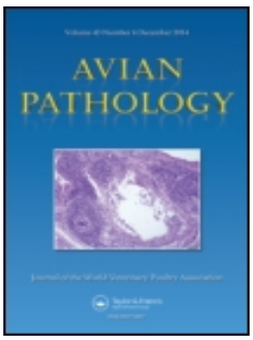

Avian Pathology

ISSN: 0307-9457 (Print) 1465-3338 (Online) Journal homepage: http://www.tandfonline.com/loi/cavp20

Bacillus spp. as direct-fed microbial antibiotic alternatives to enhance growth, immunity, and gut health in poultry

Ar'Quette Grant, Cyril G. Gay & Hyun S. Lillehoj

To cite this article: Ar'Quette Grant, Cyril G. Gay & Hyun S. Lillehoj (2018): Bacillus spp. as direct-fed microbial antibiotic alternatives to enhance growth, immunity, and gut health in poultry, Avian Pathology, DOI: 10.1080/03079457.2018.1464117

To link to this article: https://doi.org/10.1080/03079457.2018.1464117

Accepted author version posted online: 11 Apr 2018.
Published online: 02 May 2018.

AVIAN PATHOLOGY, 2018
https://doi.org/10.1080/03079457.2018.1464117

Taylor & Francis
Taylor & Francis Group

REVIEW ARTICLE

Bacillus spp. as direct-fed microbial antibiotic alternatives to enhance growth, immunity, and gut health in poultry

Ar'Quette Grant[a], Cyril G. Gay[b] and Hyun S. Lillehoj[a]

[a]Animal Biosciences and Biotechnology Laboratory, Agricultural Research Service, US Department of Agriculture, Beltsville, MD, USA; [b]National Program Staff – Animal Health, Agricultural Research Service, US Department of Agriculture, Beltsville, MD, USA

ABSTRACT

The increasing occurrence of antibiotic-resistant bacteria combined with regulatory pressure and consumer demands for foods produced without antibiotics has caused the agricultural industry to restrict its practice of using antibiotic growth promoters (AGP) in food animals. The poultry industry is not immune to this trend, and has been actively seeking natural alternatives to AGP that will improve the health and growth performance of commercial poultry flocks. *Bacillus* probiotics have been gaining in popularity in recent years as an AGP alternative because of their health-promoting benefits and ability to survive the harsh manufacturing conditions of chicken feed production. This review provides an overview of several modes of action of some *Bacillus* direct-fed microbials as probiotics. Among the benefits of these direct-fed microbials are their production of naturally synthesized antimicrobial peptides, gut flora modulation to promote beneficial microbiota along the gastrointestinal tract, and various immunological and gut morphological alterations. The modes of action for increased performance are not well defined, and growth promotion is not equal across all *Bacillus* species or within strains. Appropriate screening and characterization of *Bacillus* isolates prior to commercialization are necessary to maximize poultry growth to meet the ultimate goal of eliminating AGP usage in animal husbandry.

ARTICLE HISTORY
Received 24 January 2018
Accepted 4 April 2018

KEYWORDS
Bacillus; direct-fed microbials; antibiotic growth promoter; poultry; feed additive; gut health

Introduction

Recovery of multi-drug resistant bacteria ("superbugs") has been increasing over the past several decades, causing what some public health organizations consider a crisis with potentially "catastrophic consequences" as the world enters the post-antibiotic era (CDC, 2013a, b). The World Health Organization (WHO) has warned that superbugs are reaching dangerous levels worldwide, a problem that is exacerbated by the overuse and abuse of antibiotics. The WHO has specifically called on individuals, healthcare professionals, policy makers, and agricultural industries to make changes to prevent the spread of antibiotic resistance (WHO, 2017). In response, the United States Food and Drug Administration (FDA) has requested that drug companies voluntarily discontinue labelling antimicrobials for growth promotion in agricultural animals (Guidance #213), and antibiotics should only be prescribed for therapeutic uses through veterinary oversight. This voluntary approach would allow industry constituents to collaborate and cooperate with the FDA to quickly achieve a positive public health outcome rather than be mandated which would take more time and resources (US FDA, 2013, 2017).

In the United States, sub-therapeutic doses of antibiotics have been supplemented into the feed of animals intended for human consumption for over 60 years since the first discovery of their growth-promoting effect (Moore *et al.*, 1946). Supplementation of streptomycin and sulfasuxidine along with folic acid in the feed of day-old chickens resulted in significantly increased growth. This growth-promoting result was confirmed by Jukes *et al.* (1950). Shortly thereafter, antibiotic-resistant bacteria were recovered from food animals given antibiotic growth promoters (AGP). Starr and Reynolds (1951) reported that coliform bacteria isolated from turkeys experimentally fed with streptomycin as a growth promoter were resistant to the antibiotic effect of streptomycin. Other studies reported the recovery of tetracycline-resistant bacteria from chickens fed sub-therapeutic levels of tetracycline (Elliot & Barnes, 1959; Dibner & Richards, 2005). Although AGP were classically used to promote the health of feed animals and have substantially improved their economic value by increasing growth and feed efficiency, the mounting concern of antibiotic resistance has forced the agricultural industry to seek alternatives to replace AGP in food bird production.

One promising antibiotic replacement is the incorporation of probiotics into feed to maintain bird health and promote growth. In the early 1900s, Nobel Prize winner Elie Metchnikoff established the groundwork

CONTACT Hyun S. Lillehoj hyun.lillehoj@ars.usda.gov

for the modern-day theory of probiotics (Mackowiak, 2013). Since then, the scientific community has repeatedly verified the health- and growth-promoting benefits of probiotics (Vila *et al.*, 2010; Mackowiak, 2013). In agriculture, popular probiotics, or direct-fed microbials (DFM), include *Lactobacillus* spp. and *Bifidobacterium* spp. Lactobacilli act by competitively excluding pathogenic bacteria from adhering to and invading the gut epithelium (Wine *et al.*, 2009), and by improved feed digestibility (Zhao & Kim, 2015). Giang *et al.* (2010) found greater digestibility of crude protein within the first two weeks of weaning when the piglet weanlings' feed was supplemented with *L. acidophilus*, *L. plantarum*, and *L. plantarum* at a rate of 4×10^9, 2×10^9, and 7×10^9 CFU/kg, respectively. *Bifidobacterium* is associated with improved gut health by increasing intestinal immunostimulation and producing volatile fatty acids that are beneficial to its host (Williams *et al.*, 1994; Haghighi *et al.*, 2005). Although promising, there are many challenges associated with industrial scale agricultural incorporation of these two bacteria. Lactobacilli and bifidobacteria are either microaerophilic or strict anaerobes, which makes their mass production and handling in an agricultural setting very difficult. The growth of both organisms is very slow, and they are sensitive to high temperatures that would be encountered during milling and pelletizing of feed production (Silva *et al.*, 2015; Quartieri *et al.*, 2016). Further, both species are unable to survive the acidic conditions of the gastric environment, minimizing the number of cells able to colonize the small intestine. Santini *et al.* (2010) reported that only two of 11 different *Lactobacillus* and *Bifidobacterium* tested survived a simulated gastric environment with a pH of 2.5 after 1 h, and only one was recovered after 3 h, although its viability was greatly diminished.

In general, *Bacillus* spp. have a distinct advantage over *Lactobacillus* and *Bifidobacterium* as DFM. As a spore-forming facultative anaerobe, it can withstand temperatures up to 113°C for 8 min, which makes it easier to manipulate and increases its likelihood of surviving feed processing steps. In addition, *Bacillus* spores are resistant to low pH, bile salts, and other harsh conditions encountered in the gastric environment (Barbosa *et al.*, 2005; Guo *et al.*, 2006; Setlow, 2006; Chaiyawan *et al.*, 2010; Shivaramaiah *et al.*, 2011). *Bacillus* spores promote gut health not only by competitive exclusion, but by producing antimicrobial peptides (AMP) that are cytotoxic to bacterial pathogens and reduce signs associated with enteric infectious diseases, such as avian coccidiosis (La Ragione & Woodward, 2003; Lee *et al.*, 2010b; Knap *et al.*, 2011; Sumi *et al.*, 2015). *Bacillus* DFM also improve gut health through the production of beneficial metabolites via alterations in the gut microflora. *Bacillus* stimulates the intestinal immune system by increasing the levels of cytokines and chemokines such as interleukin-1β (IL-1β) and interferon-γ (IFNγ) in the chicken gut (Lee *et al.*, 2013), and some *Bacillus* probiotics have been used clinically to help alleviate gastric disorders in humans (Hun, 2009; Gareau *et al.*, 2010). In fact, several studies showed that certain strains of *B. subtilis* promote the growth of chickens to a greater extent than AGP (Opalinski *et al.*, 2007; Gadde *et al.*, 2017b). Thus, *Bacillus* spp. have great potential to replace AGP and several commercial products are currently available (Table 1).

The objective of this review is to provide a collective overview of the direct and indirect mechanisms used by *Bacillus* spp. to improve gut health, immunity, and function as a probiotic growth promoter in poultry, more specifically broiler chickens. Although other non-poultry animals that incorporate *Bacillus* will be sparingly used as examples throughout this review, they will only serve to supplement and emphasize the various topics.

Bacillus-produced AMP

Bacillus has been a recognized producer of AMP for over 50 years, devoting up to 5% of its genome to AMP production, and produces at least 66 different AMP, of which several have been purified and commercialized (Stein, 2005; Sumi *et al.*, 2015; Suva *et al.*,

Table 1. Non-comprehensive list of commercially available *Bacillus* DFM marketed for agricultural poultry use.

Brand name	Bacillus strain(s)	Manufacturer	Notes
POULTRY-FEED	*Bacillus licheniformis* *Bacillus subtilis*	Bionetix-International, www.bionetix-international.com	Also contains *S. cerevisiae* and *E. faecium*
GALLIPRO® MS	*Bacillus subtilis* DSM5750 *Bacillus licheniformis* DSM5749	Chr. Hansen Holdings, www.chr-hansen.com/	Also produce GALLIPRO Fit, GALLIPRO Max and GALLIPRO Tech
B-Act®	*Bacillus licheniformis*	AgriHealth, www.agrihealth.co.nz/	Provided at a concentration of 3.2×10^9 viable spores/gram
FloraFix-BIRDS	*Bacillus subtilis*	BioGrow Australia, biogrowcompany.com/australia	Also contains *B. longum*, *E. faecium*, *L. acidophilus*, and *L. casei*
GUT START® – T	*Bacillus subtilis*	Agro BioSciences, Inc., www.agro-biosciences.com/	Also contains *Lactobacillus salivarius*, *L. plantarum* final concentration of 2.75×10^{10} CFU/g
Alterion®	*Bacillus subtilis*	Adisseo-Novozyme, http://feedsolutions.adisseo.com/en/	Supplied at a concentration of 1×10^{10} CFU/g
Enviva®	*Bacillus amyloliquefaciens*	Dupont-Danisco, http://animalnutrition.dupont.com/	Recommended dose is 7.5×10^7 CFU/kg
SPORULIN®	*Bacillus subtilis*	Novus International, http://www.novusint.com/	Provided at no less than 4×10^9 CFU/g

2016). A number of these AMP have been well reviewed by Sumi *et al.* (2015). Bacteria can produce AMP both ribosomally, where AMP have a narrow antimicrobial range against closely related organisms, and non-ribosomally, where gene encoded precursor molecules are post-translationally assembled by enzymes to exert a broader antimicrobial range (Sumi *et al.*, 2015). The activity of these small, positively-charged peptides is mediated through the disruption of bacterial membranes, making the development of resistance more difficult compared with traditional antibiotics that target enzymatic processes (Sang & Blecha, 2008). Andersson *et al.* (2016) thoroughly reviewed the potential for pathogens to become resistant to eukaryotic AMP in a clinical setting, and selection for phenotypes resistant to eukaryotic AMP could easily be acquired *in vitro*. Although not impossible, bacterial resistance to prokaryotic AMP *in vivo* could prove more difficult due to several factors, including the large variety of AMP produced and their primary mode of action of cell wall disruption. Maroti *et al.* (2011) described the interaction of AMP-sensitive and resistant bacteria within the gut microbial environment as a game of "rock-paper-scissors", where AMP-producing bacteria kill the AMP-sensitive strains, but are outcompeted in their growth by AMP-resistant strains. AMP-resistant bacteria, on the other hand, are then outcompeted by the growth of AMP-sensitive bacteria due to the lack of toxins in the environment, thus producing a balanced microbial microenvironment.

Ribosomally produced AMP

The major ribosomally produced AMP are bacteriocins which fall into three main classes (Zhao & Kuipers, 2016). Class I are the lantibiotics containing the modified amino acids lanthionine and methyllanthionine, class II are low molecular weight (<30 kDa) non-modified bacteriocins, and class III are non-heat tolerant high molecular weight proteins (>30 kDa). Bacteriocins form pores in the cell wall of bacteria, initially by attraction to the negatively charged cardiolipins, phosphatidylserine, or phosphatidylglycerol. After attaching to specific cell wall receptors, the spectrum of the antimicrobial activity is dependent on the peptide (Lee & Kim, 2011). The mechanisms and functions of the various bacteriocins produced by *Bacillus* are extensive (Sumi *et al.*, 2015); the bacteriocins considered in this review will be limited to the amphiphilic *B. subtilis*-produced lantibiotic, subtilin (class I), subtilin-like entainin (class I), and the *B. thuringiensis*-produced thuricin and bacthuricin (class II).

Bacillus ribosomally produced AMP are mainly effective against Gram-positive bacteria that are closely related to it. For instance, Rea *et al.* (2010) identified a two-component thuricin which was effective against clinical strains of *Clostridium difficile*. These two-components, Trn-α and Trn-β, worked synergistically to reduce *C. difficile* from approximately 10^6 CFU/ml to below the limits of detection within 3 h. It is just as important to note that, at similar concentrations used to reduce *C. difficile*, this thuricin had no effect against *L. casei* and *B. lactis* which suggests that thuricin would have little effect against other commensal gut bacteria. It is important to note that *C. difficile* is a foodborne pathogen that is readily isolated in poultry, and studies have found genotypically identical strains of this organism between animals, food, and clinical strains isolated in the same geographical area (Harvey *et al.*, 2011; Lund & Peck, 2015). Bacthuricin was also shown to be effective against food pathogens *Listeria monocytogenes* and *B. cereus*. The well-diffusion technique was used to demonstrate that Bacthuricin F4 greatly inhibited *B. cereus* after 24 h (Kamoun *et al.*, 2005). Kamoun *et al.* (2011) showed similar results where Bacthuricin F103 was purified and characterized prior to applying 50 AU to 1×10^5 cells of *B. cereus* ATCC 14579, *in vitro*. They reported a reduction of 1.2 Log CFU/ml within the first 5 min, and this decline remained steady for 3 h resulting in a 4 Log reduction. This same study then applied 500 AU of Bacthuricin F103 to beef that was artificially contaminated with 2×10^2 CFU/g of *L. monocytogenes*, and after 6 days the pathogenic load decreased by 1.61 Log CFU/g and was undetectable after 10 days showing its versatility *in vitro* as well as in a meat model. Another study reported that after purification, an unsuccinylated entainin, a subtilin-like lantibiotic, was effective against *Staphylococcus aureus* (MIC between 4 and 8 μg/ml), and *Enterococcus faecalis* (MIC between 8 and 16 μg/ml) (Fuchs *et al.*, 2011). Both organisms are Gram-positive pathogens of interest commonly found in poultry (Persoons *et al.*, 2009; Lee *et al.*, 2010a), and entainin reduced them to levels similar to nisin A. This entainin was produced by *B. subtilis* DSM 15029, and it contained a 3-amino acid difference to the subtilin produced by *B. subtilis* ATCC 6633, and the ATCC 6633 strain did not produce entainin in discernable amounts (Fuchs *et al.*, 2011). This suggests that different *Bacillus* strains can produce differently structured AMP, and it suggests *Bacillus* strains have the potential to evolve and produce different AMP that are like previously produced peptides but more effective.

A major class of ribosomally produced AMP that has been widely studied and has been used in agriculture production are the lantibiotic class of bacteriocins, more specifically subtilin (Lee & Kim, 2011). When mature, this *B. subtilis*-produced AMP contains 32 amino acids, a *meso*-lanthionine ring, and four methyllanthionine rings, a structure that closely resembles nisin, a *Lactococcus* antimicrobial that has been used in the dairy and cheese industry. Subtilin binds to

bactoprenyl pyrophosphate in lipid II, and initiates a cascade of downstream reactions which results in disrupted cell barrier function and bacterial death (Chan *et al.*, 1989; Parisot *et al.*, 2008). The thick cell walls of Gram-positive organisms are most susceptible to subtilin. A suspension of *B. cereus* was significantly reduced after 4 h by 1 μg/ml of subtilin in a study conducted by Liu and Hansen (1992). Interestingly, this same study showed a site mutation at position 4 from GLU to ILE resulted in subtilin reducing *B. cereus* at lower concentrations, 0.3 μg/ml, compared to the wild-type which lends weight to the theory that slight mutations to the peptide's structure might enhance the lethality of *Bacillus*-produced AMP. Like other bacteriocins, the production of subtilin is based on cell-density signalling, or quorum sensing, as an adaptive response to environmental stress including decreased availability of nutrients (Abriouel *et al.*, 2011). Initiating subtilin production reduces the competition from the surrounding biota, and increases the available nutrients in the gastrointestinal (GI) environment, so more of these nutrients are readily available to the host and, in poultry production, this can equate to better feed efficiency. Subtilin does not cause complete bacterial interruption, and some beneficial organisms can be promoted in the presence of subtilin (Hosoi *et al.*, 2000).

Non-ribosomally produced AMP

Bacillus also produces a series of non-ribosomally synthesized AMP through detailed mechanisms of assembly from over 300 different precursors mediated by a series of peptide synthases. These AMP have a broader range of microbial inhibitions and are effective against both Gram-positive and -negative bacteria, as well as viruses, fungi, and yeasts (Hancock & Chapple, 1999). Condensation of these molecules is catalysed by a thioesterase, and elongation typically occurs with three domains: an adenylation domain, a thiolation carrier domain, and a condensation domain (Stein, 2005). The most well-studied non-ribosomal AMP produced by *Bacillus* include bacitracin and gramicidin, which are both popular antimicrobials used in the medical field. Other non-ribosomal AMP produced by *Bacillus* include iturins and fengycins; these lipopeptides exhibit strong antifungal activity (Maget-Dana & Peypoux, 1994; Deleu *et al.*, 2008).

A lipoheptapeptide produced by *B. subtilis*, surfactin, is one of the most powerful surfactants known, which interferes with biological membranes of bacteria, viruses, and mycoplasmas. Bio-surfactants have low toxicity and are biodegradable which makes them advantageous in agriculture, because pharmaceutical antibiotics may persist in the environment for long periods of time which increases the stability of resistance phenotypes in bacteria (Pérez-García *et al.*, 2011). Fernandes *et al.* (2007) applied two surfactins, isolated from *B. subtilis* R14, to 29 multi-drug resistant bacteria and the surfactins were found to be effective against them all. One of the bacteria was a multi-drug resistant *E. coli* and, although *E. coli* is a commensal in the chicken gut, overgrowth of this organism can result in significant economic losses caused by colibacillosis and airsacculitis in poultry (Diarra *et al.*, 2007). Surfactin disintegrated the cell membrane of several *Mycoplasma* spp. indicating its potential effectiveness against a similar species *Mycoplasma gallisepticum*, the causative agent of chronic respiratory disease in chickens (Vollenbroich *et al.*, 1997). However, this theory requires further research.

The AMP discussed were applied to organisms *in vitro* or after the antimicrobial was purified. However, during large-scale poultry production, *Bacillus* spores would be supplemented in feed and/or water and the multitude of AMP produced by single or multiple strains would affect the gut community, *in vivo*, and in the presence of other microbiome interactions. In other words, the *in vitro* results of these studies may not translate directly with what occurs in the gut. Resistance to antimicrobials of human and veterinary importance is a risk when supplementing with whole bacterial organisms. In order to be considered suitable DFM, bacteria must show susceptibility to the following antibiotics: ampicillin, vancomycin, gentamicin, kanamycin, streptomycin, neomycin, erythromycin, clindamycin, quinupristin + dalfopristin, tetracycline, chloramphenicol, trimethoprim, and linezolid (Anadón *et al.*, 2006). *Bacillus* spp. must also test negative for the toxigenic genes: haemolytic enterotoxin (*nhe*), haemolysin BL (*hbl*), cytotoxin K (*cytK*), and cereulid (*ces*) (European Food Safety Authority, 2014).

Bacillus-induced gut microflora modulation and resulting metabolite production

Table 2 summarizes the results of some published studies that have examined the effects of *Bacillus* DFM on chicken growth performance. Although the exact mechanisms are unknown, it has been suggested that *Bacillus* supplementation as a DFM alters the gut microbiota, reduces the competition for nutrients between microbes and the host, and improves GI health. Microbial colonization of the chicken GI tract begins at hatch and rapidly increases. Within 24 h, the microbial load in the proximal and distal intestine can reach 10^8 and 10^{10} cells/g, respectively (Apajalahti *et al.*, 2004). The composition of the intestinal microflora is heavily influenced by the litter left from the previous flock (Lee *et al.*, 2013). Early application of *Bacillus* spp. as a DFM is crucial if it is to maintain a lasting presence within the gut community. Studies have shown that supplementation of *Bacillus* spp. as a DFM improves overall intestinal health and growth

Table 2. Effect of *Bacillus* DFM on chicken growth performance.

Bacillus strain	Dosage	Length of study	Pathogen challenge	Growth performance	Reference
B. subtilis	10^8 CFU/t 10^9 CFU/t	42 days	No No	None None	Teo and Tan (2007)
B. subtilis Calsporin	10^9 CFU/g	35 days	No	Reported improved ADG but did not report final weight	Jeong and Kim (2014)
B. subtilis DSM17299	8×10^5 CFU/g	42 days	Yes, *Salmonella* Heidelberg	Weight gain was numerically significant but not statistically significant	Knap *et al.* (2011)
B. licheniformis (DSM17236)	8×10^5 CFU/g 8×10^6 CFU/g 8×10^7 CFU/g	28 days	Yes, *Clostridium perfringens*	All three *B. licheniformis* concentrations caused a weight gain significantly greater than the challenged control	Knap *et al.* (2010)
B. subtilis C-301 (Calsporin)	30 g/t feed	42 days	No	Yes, significant growth was reported in the *B. subtilis* group compared to the non-treated control	Fritts *et al.* (2000)
B. subtilis 1781	1.5×10^5 CFU/g	14 days	No	Body weight gain was significant compared to control and two other *Bacillus* DFM treatment groups	Gadde *et al.* (2017b)
B. subtilis (Avicorr™)	1.5×10^5 CFU/g	28 days	Yes, broilers were reared on *Eimeria*-positive used litter	Significantly greater body weights were reported in the *B. subtilis*-treated group compared to the controls	Lee *et al.* (2015)
B. subtilis Calsporin (Calpis Co.)	1×10^{10} CFU/g	42 days	No	Significantly greater body weights were reported compared to the control and similar body weights as the LAB-treated group	Aliakbarpour *et al.* (2012)
B. subtilis (Bs27) *B. subtilis* (LSSAO1) *B. subtilis* (3AP4) *B. subtilis* (BS18) *B. subtilis* (15AP4) *B. subtilis* (22CP1) *B. subtilis* (Bs27) *B. subtilis* (Bs278) *B. subtilis* (Avicorr™)	1.5×10^5 CFU/g	21 days	No	Increased body weights for five of the nine tested strains compared to control but none were significant: Bs27, LSSAO1, 3AP4, 15AP4, Avicorr™	Lee *et al.* (2010a)
B. licheniformis H2	1×10^6 CFU/g	22 days	Yes, *Clostridium perfringens*	Significantly increased body weights compared to *C. perfringens* infected controls	Lin *et al.* (2017)

in chickens but, again, the exact mechanisms are unknown. *Bacillus* spores are not reactive prior to ingestion. However, they quickly germinate once inside the chickens' GI environment and vegetative cells can outnumber spores within 20 h of oral administration and could be detected along the small intestine, caeca, and colon (Cartman *et al.*, 2008). *Bacillus* DFM could affect the distribution and colonization of the innate microflora along the GI tract and promote the growth and proliferation of other good symbiotic bacteria. One theory posits that the growth-promoting effects of DFM in poultry are linked to reduced number and diversity of the natural microbiota, thus allowing for increased nutrient utilization by host intestinal epithelial cells and reduced effects of detrimental microbial metabolites (Gadde *et al.*, 2017a).

In general, lactobacilli and other bacteria of the phylum Firmicutes comprise 80–90% of the total small intestinal microflora, followed by members of Proteobacteria and Bacteroidetes phyla. The intestinal duodenum is predominated by *Lactobacillus*, *Streptococcus*, and coliforms, while the ileum houses mostly *Lactobacillus*, *Streptococcus*, coliforms, and members of the Enterobacteriaceae and Clostridiaceae families. Two bacteria known for their ability to improve chicken gut health are *Lactobacillus* spp. and *Bifidobacterium*, a genus of the phylum Actinobacteria. Both microorganisms ferment simple sugars, and both are relatively unaffected by *Bacillus* DFMs (Kaplan & Hutkins, 2003; Moura *et al.*, 2007; Rinttilä & Apajalahti, 2013; Choi *et al.*, 2014). For instance, Teo and Tan (2007) recovered similar levels of *Lactobacillus* and *Bifidobacterium* spp. from the ileum of broilers given feed supplemented with 10^9 CFU/ml *B. subtilis* PB6 when compared to the negative control. In another study, *B. subtilis* (*natto*) enhanced the growth of beneficial bacteria *L. reuteri* and *L. acidophillus* (Hosoi *et al.*, 2000), a result that was echoed by Jeong and Kim (2014) who reported a significant increase in *Lactobacillus* in the ilea and caeca after supplementing chicken feed with *B. subtilis* C-3102. The host can recover some of the energy lost through competition with microbes in this part of the gut by absorbing bacterially-produced nutrients and metabolites, such as lactic acid and volatile fatty acids, from fermenting bacteria. Some *Bacillus* DFM can facilitate the increase in beneficial gut biota in the upper GI tract to maintain or even increase the production of these nutrients.

Diets rich in carbohydrates, such as the corn- and soy-based diets typically given to production chickens, provide materials for fermenting biota in the upper gut, and those bacteria produce fermentation intermediates that include lactate, fumarate, and succinate. These metabolites are either absorbed by the host or used by other biota to produce other end products. After supplementing water with commercial organic acids, Chaveerach *et al.* (2004) reported greatly reduced amounts of lactate in the caecum compared with the crop, suggesting either lactate absorption by the host or utilization by other biota which could lead to the production of beneficial end

products, such as the short chain fatty acid (SCFA), butyrate. In an *in vitro* co-culture study, *B. longum* produced lactate in the presence of fructooligosaccharides and *Eubacterium hallii* fermented the lactate to produce significant amounts of butyrate. *B. longum* alone did not produce significant amounts of butyrate and *E. hallii* could not grow on fructooligosaccharides (Belenguer *et al.*, 2006). Although this study was *in vitro*, it is likely that a similar microflora metabolite utilization and production occur along the gut, especially with increased levels of certain fermenting bacteria. Increased levels of fermenting bacteria in the families Bifidobacteriaceae, Bacteroidaceae, and Lactobacillaceae strongly correlate with increased levels of β-glucosidase in the avian gut of grain-fed birds (Waite & Taylor, 2014). Deficiencies in β-glucosidase have been associated with increases of *Bifidobacterium* in the stool of healthy humans (Depeint *et al.*, 2008); however, more research is needed to understand its exact impact on poultry.

Competition between host and gut biota occurs in the small intestine, mainly by the *Lactobacillus* spp. that is estimated to utilize approximately 3–6% of total dietary protein; however, this estimate can vary greatly depending on bacterial density and the host absorbs much of their needed amino acids in the proximal small intestine where bacterial densities are lower (Apajalahti & Vienola, 2016). *Lactobacillus* spp. and other protein-assimilating bacteria in the small intestine act as a filter to prevent the passage of amino acids to the caeca where they would be further fermented into toxic by-products, such as indoles and ammonia, by putrefying bacteria (Searle *et al.*, 2009; Apajalahti & Vienola, 2016; Ghasemian & Jahanian, 2016). Indole toxicity uncouples the protein gradient across biological membranes and inhibits ATP production (Chimerel *et al.*, 2013), and deamination production of ammonia adversely affects gut epithelial cell morphology, DNA synthesis, and metabolism, all of which result in decreased health and growth (Clausen & Mortensen, 1992; Apajalahti & Vienola, 2016). Greater nutrient absorption could lead to increased growth in poultry; however, this growth promotion is not guaranteed. Some studies, like Jeong and Kim (2014), Fritts *et al.* (2000), Gadde *et al.* (2017b), and Hosoi *et al.* (2000), reported improved growth performance that was either equal to or greater than the controls, while others report that their *Bacillus* DFM either had no difference in weight gain or caused a decrease in body weight, such as Teo and Tan (2007) (Table 2). Variation of DFM performance can even occur within the same study. Although Fritts *et al.* (2000) reported improved weight gain, this was only in one trial within the study. This same study also reported that *B. subtilis* C-301 did not improve growth in a separate independent trial under the same experimental conditions, which led the researchers to infer that heat stress may have reduced broiler performance, because the underperforming trial was conducted during a warm season.

The caeca have the highest bacterial density along the chicken gut, housing upwards of 10^{11} cells/g digesta, and the longest residence time of 12–20 h. The primary phyla in the caeca belong to Firmicutes, Bacteroides, and Proteobacteria with the order Clostridiales representing more than 50%. This organ is responsible for water regulation and carbohydrate fermentation and it is the largest SCFA producing organ in the chicken (Torok *et al.*, 2011; Oakley *et al.*, 2014). SCFA are necessary for epithelial cell maintenance and the inhibition of pathogenic organisms. Butyrate has been of interest to the poultry industry because it is the preferred energy source for enterocytes and colonocytes to maintain the integrity of the gut lining and effectively ward off *Salmonella* colonization (Van Immerseel *et al.*, 2003). Supplementation with *Bacillus* has been reported to increase SCFA production either directly or indirectly by modulating SCFA producing bacteria. For instance, butyrate was significantly increased when chicken feed was co-supplemented with 5000 U/kg of serine protease and phytase along with 8×10^5 CFU/g of *B. licheniformis* (Murugesan *et al.*, 2014). It is worth mentioning that SCFA and nutrient production when supplemented with *Bacillus* is not equal and can differ from study to study. Novak *et al.* (2011) reported a decrease in butyrate and other SCFA produced when individually supplementing with *B. subtilis* and *B. licheniformis* while using a slow growing chicken breed in their model. Host breed, genomic and metabolic changes can influence gut microbial response to nutrient metabolism, as is evident in a study by Kim *et al.* (2015). Also, very few nutrients are absorbed in the caeca. When the caecal microflora produces SCFA and other nutrients such as vitamins B, possibly in response to *Bacillus* DFM supplementation, a large portion would be excreted in the faeces. However, the coprophagic nature of chickens allows them to ingest nutrients from the excrement of other birds and benefit from the nutrients produced by another bird's microflora. This was evident from caged chickens having vitamin deficiencies which were absent from chickens raised on hard floors (Pan & Yu, 2014). Access to faeces is another factor that causes study-to-study variation during DFM research. *Bacillus* should be thoroughly screened prior to implementation as a DFM because of the strain-to-strain variation. Not much is known about the various metabolites that are affected in response to *Bacillus* DFM supplementation and, to date, no study exists that correlates metabolomic production to gut flora modulation in the presence of *Bacillus* DFM. This novel area of study could lead researchers to possibly replace DFM with the beneficial metabolites they produce, which could decrease the variability that is often observed with DFM.

Pathogenic control by *Bacillus* DFMs

Pathogen reduction and/or suppression by the GI commensal microflora is the result of multiple factors. Although the exact mechanisms are unknown, *Bacillus* as DFM could prevent pathogenicity by direct inhibition of pathogens through mechanisms of competitive exclusion such as the production of AMP or by enhancing the intestinal mucosal layer to prevent microbial diffusion across the membrane (Johansson *et al*., 2008). Pathogenic control is a major concern for the poultry industry from an economic and public health perspective. *Salmonella* is classically associated with poultry and causes approximately one million illnesses in the United States annually, and zoonotic pathogenic *Clostridium* spp. cost the American poultry industry roughly $6 billion annually (Wade & Keyburn, 2015; CDC, 2017). Studies show that *Bacillus* strains can reduce or inhibit poultry pathogens, both *in vitro* and as DFM. For instance, Teo and Tan (2007) showed that two strains of *B. subtilis* isolated from the gut of a chicken were antagonistic against *C. perfringens* ATCC 13124 after 24 h of incubation under anaerobic conditions, and Knap *et al.* (2010) reduced necrotic enteritis (NE) caused by *C. perfringens* in chickens with three concentrations of *B. licheniformis* (8×10^5 CFU/g feed, 8×10^6 CFU/g feed, and 8×10^7 CFU/g feed). All three concentrations reduced clinical signs of NE to levels similar to growth-promoting doses of virginiamycin (50 g/ton) and they maintained similar body weight and feed conversion ratios (FCR) as the virginiamycin-treated group. Park and Kim (2014) reported that intestinal concentrations of *Salmonella* Typhimurium were significantly decreased in the presence of three concentrations of *B. subtilis* B2A (1×10^4 CFU/g, 1×10^5 CFU/g, and 1×10^6 CFU/g). Although the weight gain of these birds was not significant, the *B. subtilis* B2A-fed group had less feed intake and better FCR, meaning they ate less to weigh the same.

In addition to controlling bacterial pathogens, *Bacillus* DFM has reduced the effects of coccidiosis, a disease caused by *Eimeria* spp. This parasite contributes to an estimated $3 billion annual loss, worldwide, and seven distinct species infect avian intestinal mucosa (Lillehoj & Trout, 1996; Shirley & Lillehoj, 2012). In an earlier study that tested eight individual strains of *B. subtilis* as a DFM against the clinical signs of coccidiosis, three *Bacillus* strains showed significant reduction in intestinal lesion scores, which is a *post mortem* observation of coccidiosis, and two of the three strains did not result in reduced body weight gain, also a clinical sign of coccidiosis (Lee *et al*., 2010b). Another study resonated these results and, after *Eimeria* infection, the treatment group that received *B. subtilis* maintained a body weight that was similar to the uninfected control and greater than the *Eimeria* infected group that was not alleviated with *B. subtilis* supplementation. This study also reported that, in addition to reducing the *post mortem* observations of coccidiosis, the *Bacillus*-treated group had enhanced immunological response towards the infection, marked by substantial up-regulation of proinflammatory cytokines and intestinal epithelial lymphocytes (Gadde *et al*., 2017c).

Rarely, if ever, is coccidiosis a unilateral parasite in poultry production, meaning *Eimeria* spp. typically promotes infections caused by the opportunistic pathogen *C. perfringens* to magnify the signs of NE (Park *et al*., 2008). Model development studies indicated that *C. perfringens* alone was not enough to cause NE, and intestinal lesion scores and body weight loss, the typical clinical signs in infected broilers, were mild or akin to the non-infected controls. However, after the addition of *Eimeria* spp., NE signs were dramatically worse resulting in broilers with noticeable intestinal lesion scores and markedly reduced body weights. After treating broilers with a cocktail of *B. subtilis* DFM for 28 days, there was a significant increase in body weight gain in the DFM-fed group compared to the salinomycin-fed group but not the controls following *Eimeria* and *C. perfringens* challenge infection. *Bacillus* DFM improve immunity by increasing antibodies against *Eimeria* spp. and serum nitric oxide (NO) levels; even if enhanced growth was not affected, these birds were more robust to fight infections and the symptoms associated with bacterial and parasitic pathogens, as is detailed in a study by Lee *et al.* (2014). A separate study had similar findings when using *B. licheniformis* to reduce NE infections in a dual *C. perfringens-Eimeria* model; however, this study took their analysis in another direction and used genomic sequencing to investigate the caecal microbial changes caused by DFM supplementation. In this case, *B. licheniformis* fed chickens had caecal profiles similar to the negative control group with *Bacteroides* being one of the most dominant taxa. However, *Bacteroides* was the lowest in the infection group that did not receive *B. licheniformis* alleviation. This study also noted a growth promotion response in the *B. licheniformis*-supplemented group (Lin *et al*., 2017).

Although performance variations depend on the supplemented DFM strain, the literature consistently reveals improved performance in the presence of a pathogen, either bacterial or parasitic (Ducatelle *et al*., 2014). Constant dosing with *Bacillus* as DFM is necessary for it to maintain its anti-pathogenic properties. Although *Bacillus* can survive in the gut, it is mainly a transient member and most of the cells are shed in faeces and must be continuously supplied in feed and water shortly after hatch through to harvest. In a study that compared the persistence of *S.* Enteritidis after a single dose of *B. subtilis* (1×10^9 cells) followed by a single dose of *S.* Enteritidis (1×10^5 cells),

there was mostly no significant reduction in the recovery of *S.* Enteritidis after 36 days in the liver, spleen, duodenum, jejunum, ileum, caeca, and colon. Inoculation with these organisms occurred within 48 h post-hatch, and the only observed reduction was very slight ($P = 0.035$) and was only on the first post-infection day.

Gut morphological and immunological changes induced by *Bacillus* DFM

Bacillus DFM interact directly with the host to produce a beneficial response to the chickens' intestinal epithelium. This immunological and physiological response is critical to successful poultry production, because it directly correlates to improved growth and performance. Many factors related to disease and stress can cause an interruption to intestinal epithelial integrity which reduces nutrient absorption, increases pathogenic invasion, and increases inflammatory diseases, all leading to reduced growth performance (Yegani & Korver, 2008). Gut barrier function must be maintained if the body is to shield itself from unwanted biological and chemical invasions, and it does this with two major mechanisms. The first is the secretion of the mucous blanket by goblet cells that are dispersed throughout the small intestine luminal epithelium (Chichlowski *et al.*, 2007). The mucus consists predominantly of mucin and is supported by other proteins, lipids, glycoproteins, and glycolipids. DFM, such as *Bacillus*, can up-regulate the mucin-producing gene, MUC2, to counteract the inflammation caused by pathogens. In a study by Gadde *et al.* (2017c), chickens were given intraperitoneal injections of *E. coli* LPS to induce an inflammatory response. The group that was given feed supplemented with *B. subtilis* strain 1781 showed significantly increased ileum levels of MUC2 and showed markedly lower signs of inflammation as noted by reduced α-1-acid glycoprotein, which is often associated with acute colitis (Hochepied *et al.*, 2002). This study also showed that up-regulation of MUC2 can happen in response to LPS circulating in the blood and not just as a reaction to direct contact with the intestinal epithelium. In other words, continual dosing with *Bacillus* DFM could combat residual infections or the pathogens that may have bypassed host defences and remain in circulation after the initial infection. An increase in MUC2 was observed in other studies that supplemented with *Bacillus* DFM in the absence of infection showing the ability of *Bacillus* to promote preventative gut infections (Lee *et al.*, 2010a). Aliakbarpour *et al.* (2012) reported that *Bacillus* DFM-treated broilers produced significantly more mucin in the jejunum than the control and was comparable to the group that received a treatment of lactic acid bacteria cocktail containing *L. casei*, *L. acidophilus*, *B. thermophilum*, and *E. faecium* (Aliakbarpour *et al.*, 2012). Both DFM-treated groups showed greater body weight than the control although all three groups consumed similar amounts of feed.

The second mechanism is the enhancement of the epithelial barrier integrity by increasing the regulation of tight junction proteins that bind to one another forming a continuous barrier that is impenetrable to pathogens and large molecules (Chichlowski *et al.*, 2007). Shen *et al.* (2006) used electron microscopy to demonstrate some of the primary molecules involved in maintaining the integrity of this barrier which include junction adhesion molecule 2 (JAM2), occludin, and zona occludens 1 (ZO1) as well as the physiological response of increased intestinal villi crypt height. Gadde *et al.* (2017c) echoed similar findings and reported a distinct increase in tight junction genes JAM2, ZO1 and occludin in the ileum when chickens challenged with LPS were given *B. subtilis* as a DFM. Greater crypt depth increases the intestinal epithelial surface area making the organ better suited for nutrient absorption which could translate into growth promotion. When broilers were fed gradually increasing amounts of *B. subtilis* LS 1-2 over 35 days, not only were villus height and crypt depth significantly better in the duodenum and ileum, but the *B. subtilis* LS 1-2-treated group weighed significantly more and had decreased caecal *Clostridium* and coliforms compared to the control (Sen *et al.*, 2012). In a *C. perfringens* challenge study, *B. subtilis* PB6 supplemented broilers had significant FCR and increased intestinal villi length of between 10.88% and 30.46% compared to infected controls demonstrating that *Bacillus* supplementation can improve host gut physiology and intestinal health in the presence of pathogens (Jayaraman *et al.*, 2013). Avian physiological responses to *Bacillus* DFM cause an increase in gut barrier protein production, crypt height, and immune modulation via cytokine and defence molecule production.

Macrophages function as an important initiator and mediator in innate and adaptive immunity by recognizing pathogens and eliminating them via phagocytosis and cytotoxicity response which includes the production of proinflammatory cytokines: IL-1β, IL-6, IL-8, and TL1A (the chicken homologue of TNF-α (Takimoto *et al.*, 2008)) and T-helper cytokines: IL-2 and IFNγ. Proinflammatory cytokines cause the production of defence molecules, NO, and inducible nitric oxide synthase (iNOS) that, at low concentrations, stimulate the maturation and activity of immune cells while, at higher concentrations, NO irreversibly binds to DNA, lipids, and proteins effectively killing the pathogen (Privett *et al.*, 2012). *Bacillus* DFM have been marked by immunostimulatory production of proinflammatory cytokines and macrophage activation without causing cytotoxicity. In the presence of pathogens, *Bacillus* DFM cause a significantly noticeable increase in the up-regulation of cytokine production,

NO, and iNOS. One study reported a 1.5-fold increase in NO concentrations with *Bacillus* DFM in the presence of *E. coli* LPS, and an other study reported broilers raised on chicken litter that was positive for *Clostridium* caused gangrenous dermatitis; IL-1β was up-regulated along with IFNγ compared to the controls that did not receive *Bacillus* treatment (Lee *et al.*, 2011, 2013). To understand the global gene expression that occurred in the presence of *B. subtilis* DFM and dual pathogens *Eimeria* spp./*C. perfringens*, one study utilized gene array techniques and identified 37 genes which were significantly related to the inflammatory response and were up- or down-regulated. This same study also reported an up-regulation of intestinal expression of IFNγ, IL-1β, and IL-12 (Lee *et al.*, 2015). Augmentation of macrophage function is one way *Bacillus* DFM enhance immunity. It has also been reported to support the proliferation of lymphoid follicles along the intestinal mucosa and support the development of gut-associated lymphoid tissue thus increasing its immunoregulatory capacity (Rhee *et al.*, 2004; Molnár *et al.*, 2011). The up- or down-regulation of cytokines depends heavily on the strain of *Bacillus* DFM, especially when administered in the absence of a pathogen. Lee *et al.* (2010a) tested eight independent isolates of *Bacillus* and one commercially available cocktail of multiple strains in a non-pathogenic study and reported an up-regulation in expression of circulating IL-6 and IL-8, a result that was reiterated in a separate independent study where *B. subtilis* (*natto*) B4 spores induced several proinflammatory cytokines including IL-1β and IL-6, and IFNγ as well as an increase in macrophage NO and iNOS production. However, Lee *et al.* (2010a) also reported only one of the strains up-regulated IFNγ gene expression, whereas the remaining strains had decreased levels and two strains down-regulated it, two strains up-regulated IL-1β and one strain down-regulated it when compared to the control. These results further emphasize effective screening when selecting strains as DFM in poultry production because of the strain-to-strain variation, dose of the given DFM, and the persistence of that strain in the GI environment, a topic that is well reviewed by Huyghebaert *et al.* (2011).

Notably, *B. subtilis* also causes the production of the anti-inflammatory, or regulatory, cytokines IL-10, and IL-4 in response to increasing levels of proinflammatory cytokines as an autoregulatory negative feedback to control the acute inflammatory response (Platzer *et al.*, 1995; Xu *et al.*, 2012). Prolonged exposure to IL-1, TL1A, and IL-6 cytokines caused reductions in muscle cell translational efficiency which resulted in muscle proteolysis effectively destroying muscle mass (Fanzani *et al.*, 2012). The chronic circulation of proinflammatory cytokines inhibits myogenic differentiation which could lead to diminished muscle growth. It could be argued that one of the multiple mechanisms by which *Bacillus* DFM could promote growth is by supporting muscle mass development through the reduction of prolonged proinflammatory cytokine production; however, this theory warrants greater study.

Conclusion

The use of *Bacillus* DFM in poultry is rapidly expanding as noted by the increase in research that studies its gut flora modulation and immune stimulation. This organism's ability to survive feed processing and administer its benefits to the gut is an advantage that *Bacillus* has over some other commonly studied DFM, and several *Bacillus* DFM strains that are commercially available for use in poultry. There is evidence that *Bacillus* DFM reduce competition for nutrients by reducing the overgrowth of bacteria in the small intestine through the production of AMP, they promote the proliferation and production of beneficial bacteria and metabolites, and they alter the immune response towards the benefit of the host, all of which are modes of action that culminate to promote growth. Although the benefits of different *Bacillus* bacteria are not equal, the next steps of research could include the identification of global metabolites that are produced in the presence of well-performing *Bacillus* DFM to comprehend the interaction between bacteria and host.

Acknowledgements

The authors would like to thank Dr Erik Lillehoj (University of Maryland School of Medicine, USA) for his valuable comments and edits on the manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

Abriouel, H., Franz, C., Omar, N.B. & Gálvez, A. (2011). Diversity and applications of *Bacillus* bacteriocins. *FEMS Microbiology Reviews*, 35, 201–232.

Aliakbarpour, H.R., Chamani, M., Rahimi, G., Sadeghi, A.A. & Qujeq, D. (2012). The *Bacillus subtilis* and lactic acid bacteria probiotics influences intestinal mucin gene expression, histomorphology and growth performance in broilers. *Asian-Australasian Journal of Animal Sciences*, 25, 1285–1293.

Anadón, A., Rosa Martínez-Larrañaga, M. & Aranzazu Martínez, M. (2006). Probiotics for animal nutrition in the European Union. Regulation and safety assessment. *Regulatory Toxicology and Pharmacology*, 45, 91–95.

Andersson, D.I., Hughes, D. & Kubicek-Sutherland, J.Z. (2016). Mechanisms and consequences of bacterial resistance to antimicrobial peptides. *Drug Resistance Updates*, 26, 43–57.

Apajalahti, J., Kettunen, A. & Graham, H. (2004). Characteristics of the gastrointestinal microbial

communities, with special reference to the chicken. *World's Poultry Science Journal*, 60, 223–232.
Apajalahti, J. & Vienola, K. (2016). Interaction between chicken intestinal microbiota and protein digestion. *Animal Feed Science and Technology*, 221, 323–330.
Barbosa, T., Serra, C., La Ragione, R., Woodward, M. & Henriques, A. (2005). Screening for *Bacillus* isolates in the broiler gastrointestinal tract. *Applied and Environmental Microbiology*, 71, 968–978.
Belenguer, A., Duncan, S.H., Calder, A.G., Holtrop, G., Louis, P., Lobley, G.E. & Flint, H.J. (2006). Two routes of metabolic cross-feeding between bifidobacterium adolescentis and butyrate-producing anaerobes from the human gut. *Applied and Environmental Microbiology*, 72, 3593–3599.
Cartman, S.T., La Ragione, R.M. & Woodward, M.J. (2008). *Bacillus subtilis* spores germinate in the chicken gastrointestinal tract. *Applied and Environmental Microbiology*, 74, 5254-5258.
CDC. (2013a). *Antibiotic resistance threats in the United States*.
CDC. (2013b). *CDC telebriefing on today's drug-resistant health threats*.
CDC. (2017). *Foodborne germs and illnesses*.
Chaiyawan, N., Taveeteptaikul, P., Wannissorn, B., Ruengsomwong, S., Klungsupya, P., Buaban, W. & Itsaranuwat, P. (2010). Characterization and probiotic properties of *Bacillus* strains isolated from broiler. *Thai Journal of Veterinary Medicine*, 40, 207–214.
Chan, W.C., Lian, L.-Y., Bycroft, B.W. & Roberts, G.C.K. (1989). Confirmation of the structure of nisin by complete 1H n.m.r. resonance assignment in aqueous and dimethyl sulphoxide solution. *Journal of the Chemical Society, Perkin Transactions*, 1, 2359–2367.
Chaveerach, P., Keuzenkamp, D.A., Lipman, L.J.A. & Van Knapen, F. (2004). Effect of organic acids in drinking water for young broilers on *Campylobacter* infection, volatile fatty acid production, gut microflora and histological cell changes. *Poultry Science*, 83, 330–334.
Chichlowski, M., Croom, J., McBride, B.W., Havenstein, G.B. & Koci, M.D. (2007). Metabolic and physiological impact of probiotics or direct-fed-microbials on poultry: a brief review of current knowledge. *International Journal of Poultry Science*, 6, 694–704.
Chimerel, C., Murray, A.J., Oldewurtel, E.R., Summers, D.K. & Keyser, U.F. (2013). The effect of bacterial signal indole on the electrical properties of lipid membranes. *ChemPhysChem*, 14, 417–423.
Choi, J.H., Kim, G.B. & Cha, C.J. (2014). Spatial heterogeneity and stability of bacterial community in the gastrointestinal tracts of broiler chickens. *Poultry Science*, 93, 1942–1950.
Clausen, M.R. & Mortensen, P.B. (1992). Fecal ammonia in patients with adenomatous polyps and cancer of the colon. *Nutrition and Cancer*, 18, 175–180.
Deleu, M., Paquot, M. & Nylander, T. (2008). Effect of fengycin, a lipopeptide produced by *Bacillus subtilis*, on model biomembranes. *Biophysical Journal*, 94, 2667–2679.
Depeint, F., Tzortzis, G., Vulevic, J., I'Anson, K. & Gibson, G.R. (2008). Prebiotic evaluation of a novel galactooligosaccharide mixture produced by the enzymatic activity of *Bifidobacterium bifidum* NCIMB 41171, in healthy humans: a randomized, double-blind, crossover, placebo-controlled intervention study. *The American Journal of Clinical Nutrition*, 87, 785–791.
Diarra, M.S., Silversides, F.G., Diarrassouba, F., Pritchard, J., Masson, L., Brousseau, R., Bonnet, C., Delaquis, P., Bach, S., Skura, B.J. & Topp, E. (2007). Impact of feed supplementation with antimicrobial agents on growth performance of broiler chickens, *Clostridium perfringens* and enterococcus counts, and antibiotic resistance phenotypes and distribution of antimicrobial resistance determinants in *Eschericia coli* isolates. *Applied and Environmental Microbiology*, 73, 6566–6576.
Dibner, J.J. & Richards, J.D. (2005). Antibiotic growth promoters in agriculture: history and mode of action. *Poultry Science*, 84, 634–643.
Ducatelle, R., Eeckhaut, V., Haesebrouck, F. & Van Immerseel, F. (2014). A review on prebiotics and probiotics for the control of dysbiosis: present status and future perspectives. *Animal*, 9, 43–48.
Elliot, S.D. & Barnes, E.M. (1959). Changes in serological type and antibiotic resistance on Lancefield group D streptococci in chickens receiving dietary chlortetracycline. *Journal of General Microbiology*, 20, 426–433.
European Food Safety Authority. (2014). Guidance on the assessment of the toxigenic potential of *Bacillus* species used in animal nutrition. *EFSA Journal*, 12, 3665.
Fanzani, A., Conraads, V.M., Penna, F. & Martinet, W. (2012). Molecular and cellular mechanisms of skeletal muscle atrophy: an update. *Journal of Cachexia, Sarcopenia and Muscle*, 3, 163–179.
Fernandes, P.A.V., Arruda, I.R., Santos, A.F.A.B., Araújo, A.A., Maior, A.M.S. & Ximenes, E.A. (2007). Antimicrobial activity of surfactants produced by *Bacillus subtilis* R14 against multidrug-resistant bacteria. *Brazilian Journal of Microbiology*, 38, 704–709.
Fritts, C.A., Kersey, J.H., Motl, M.A., Kroger, E.C., Yan, F., Si, J., Jiang, Q., Campos, M.M., Waldroup, A.L. & Waldroup, P.W. (2000). *Bacillus subtilis* C-3102 (calsporin) improves live performance and microbiological status of broiler chickens. *The Journal of Applied Poultry Research*, 9, 149–155.
Fuchs, S.W., Jaskolla, T.W., Bochmann, S., Kötter, P., Wichelhaus, T., Karas, M., Stein, T. & Entian, K.-D. (2011). Entianin, a novel subtilin-like antibiotic from *Bacillus subtilis* subsp. spizizenii DSM 15029T with high antimicrobial activity. *Applied and Environmental Microbiology*, 77, 1698–1707.
Gadde, U., Kim, W.H., Oh, S.T. & Lillehoj, H.S. (2017a). Alternatives to antibiotics for maximizing growth performance and feed efficiency in poultry: a review. *Animal Health Research Reviews*, 18, 26–45.
Gadde, U., Oh, S.T., Lee, Y.S., Davis, E., Zimmerman, N., Rehberger, T. & Lillehoj, H.S. (2017b). The effects of direct-fed microbial supplementation, as an alternative to antibiotics, on growth performance, intestinal immune status, and epithelial barrier gene expression in broiler chickens. *Probiotics and Antimicrobial Proteins*, 9, 1–9.
Gadde, U.D., Oh, S., Lee, Y., Davis, E., Zimmerman, N., Rehberger, T. & Lillehoj, H.S. (2017c). Dietary *Bacillus subtilis*-based direct-fed microbials alleviate LPS-induced intestinal immunological stress and improve intestinal barrier gene expression in commercial broiler chickens. *Research in Veterinary Science*, 114, 236–243.
Gareau, M.G., Sherman, P.M. & Walker, W.A. (2010). Probiotics and the gut microbiota in intestinal health and disease. *Nature Reviews Gastroenterology & Hepatology*, 7, 503–514.
Ghasemian, M. & Jahanian, R. (2016). Dietary mannan-oligosaccharides supplementation could affect performance, immunocompetence, serum lipid metabolites, intestinal bacterial populations, and ileal nutrient digestibility

in aged laying hens. *Animal Feed Science and Technology*, 213, 81–89.

Giang, H.H., Viet, T.Q., Ogle, B. & Lindberg, J.E. (2010). Growth performance, digestibility, gut environment and health status in weaned piglets fed a diet supplemented with potentially probiotic complexes of lactic acid bacteria. *Livestock Science*, 129, 95–103.

Guo, X., Li, D., Lu, W., Piao, X. & Chen, X. (2006). Screening of *Bacillus* strains as potential probiotics and subsequent confirmation of the *in vivo* effectiveness of *Bacillus subtilis* MA139 in pigs. *Antonie van Leeuwenhoek*, 90, 139–146.

Haghighi, H.R., Gong, J., Gyles, C.L., Hayes, M.A., Sanei, B., Parvizi, P., Gisavi, H., Chambers, J.R. & Sharif, S. (2005). Modulation of antibody-mediated immune response by probiotics in chickens. *Clinical and Diagnostic Laboratory Immunology*, 12, 1387–1392.

Hancock, R.E.W. & Chapple, D.S. (1999). Peptide antibiotics. *Antimicrobial Agents and Chemotherapy*, 43, 1317–1323.

Harvey, R., Norman, K., Andrews, K., Hume, M., Scanlan, C., Callaway, T., Anderson, R. & Nisbet, D. (2011). *Clostridium difficile* in poultry and poultry meat. *Foodborne Pathogens and Disease*, 8, 1321–1323.

Hochepied, T., Wullaert, A., Berger, F.G., Baumann, H., Brouckaert, P., Steidler, L. & Libert, C. (2002). Overexpression of α(1)-acid glycoprotein in transgenic mice leads to sensitisation to acute colitis. *Gut*, 51, 398–404.

Hosoi, T., Amentani, A., Kiuchi, K. & Kaminogawa, S. (2000). Improved growth and viability of lactobacilli in the presence of *Bacillus subtilis* (natto), catalase, or subtilisin. *Canadian Journal of Microbiology*, 46, 892–897.

Hun, L. (2009). *Bacillus coagulans* significantly improved abdominal pain and bloating in patients with IBS. *Postgraduate Medicine*, 121, 119–124.

Huyghebaert, G., Ducatelle, R. & Van Immerseel, F. (2011). An update on alternatives to antimicrobial growth promoters for broilers. *The Veterinary Journal*, 187, 182–188.

Jayaraman, S., Thangavel, G., Kurian, H., Mani, R., Mukkalil, R. & Chirakkal, H. (2013). *Bacillus subtilis* PB6 improves intestinal health of broiler chickens challenged with *Clostridium perfringens*-induced necrotic enteritis. *Poultry Science*, 92, 370–374.

Jeong, J.S. & Kim, I.H. (2014). Effect of *Bacillus subtilis* C-3102 spores as a probiotic feed supplement on growth performance, noxious gas emission, and intestinal microflora in broilers. *Poultry Science*, 93, 3097–3103.

Johansson, M.E.V., Phillipson, M., Petersson, J., Velcich, A., Holm, L. & Hansson, G.C. (2008). The inner of the two Muc2 mucin-dependent mucus layers in colon is devoid of bacteria. *Proceedings of the National Academy of Sciences of the United States of America*, 105, 15064–15069.

Jukes, T.H., Stokstad, E.L.R., Tayloe, R.R., Cunha, T.J., Edwards, H.M. & Meadows, G.B. (1950). Growth-promoting effect of aureomycin on pigs. *Archives of Biochemistry*, 26, 324–325.

Kamoun, F., Fguira, I.B., Hassen, N., Mejdoub, H., Lereclus, D. & Jaoua, S. (2011). Purification and characterization of a new *Bacillus thuringiensis* bacteriocin active against *Listeria monocytogenes, Bacillus cereus* and *Agrobacterium tumefaciens*. *Applied Biochemistry and Biotechnology*, 165, 300–314.

Kamoun, F., Mejdoub, H., Aouissaoui, H., Reinbolt, J., Hammami, A. & Jaoua, S. (2005). Purification, amino acid sequence and characterization of bacthuricin F4, a new bacteriocin produced by *Bacillus thuringiensis*. *Journal of Applied Microbiology*, 98, 881–888.

Kaplan, H. & Hutkins, R.W. (2003). Metabolism of fructooligosaccharides by *Lactobacillus paracasei* 1195. *Applied and Environmental Microbiology*, 69, 2217–2222.

Kim, D.K., Lillehoj, H.S., Jang, S.I., Lee, S.H., Hong, Y.H. & Lamont, S.J. (2015). Genetically disparate Fayoumi chicken lines show different response to avian necrotic enteritis. *Journal of Poultry Science*, 52, 245–252.

Knap, I., Kehlet, A.B., Bennedsen, M., Mathis, G.F., Hofacre, C.L., Lumpkins, B.S., Jensen, M.M., Raun, M. & Lay, A. (2011). *Bacillus subtilis* (DSM17299) significantly reduces *Salmonella* in broilers. *Poultry Science*, 90, 1690–1694.

Knap, I., Lund, B., Kehlet, A.B., Hofacre, C. & Mathis, G. (2010). *Bacillus licheniformis* prevents necrotic enteritis in broiler chickens. *Avian Diseases*, 54, 931–935.

La Ragione, R.M. & Woodward, M.J. (2003). Competitive exclusion by *Bacillus subtilis* spores of *Salmonella enterica* serotype Enteritidis and *Clostridium perfringens* in young chickens. *Veterinary Microbiology*, 94, 245–256.

Lee, H. & Kim, H.Y. (2011). Lantibiotics, class I bacteriocins from the genus *Bacillus*. *Journal of Microbiology and Biotechnology*, 21, 229–235.

Lee, K., Kyung, D., Lillehoj, H.S., Jang, S.I. & Lee, S.-h. (2015). Immune modulation by *Bacillus subtilis*-based direct-fed microbials in commercial broiler chickens. *Animal Feed Sceince and Technology*, 200, 76–85.

Lee, K., Lee, S.H., Lillehoj, H.S., Li, G., Jang, S.I., Babu, U.S., Park, M.S., Kim, D.K., Lillehoj, E.P., Neumann, A.P., Rehberger, T.G. & Siragusa, G.R. (2010a). Effects of direct-fed microbials on growth performance, gut morphometry, and immune characteristics in broiler chickens. *Poultry Science*, 89, 203–216.

Lee, K., Li, G., Lillehoj, H.S., Lee, S.H., Jang, S.I., Babu, U.S., Lillehoj, E.P., Neumann, A.P. & Siragusa, G.R. (2011). *Bacillus subtilis*-based direct-fed microbials augment macrophage function in broiler chickens. *Research in Veterinary Science*, 91, e87–91.

Lee, K., Lillehoj, H.S., Jang, S.I. & Lee, S.-H. (2014). Effects of salinomycin and *Bacillus subtilis* on growth performance and immune responses in broiler chickens. *Research in Veterinary Science*, 97, 304–308.

Lee, K., Lillehoj, H.S., Jang, S.I., Lee, S.-H., Bautista, D. A. & Siragusa, G. R. (2013). Effect of *Bacillus subtilis*-based direct-fed microbials on immune status in broiler chickens raised on fresh or used litter. *Asian-Australasian Journal of Animal Sciences*, 26, 1592–1597.

Lee, K., Lillehoj, H.S., Jang, S.I., Li, G., Lee, S.-H., Lillehoj, E.P. & Siragusa, G.R. (2010b). Effect of *Bacillus*-based direct-fed microbials on *Eimeria maxima* infection in broiler chickens. *Comparative Immunology, Microbiology and Infectious Diseases*, 33, e105–e110.

Lillehoj, H.S. & Trout, J.M. (1996). Avian gut-associated lymphoid tissues and intestinal immune responses to *Eimeria* parasites. *Clinical Microbiology Reviews*, 9, 349–360.

Lin, Y., Xu, S., Zeng, D., Ni, X., Zhou, M., Zeng, Y., Wang, H., Zhou, Y., Zhu, H., Pan, K. & Li, G. (2017). Disruption in the cecal microbiota of chickens challenged with *Clostridium perfringens* and other factors was alleviated by *Bacillus licheniformis* supplementation. *Plos One*, 12, e0182426.

Liu, W., Hansen, J.N. (1992). Enhancement of the chemical and antimicrobial properties of subtilin by site-directed mutagenesis. *Journal of Biological Chemistry*, 267, 25078–25085.

Lund, B.M. & Peck, M.W. (2015). A possible route for foodborne transmission of *Clostridium difficile? Foodborne Pathogens and Disease*, 12, 177–182.
Mackowiak, P.A. (2013). Recycling Metchnikoff: probiotics, the intestinal microbiome and the quest for long-life. *Frontiers in Public Health*, 1, 52, 1–3.
Maget-Dana, R. & Peypoux, F. (1994). Iturins, a special class of pore-forming lipopeptides: biological and physicochemical properties. *Toxicology*, 87, 151–174.
Maróti, G., Kereszt, A., Kondorosi, É., & Mergaert, P. (2011). Natural roles of antimicrobial peptides in microbes, plants and animals. *Research in Microbiology*, 162, 363–374.
Molnár, A.K., Podmaniczky, B., Kürti, P., Tenk, I., Glávits, R., Virág, G. & Szabó, Z. (2011). Effect of different concentrations of *Bacillus subtilis* on growth performance, carcase quality, gut microflora and immune response of broiler chickens. *British Poultry Science*, 52, 658–665.
Moore, P.R., Evenson, A., Luckey, T.D., McCoy, E., Elvehjem, C.A. & Hart, E.B. (1946). Use of sulfasuxidine, streptothricin, and streptomycin in nutritional studies with the chick. *Journal of Biological Chemistry*, 165, 437–441.
Moura, P., Barata, R., Carvalheiro, F., Gírio, F., Loureiro-Dias, M.C. & Esteves, M.P. (2007). *In vitro* fermentation of xylo-oligosaccharides from corn cobs autohydrolysis by *Bifidobacterium* and *Lactobacillus* strains. *LWT – Food Science and Technology*, 40, 963–972.
Murugesan, G.R., Romero, L.F. & Persia, M.E. (2014). Effects of protease, phytase and a *Bacillus* sp. direct-fed microbial on nutrient and energy digestibility, ileal brush border digestive enzyme activity and cecal short-chain fatty acid concentration in broiler chickens. *Plos One*, 9, 1–7.
Novak, R., Bogovič Matijašić, B., Terčič, D., Červek, M., Gorjanc, G., Holcman, A., Levart, A. & Rogelj, I. (2011). Effects of two probiotic additives containing *Bacillus* spores on carcass characteristics, blood lipids and cecal volatile fatty acids in meat type chickens. *Journal of Animal Physiology and Animal Nutrition*, 95, 424–433.
Oakley, B.B., Lillehoj, H.S., Kogut, M.H., Kim, W.K., Maurer, J.J., Pedroso, A., Lee, M.D., Collett, S.R., Johnson, T.J. & Cox, N.A. (2014). The chicken gastrointestinal microbiome. *FEMS Microbiology Letters*, 360, 100–112.
Opalinski, M., Maiorka, A., Dahlke, F., Cunha, F., Vargas, F.S.C. & Cardozo, E. (2007). On the use of a probiotic (*Bacillus subtilis*-strain DSM 17299) as growth promoter in broiler diets. *Brazilian Journal of Poultry Science*, 9, 99–103.
Pan, D. & Yu, Z. (2014). Intestinal microbiome of poultry and its interaction with host and diet *Gut Microbes*, 5, 108–119.
Parisot, J., Carey, S., Breukink, E., Chan, W.C., Narbad, A. & Bonev, B. (2008). Molecular mechanism of target recognition by subtilin, a class I lanthionine antibiotic. *Antimicrobial Agents and Chemotherapy*, 52, 612–618.
Park, J.H. & Kim, I.H. (2014). Supplemental effect of probiotic *Bacillus subtilis* B2A on productivity, organ weight, intestinal *Salmonella* microflora, and breast meat quality of growing broiler chicks. *Poultry Science*, 93, 2054–2059.
Park, S.S., Lillehoj, H.S., Allen, P.C., Park, D.W., FitzCoy, S., Bautista, D.A. & Lillehoj, E.P. (2008). Immunopathology and cytokine responses in broiler chickens coinfected with *Eimeria maxima* and *Clostridium perfringens* with the use of an animal model of necrotic enteritis. *Avian Diseases*, 52, 14–22.
Pérez-García, A., Romero, D. & de Vicente, A. (2011). Plant protection and growth stimulation by microorganisms: biotechnological applications of bacilli in agriculture. *Current Opinion in Biotechnology*, 22, 187–193.
Persoons, D., Van Hoorebeke, S., Hermans, K., Butaye, P., de Kruif, A., Haesebrouck, F. & Dewulf, J. (2009). Methicillin-resistant *Staphylococcus aureus* in poultry. *Emerging Infectious Diseases*, 15, 452–453.
Platzer, C., Meisel, C., Vogt, K., Platzer, M., Volk, H.D. (1995). Up-regulation of monocytic IL-10 by tumor necrosis factor-alpha and cAMP elevating drugs. *International Immunology*, 7, 517–523.
Privett, B.J., Broadnax, A.D., Bauman, S.J., Riccio, D.A. & Schoenfisch, M.H. (2012). Examination of bacterial resistance to exogenous nitric oxide. *Nitric Oxide*, 26, 169–173.
Quartieri, A., Simone, M., Gozzoli, C., Popovic, M., D'Auria, G., Amaretti, A., Raimondi, S. & Rossi, M. (2016). Comparison of culture-dependent and independent approaches to characterize fecal bifidobacteria and lactobacilli. *Anaerobe*, 38, 130–137.
Rea, M.C., Sit, C.S., Clayton, E., O'Connor, P.M., Whittal, R.M., Zheng, J., Vederas, J.C., Ross, R.P. & Hill, C. (2010). Thuricin CD, a posttranslationally modified bacteriocin with a narrow spectrum of activity against *Clostridium difficile*. *Proceedings of the National Academy of Sciences*, 107, 9352–9357.
Rhee, K.J., Sethupathi, P., Driks, A., Lanning, D.K. & Knight, K.L. (2004). Role of commensal bacteria in development of gut-associated lymphoid tissues and preimmune antibody repertoire. *The Journal of Immunology*, 172, 1118–1124.
Rinttilä, T. & Apajalahti, J. (2013). Intestinal microbiota and metabolites—implications for broiler chicken health and performance. *The Journal of Applied Poultry Research*, 22, 647–658.
Sang, Y. & Blecha, F. (2008). Antimicrobial peptides and bacteriocins: alternatives to traditional antibiotics. *Animal Health Research Reviews*, 9, 227–235.
Santini, C., Baffoni, L., Gaggia, F., Granata, M., Gasbarri, R., Di Gioia, D. & Biavati, B. (2010). Characterization of probiotic strains: an application as feed additives in poultry against *Campylobacter jejuni*. *International Journal of Food Microbiology*, 141, S98–S108.
Searle, L.E.J., Best, A., Nunez, A., Salguero, F.J., Johnson, L., Weyer, U., Dugdale, A.H., Cooley, W.A., Carter, B., Jones, G., Tzortzis, G., Woodward, M.J. & La Ragione, R.M. (2009). A mixture containing galactooligosaccharide, produced by the enzymic activity of *Bifidobacterium bifidum*, reduces *Salmonella enterica* serovar Typhimurium infection in mice. *Journal of Medical Microbiology*, 58, 37–48.
Sen, S., Ingale, S.L., Kim, Y.W., Kim, J.S., Kim, K.H., Lohakare, J.D., Kim, E.K., Kim, H.S., Ryu, M.H., Kwon, I.K. & Chae, B.J. (2012). Effect of supplementation of *Bacillus subtilis* LS 1-2 to broiler diets on growth performance, nutrient retention, caecal microbiology and small intestinal morphology. *Research in Veterinary Science*, 93, 264–268.
Setlow, P. (2006). Spores of *Bacillus subtilis*: their resistance to and killing by radiation, heat and chemicals. *Journal of Applied Microbiology*, 101, 514–525.
Shen, T.-Y., Qin, H.-L., Gao, Z.-G., Fan, X.-B., Hang, X.-M. & Jiang, Y.-Q. (2006). Influences of enteral nutrition combined with probiotics on gut microflora and barrier function of rats with abdominal infection. *World Journal of Gastroenterology*, 12, 4352–4358.
Shirley, M.W. & Lillehoj, H.S. (2012). The long view: a selective review of 40 years of coccidiosis research. *Avian Pathology*, 41, 111–121.
Shivaramaiah, S., Pumford, N.R., Morgan, M.J., Wolfenden, R.E., Wolfenden, A.D., Torres-Rodríguez, A., Hargis, B.M.

& Téllez, G. (2011). Evaluation of *Bacillus* species as potential candidates for direct-fed microbials in commercial poultry. *Poultry Science*, 90, 1574–1580.

Silva, P.T., Fries, L.L.M., Menezes, C.R., Silva, C.B., Soriani, H.H., Bastos, J.O., Motta, M.H. & Ribeiro, R.F. (2015). Microencapsulation of probiotics by spray drying: evaluation of survival in simulated gastrointestinal conditions and availability under different storage temperatures. *Ciência Rural*, 45, 1342–1347.

Starr, M.P. & Reynolds, D.M. (1951). Streptomycin resistance of coliform bacteria from turkeys fed streptomycin. *American Journal of Public Health and the Nations Health*, 41, 1375–1380.

Stein, T. (2005). Bacillus subtilis antibiotics: structures, syntheses and specific functions. *Molecular Microbiology*, 56, 845–857.

Sumi, C., Yang, B., Yeo, I.-C. & Hahm, Y. (2015). Antimicrobial peptides of the genus *Bacillus*: a new era for antibiotics. *Canadian Journal of Microbiology*, 61, 93–103.

Suva, M., Sureja, V. & Kheni, D. (2016). Novel insight on probiotic *Bacillus subtilis*: mechanism of action and clinical applications. *Journal of Current Research in Scientific Medicine*, 2, 65–72.

Takimoto, T., Sato, K., Akiba, Y. & Takahashi, K. (2008). Role of chicken TL1A on inflammatory responses and partial characterization of its receptor. *The Journal of Immunology*, 180, 8327–8332.

Teo, a.Y. & Tan, H.M. (2007). Evaluation of the performance and intestinal gut microflora of broilers fed on corn-soy diets supplemented with *Bacillus subtilis* PB6 (CloSTAT). *Journal of Applied Poultry Research*, 16, 296–303.

Torok, V.A., Hughes, R.J., Mikkelsen, L.L., Perez-Maldonado, R., Balding, K., MacAlpine, R., Percy, N.J. & Ophel-Keller, K. (2011). Identification and characterization of potential performance-related gut microbiotas in broiler chickens across various feeding trials. *Applied and Environmental Microbiology*, 77, 5868–5878.

US FDA. (2013). *New animal drugs and new animal drug combination products administered in or on medicated feed or drinking water of food-producing animals: recommendations for drug sponsors for voluntarily aligning product use conditions with GFI #209*. Department of Health and Human Services.

US FDA. (2017). *FDA's strategy on antimicrobial resistance – questions and answers*.

Van Immerseel, F., De Buck, J., Pasmans, F., Velge, P., Bottreau, E., Fievez, V., Haesebrouck, F. & Ducatelle, R. (2003). Invasion of *Salmonella* Enteritidis in avian intestinal epithelial cells in vitro is influenced by short-chain fatty acids. *International Journal of Food Microbiology*, 85, 237–248.

Vila, B., Esteve-Garcia, E. & Brufau, J. (2010). Probiotic microorganisms: 100 years of innovation and efficacy; modes of action. *World's Poultry Science Journal*, 66, 369–380.

Vollenbroich, D., Pauli, G., Ozel, M. & Vater, J. (1997). Antimycoplasma properties and application in cell culture of surfactin, a lipopeptide antibiotic from *Bacillus subtilis*. *Applied and Environmental Microbiology*, 63, 44–49.

Wade, B. & Keyburn, A. (2015). The true cost of necrotic enteritis. *Poultry World*.

Waite, D.W. & Taylor, M.W. (2014). Characterizing the avian gut microbiota: membership, driving influences, and potential function. *Frontiers in Microbiology*, 5, 1–12.

WHO. (2017). *Antibiotic resistance*.

Williams, C.H., Witherly, S.A. & Buddington, R.K. (1994). Influence of dietary neosugar on selected bacterial groups of the human faecal microbiota. *Microbial Ecology in Health and Disease*, 7, 91–97.

Wine, E., Gareau, M.G., Johnson-Henry, K. & Sherman, P.M. (2009). Strain-specific probiotic (*Lactobacillus helveticus*) inhibition of *Campylobacter jejuni* invasion of human intestinal epithelial cells. *FEMS Microbiology Letter*, 300, 146–156.

Xu, X., Huang, Q., Mao, Y., Cui, Z., Li, Y., Huang, Y., Rajput, I.R., Yu, D. & Li, W. (2012). Immunomodulatory effects of *Bacillus subtilis* (natto) B4 spores on murine macrophages. *Microbiology and Immunology*, 56, 817–824.

Yegani, M. & Korver, D.R. (2008). Factors affecting intestinal health in poultry. *Poultry Science*, 87, 2052–2063.

Zhao, P.Y. & Kim, I.H. (2015). Effect of direct-fed microbial on growth performance, nutrient digestibility, fecal noxious gas emission, fecal microbial flora and diarrhea score in weanling pigs. *Animal Feed Science and Technology*, 200, 86–92.

Zhao, X. & Kuipers, O. (2016). Identification and classification of known and putative antimicrobial compounds produced by a wide variety of Bacillales species. *BMC Genomics*, 17, 1–18.