to prolong shelf-life.

Due to this restrictions, use of some spore former bacteria from the genus *Bacillus* have earned interest in the last years. As mentioned before, spores are resilient to harsh environmental conditions, and have a long shelf-life, making them feed-stable and suitable for commercialization [40, 41].

Nevertheless, it is important to understand that not all *Bacillus* species are used as direct-fed microbials; each isolate has different characteristics according to the chemical substances that produce, heat resistance temperatures, rate of growth, rate of sporulation, persistence in the GIT, and probable advantages to the host. Several studies have shown that vegetative cells of certain *Bacillus* isolates prevent colonization of the GIT by enteropathogens such as *Salmonella spp.*, *Clostridium perfringens*, and *Campylobacter jejuni* [7, 42, 43]. For instance, Shivaramaiah et al. [11], administered spores of different *Bacillus spp.* strains to evaluate their effect on *Salmonella* Typhimurium exclusion and growth performance, showing at the end a reduction in the recovery of this pathogenic bacteria from the crop and ceca of chicks and poults that consumed *Bacillus*-DFM supplemented diets in comparison to the untreated group ($P<0.01$). Additionally, it was also observed an improvement on performance parameters when broilers and poults were fed with *Bacillus*-supplemented diets compared to the control unsupplemented group ($P<0.05$) [11]. Furthermore in a different study, Knap and co-workers evaluated the effect of the inclusion of *B. licheniformis* (DSM 17236) in the diet of broiler chickens on the presentation of necrotic enteritis (*C. perfringens*) [44]. The study reported that the performance of broilers receiving the direct-fed microbial (10^6 and 10^7 cfu/g) was similar to the group of birds that consume a medicated diet (Virginiamycin 15 g/ton feed), moreover no significant difference were observed regarding to the necrotic enteritis lesion score or mortality [44]. In addition, multiple published studies support the fact that some isolates of *B. subtilis* have the ability to decrease the persistence of *C. perfringens*, avian pathogenic *Escherichia coli* and *Salmonella* serovar Enteritidis in the GIT of poultry

[45, 46]. The mode of action of *Bacillus* vegetative cells to reduce colonization of enteropathogens is not completely known.

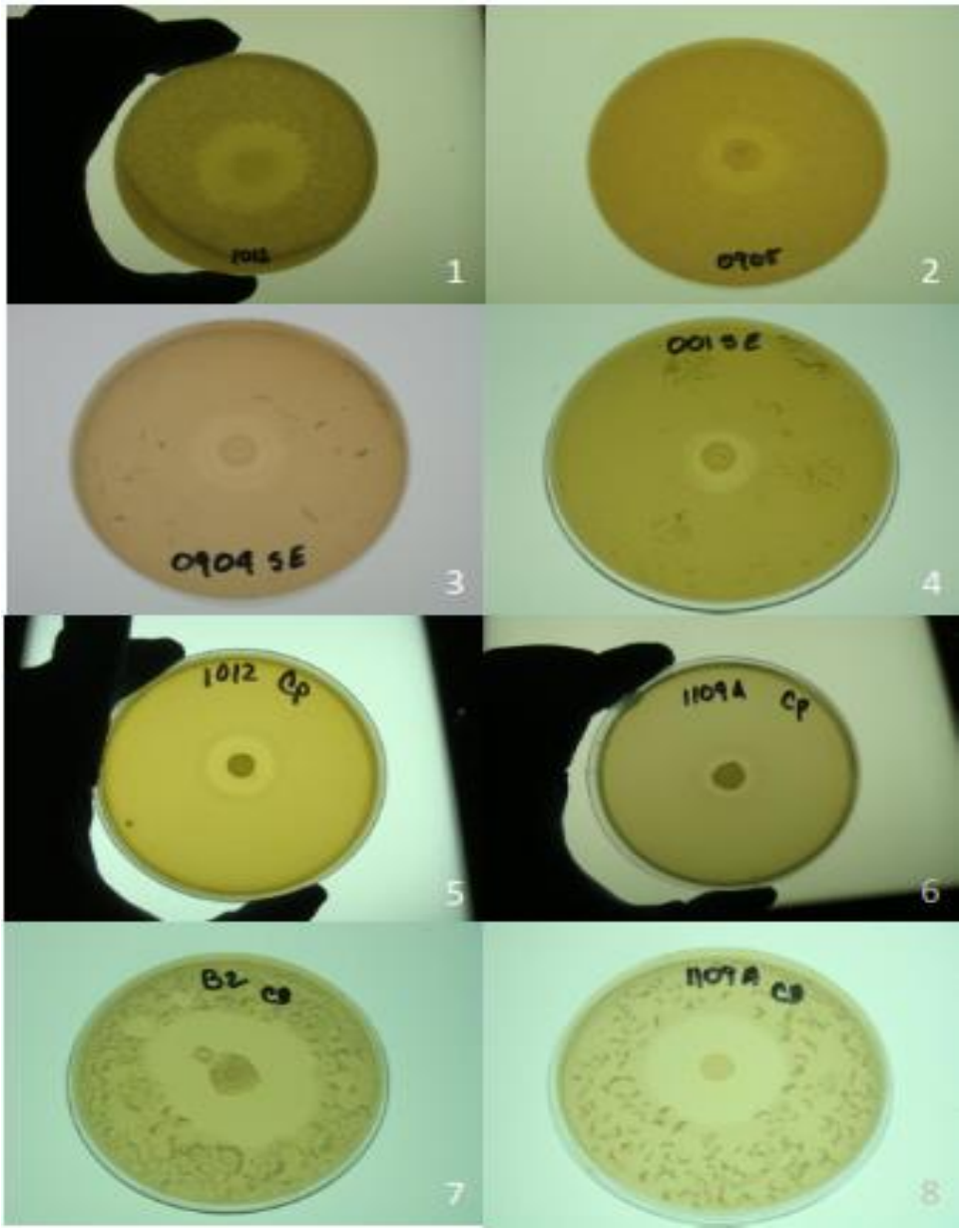


Figure 2. Evaluation of bacteriocin-like compounds synthesis from different *Bacillus spp*. as direct-fed microbial candidates using an overlay methodology. *1 E. coli* F18 – *Bacillus* 1012 and 0905 showing 10 and 7 mm of inhibition zone respectively; 2 *Salmonella* Enteritidis – *Bacillus* 0904 and 001 showing 8 and 5 mm of inhibition zone respectively; 3 *C. perfringens* – *Bacillus* 1012 and 1109A showing 7 and 3 mm of inhibition zone respectively; 4 *C. difficile* – *Bacillus* B2 and 1109A showing 13 and 12 mm of inhibition zone respectively.

Some *Bacillus* isolates have the capacity to produce antimicrobial compounds against different pathogens (Figure 2) or stimulate the immune system of the host. Moreover in the case of *C. perfringens*, due to the ability of some strains of *Bacillus* to produce proteases, could be possible that *Clostridium* toxins (α-toxin, NetB) were degraded by these enzymes [44]. On the other hand, some *Bacillus* species have also been studied for detoxification or protective effect on cases of mycotoxicosis [47, 48]. In addition to the use of *Bacillus* spores as direct-fed microbials, spore forming bacteria have also been studied as a vector for oral vaccines, providing in this way an excellent alternative combining the benefits of a probiotic with the advantages of a possible tool to increase acquire immune responses for different diseases without the presentation of vaccine reactions [49].

5. Effect of *Bacillus* Direct-Fed Microbials on Performance Parameters

In the case of poultry performance and feed formulation, one of the principal problems is the continuous utilization of cereal grains such as corn for biofuel production, therefore, affecting feedstuffs availability and feed cost, which represents around a seventy percent of the production expenses in the poultry industry. Ethanol production and variability in corn prices have led to the use of alternative and less digestible energy sources in poultry diets. Cereals such as wheat and barley, as well as by products of biofuel production (Distiller's dried grains with solubles) have become occasionally feed ingredients used in poultry diets. Unfortunately, these alternative raw materials increased the amount of less digestible non-starch polysaccharides (NSP) in the feed, which as a result generate an increment in digesta viscosity in monogastrics animals [50, 51]. Utilization of *Bacillus* direct-fed microbials is one of the alternatives to optimize the digestibility of NSP rich diets, because some isolates have been recognized as xylanase, cellulase and β-glucanase producers [16, 17, 52]. Xylanase is one of the enzymes that have shown reduction of intestinal viscosity, which is one of the factors involved in presentation of *Clostridium*-associated enteritis [53, 54]. More recently, our laboratory have demonstrated that inclusion of certain *Bacillus*-DFM candidates that produce exogenous enzymes such as xylanase in high NSP diets significantly reduced both viscosity and *C. perfringens* proliferation in an *in vitro* digestive model study simulating different compartments of GIT of poultry [55]. This results were also observed during *in vivo* experiments conducted with chickens and turkeys fed with a high NSP rye-based diet. When the *Bacillus*-DFM candidate was included in the experimental diet rye-based diet, significant improvements in intestinal viscosity, performance parameters, bacterial translocation and bone quality were observed in supplemented animals (Tables 2 and 3), suggesting that the consumption of a selected *Bacillus*-DFM producing a variable set of enzymes, could contribute to enhance nutrient digestibility and promote healthy intestinal integrity [56]. Additionally, some *Bacillus* species can also synthesize proteases, which could be used to help in the degradation of low quality proteins present in the diet, hence, preventing detrimental enteric microflora changes that could result in the proliferation of *C. perfringens*. One qualitative method to screen different *Bacillus* strains for protease activity is the utilization of milk agar medium, followed by the measurement of the zone of clearance present around the evaluated bacterial colony after 24 hours of incubation at 37^{o}C [18].

Table 2. Evaluation of body weight, digesta viscosity, and bacterial translocation to the liver in neonatal turkey poults fed with a rye-soybean based diet or rye-soybean based diet with *Bacillus* direct-fed microbial (DFM) supplementation. Adapted from Latorre et al. [56]

	Experiment 1			Experiment 2		
	Body weight[†] (g)	Digesta viscosity[‡] (cP Log_{10})	Bacterial translocation[£] (cfu Log_{10})	Body weight[†] (g)	Digesta viscosity[‡] (cP Log_{10})	Bacterial translocation[£] (cfu Log_{10})
CON[c]	65.91 ± 3.6[b]	2.03 ± 0.3[a]	3.03 ± 0.5[a]	74.47 ± 1.6[b]	1.80 ± 0.4[a]	2.13 ± 0.7[a]
TRT[d]	82.85 ± 4.2[a]	1.54 ± 0.2[b]	1.24 ± 0.5[b]	95.60 ± 2.2[a]	1.62 ± 0.5[b]	0.35 ± 0.4[b]

[a-b]Superscripts within columns indicate significant difference at $P < 0.05$.

[c]Control rye based diet without DFM.

[d]Control rye based diet with candidate DFM (10^6 spores/g of feed).

[†]Body weight n=25; Data is express as Mean ± SE.

[‡]Digesta viscosity is expressed in Log_{10} (in centipoise, cP = 1/100 dyne s/cm^2), n = 12.

[£]Liver bacterial translocation (expressed in cfu Log_{10} /g of tissue), n = 12.

Table 3. Evaluation of bone strength and bone composition in neonatal turkey poults fed with a rye-soybean based diet without or with *Bacillus* direct-fed microbial (DFM) supplementation[†]. Adapted from Latorre et al. [56]

	Tibia strength load at yield (kg/mm)	Tibia diameter (mm)	Total ash from tibia (%)	Calcium (% of ash)	Phosphorus (% of ash)
CON[c]	1.14 ± 0.2[b]	4.45 ± 0.3[b]	35.61 ± 0.8[b]	27.35 ± 0.1[b]	16.35 ± 0.5[b]
TRT[d]	2.55 ± 0.1[a]	5.82 ± 0.8[a]	50.87 ± 0.7[a]	40.31 ± 0.5[a]	22.67 ± 0.3[a]

[a-b] Superscripts within columns indicate significant difference at $P < 0.05$.

[c]Control rye based diet without DFM.

[d]Control rye based diet with candidate DFM (10^6 spores/g of feed).

[†]Tibias from twelve poults were collected to evaluate bone quality. Data is expressed as mean ± SE.

Furthermore, different studies have supported the hypothesis that incorporation of *B. subtilis* spores in the diet improved production parameters in poultry [57-61]. Samanya and Yamauchi [62] reported that the supplementation of *B. subtilis* var. *natto* in the diet of chickens increased villi height and enterocyte proliferation, showing also a decrease in blood ammonia concentration which was correlated with a better intestinal function.

Moreover, Wolfenden et al. [8] included two different *Bacillus* isolates (PHL-RW35 and PHL-RW41) at 10^7 and 10^5 spores/g of feed respectively, and obtained significant increases in body weight after 11 days of age in broiler chicks.

Similarly, in another study done by the same author [7], inclusion of a *B. subtilis* (PHL-NP122) in the diet of turkeys under commercial conditions resulted in a similar improvement on body weight at 23 days of age in comparison with a group of chickens consuming a medicated diet (Nitarsone). These results suggest that utilization of some *Bacillus* isolates

could be an effective alternative to maintain or increase production parameters without utilization of antibiotics growth promoters in the poultry industry.

CONCLUSION AND FUTURE PERSPECTIVES

Besides control of pathogen colonization of the GIT through production of bacteriocins and stimulation of the immune system, some *Bacillus* direct-fed microbials have the capacity to produce a variable set of enzymes that may contribute to enhance performance through improving digestibility, reducing intestinal viscosity and promoting healthy intestinal integrity in commercial poultry. Additionally, it has been shown that some *Bacillus* isolates are candidates to be used as vectors for oral vaccines, which add one advantage more to the set of benefits obtained by this amazing microorganism. However, there are still a lot of unknowns about physiological aspects involved in the germination and sporulation process, and also the mechanism of action used by these bacteria to control colonization of enteropathogens and improve performance parameters.

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