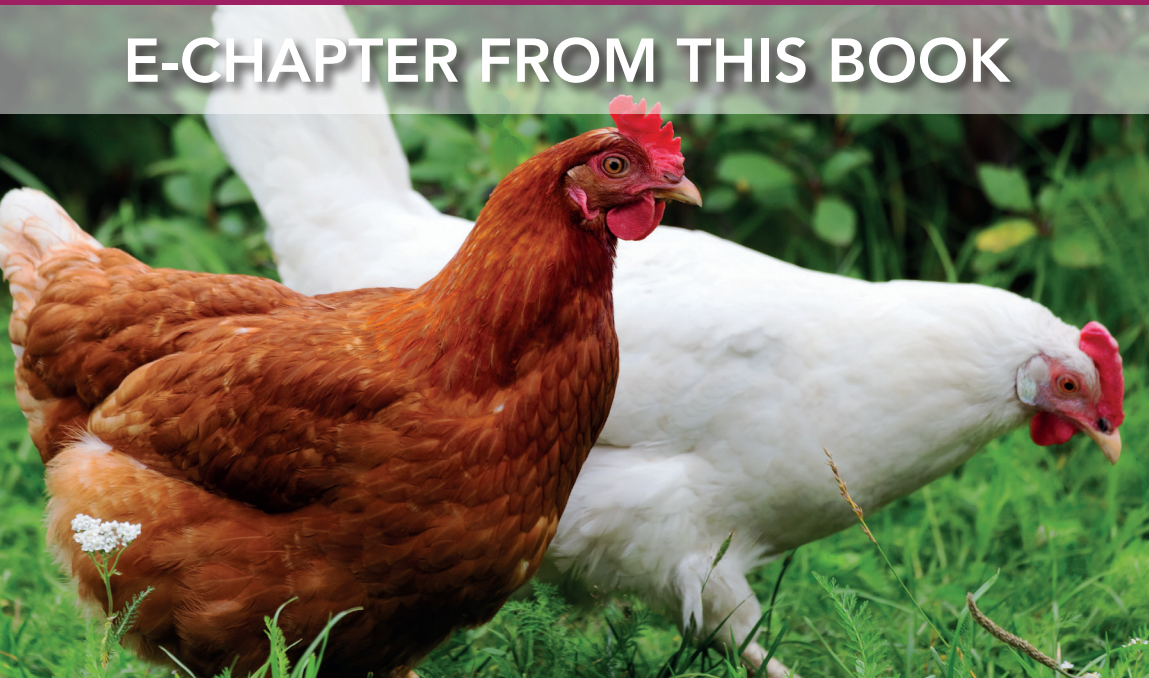


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Improving gut health in poultry

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E-CHAPTER FROM THIS BOOK



The role of probiotics in optimizing gut function in poultry

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1 Introduction

A common human propensity is to regard all microorganisms as 'harmful', in particular, equating bacteria to pathogenic germs. Nothing could be further from the truth. The number of beneficial bacterial species far exceeds the number of pathogenic species and many of the known bacteria are in fact useful or even indispensable for the continued existence of life on Earth. Prokaryotic microorganisms are widespread in all environments on Earth, establishing diverse interactions with many eukaryotic taxa (Bronstein et al., 2006). The cooperative interactions between species (mutualism) have had a central role in the generation and maintenance of life on earth (Kikuchi et al., 2009). Prokaryotes and eukaryotes are involved in diverse forms of mutualism (Saridakis and Bourtzis, 2010). Adaptive diversification is a process intrinsically tied to species interactions (Xie et al., 2010). Yet, the influence of most types of interspecific interactions on adaptive evolutionary diversification remains poorly understood. The endosymbiotic theory states that several key organelles of eukaryotes originated as symbioses between separate single-celled organisms (Degli Esposti et al., 2014). According to this theory, mitochondria and plastids (e.g. chloroplasts), and possibly other organelles, represent formerly free-living bacteria that were taken inside another cell as an endosymbiont, around

1.5 billion years ago (Gibson and Hunter, 2010). Molecular and biochemical evidence suggests that the mitochondrion developed from *Proteobacteria* and the chloroplast from *Cyanobacteria* (Mackiewicz et al., 2012). Numerous facultative heritable endosymbionts are reproductive manipulators (Saridaki and Bourtzis, 2010). Nevertheless, many do not manipulate reproduction, so they are expected to confer fitness benefits to their hosts, as has been shown in several studies that report defence against natural enemies, tolerance to environmental stress and increased fecundity (Xie et al., 2010). One example of such a beneficial group of microorganisms is the incredibly complex and abundant ensemble of microbes that harbour in the gastrointestinal tract (GIT) of metazoans. The GIT is more densely populated with microorganisms than any other organ and is an interface where the microbiota may have a pronounced impact on animal biology (Maslowski and Mackay, 2011). More than 50 genera and at least 500-1000 different species are distributed along the length of the GIT in most metazoans (Fraune and Bosch, 2010). The bacterial population of the human caecum and colon is numerically $\sim 10^{13}$ cfu/g (Neish, 2009), comprising about 40-55% of solid stool matter and weights ~ 1 kg (Blaser, 2006). Presumably, the assembly of the gut microflora is regulated by the elaborate and combinatorial host-microbial and microbial-microbial interactions predicated on principles refined over the course of evolution (Xu and Gordon, 2003). Comparison of rodents raised without exposure to any microorganisms to those colonized with an assembly of microbiota revealed a wide range of host functions affected by indigenous microbial communities. For example, the microbiota directs the assembly of the gut-associated lymphoid tissue (Martin et al., 2010), helps educate the immune system (McFall-Ngai, 2007), affects the integrity of the intestinal mucosal barrier (Duerkop et al., 2009), modulates proliferation and differentiation of its epithelial lineages (Moran, 2007), regulates angiogenesis (Sekirov et al., 2010), modifies the activity of the enteric nervous system (Tlaskalová-Hogenová et al., 2011) and plays a key role in extracting and processing nutrients consumed in the diet (Walter et al., 2011). The microflora can metabolize proteins and protein degradation products, sulphur-containing compounds, and endogenous and exogenous glycoproteins (Qiu et al., 2012). Some organisms grow on intermediate products of fermentation such as H_2 , lactate, succinate, formate and ethanol, converting these compounds to end products including short-chain fatty acids, a process which has direct benefits on digestive physiology (Dass et al., 2007). In particular with diet composition, one must conclude that metazoans literally 'become what we eat'. So, any disorders in this fragile microbial ecosystem (dysbacteriosis) may predispose the host to a whole range of chronic diseases and infections, thereby affecting the production of food animals. On the other hand, over millions of years, animals have developed various means for supporting complex and dynamic consortia of microorganisms during their life cycle (Dale and Moran, 2006). As with most

complex ecosystems, it appears that the majority of these microbial species cannot be cultured when removed from the niches in their host animals (Moran, 2007). The fragile composition of the gut microflora can be affected by various factors such as age, diet, environment, stress and medication (Bäckhed, 2011). Furthermore, many factors are involved in shaping gut microflora from infancy such as mode of delivery, type of infant feeding, hospitalization, prematurity, antibiotic use and dietary nutrient composition (Choct, 2009). Dietary ingredients have a profound effect on the composition of the gut microflora, which in turn, regulates the physiology of all animals (Fraune and Bosch, 2010). As such, nutritional components of the diet are of critical importance not only for meeting the nutrient requirements of the host but also shaping the profile of the microbiome, which in turn, will determine the balance between health and disease. As an example, several studies have shown the effect of diet composition on promoting insulin sensitivity, diabetes, cancer and other metabolic disorders (Cani and Delzenne, 2009). Some researchers believe that the alarming increase in autoimmune diseases in the West may be due to a disruption in the ancient relationship between our bodies and a healthy microbiome (Salzman, 2011). Thus, colonization of microbiomes in metazoans begins at birth and is followed by progressive assembly of a complex and dynamic microbial society maintaining a perfect harmony or homeostasis (Di Mauro et al., 2013). However, little is known about how they influence the normal development and physiology of hosts. A transcendent view of vertebrate biology, therefore, requires an understanding of the contributions of these indigenous microbial communities to host development and adult physiology.

2 Experiences of probiotics in poultry

The alarming spread of antibiotic resistance genes has created public concerns leading to new laws that restrict the use of antibiotics as growth promoters in domestic and meat-producing animals in several countries around the world. Such policies have obligated scientists to evaluate different alternatives to antibiotics. Furthermore, the satisfaction of consumer preferences has also become an important strategy for countries looking to export their animal products (Parker, 1990; Dahiya et al., 2006; You and Silbergeld, 2014). Hence, the use of probiotics as alternative tools to antibiotic growth promoters has been increasing and their efficacy has been demonstrated by many investigators around the world (Hammes and Hertel, 2002; Tellez et al., 2013). Nevertheless, some of the mechanisms by which the probiotics improve health and performance parameters in domestic animals are not well understood (Musso et al., 2010). Even though the use of probiotics is not new, it is only in the last two decades that scientists have demonstrated the potential of probiotics

to improve health and performance parameters of meat-producing animals (Isolauri et al., 2002; Salminen and Isolauri, 2006; Dominguez-Bello and Blaser, 2008). Studies have shown how probiotic bacteria regulate production of pro-inflammatory and anti-inflammatory cytokines (Borchers et al., 2009; Lyte, 2011), exert antioxidant properties (Farnell et al., 2006; Tao et al., 2006; Zareie et al., 2006; Segawa et al., 2011; Howarth and Wang, 2013) and enhance barrier integrity (Yu et al., 2012). In addition, several investigators have demonstrated the benefits of probiotics on innate immunity (Alvarez-Olmos and Oberhelman, 2001; Vanderpool et al., 2008; Molinaro et al., 2012) as well as on humoral immunity (Arvola et al., 1999; Haghghi et al., 2006; Howarth and Wang, 2013).

Our laboratory has worked to identify probiotic candidates for use in poultry such as a defined lactic acid bacteria (LAB)-based probiotic that was confirmed to increase the resistance of poultry to *Salmonella* spp. infections (Farnell et al., 2006; Higgins et al., 2007; Menconi et al., 2011; Tellez et al., 2012; Biloni et al., 2013) and reduce transit diarrhoea in turkeys (Higgins et al., 2005). In other commercial trials, administration of this probiotic mixture to turkeys and chickens increased performance and reduced costs of production (Vicente et al., 2007, 2008; Torres-Rodriguez et al., 2007). More recently, microarray analysis of caeca in chickens infected with *Salmonella* serovar Enteritidis (SE) and then treated with this probiotic showed significant differences in the expression of some inflammatory genes associated with the NF κ B complex (Higgins et al., 2011).

3 Probiotics and inflammation

Salmonellosis remains one of the most comprehensive food-borne diseases that can be transmitted to humans through animal and plant products (Hernández-Reyes and Schikora, 2013; Zheng et al., 2013; Schleker et al., 2015). Chickens infected with SE show a significant increase of heterophils-to-lymphocyte ratio (Al-Murrani et al., 2002; Shini et al., 2008). In a recent study, we have also observed a marked heterophilia and lymphopenia in chickens challenged with SE. However, these haematological changes were prevented in chickens that received a LAB probiotic 1 h after SE challenge. Furthermore, we observed that the reduction in intestinal colonization by SE was associated with a reduction in intestinal permeability of fluorescein isothiocyanate-dextran (FITC-d) in chickens (Prado-Rebolledo et al., 2017). Due to its molecular size (4kDa), FITC-d is a molecule which does not cross the intact GIT barrier. However, when conditions disturb tight junctions (TJ) between epithelial cells, FITC-d can enter the systemic circulation as demonstrated by an increase in paracellular permeability after oral administration (Yan et al., 2009; Kuttappan et al., 2015; Vicuña et al., 2015a,b). TJ act as intercellular cement between epithelial cells and regulate the permeability and dissemination of microorganisms and antigens (Ulluwishewa et al., 2011). Hence, any damage to

this fragile epithelium may lead to chronic intestinal and systemic inflammation (Steed et al., 2010). Uptake and distribution within the host of salmonellae infections are associated with disruption of the TJ complex, loss of barrier function, bacterial translocation and initiation of polymorphonuclear (PMN) cells migration across the intestinal barrier (Köhler et al., 2007). Therefore, appropriate gut barrier function is indispensable to maintain optimal health (Groschwitz and Hogan, 2009; Sharma et al., 2010; Jeon et al., 2013; Pastorelli et al., 2013). Bacterial endotoxin has been shown to activate aldose reductase (AR), a member of the aldo-keto reductase (AKR) superfamily and nuclear factor (NF- κ B), a member of the NF- κ B superfamily and nuclear factor (NF- κ B) results in the expression of several inflammatory cytokines (Ozinsky et al., 2000; Overman et al., 2012). It is well known that oxidative stress-induced inflammation is a major contributor to several diseases. AR catalytic activity plays a crucial role in some inflammatory diseases associated with disruption of TJ between epithelial cells (Srivastava et al., 2011; Yadav et al., 2011; Pastel et al., 2012). Interestingly, microarray analysis with our LAB probiotic in broiler chickens challenged with SE showed a significant reduction in intestinal gene expression associated with the NF- κ B complex and AR (Higgins et al., 2011). Hence, our studies suggest that the probiotic preserved the integrity of the intestinal epithelial cells (IEC), which represent the second layer of innate defence mechanisms of the GIT (Sakamoto et al., 2000; Johansson et al., 2010; Kim and Ho, 2010). These results are in agreement with numerous studies demonstrating that probiotics prevent *Salmonella* translocation, suppress the oxidant-induced intestinal permeability and improve intestinal barrier function (Madsen et al., 2001; Ewaschuk et al., 2007; Mennigen and Bruewer, 2009; Segawa et al., 2011; Hsieh et al., 2015).

Researchers have investigated the effects of probiotics on gene regulation associated with immune modulation, enteropathogens control and homeostasis, using *in vitro* and *in vivo* disease challenge models (Tellez et al., 1993; Timbermont et al., 2010; Kiarie et al., 2013; Sherryll Lynn et al., 2013). Metchnikoff founded the research field of beneficial microorganisms for animals and humans (probiotics), aimed at modulating the intestinal microflora (Metchnikoff and Metchnikoff, 1907). At the moment, new molecular techniques are helping us to understand how the anti-inflammatory, cell integrity and antioxidant properties of probiotics can improve gut and barrier integrity. Given the recent international legislation and domestic consumer pressure to withdraw growth-promoting antibiotics and limit antibiotics available for treatment of bacterial infections, probiotics and direct-fed microbials (DFM) can offer clear alternative options.

4 Risks of overuse of antibiotics

Fluoroquinolones are the third generation of quinolone progress. Nalidixic acid and piperidic acid are examples of the first generation and currently have

limited activity against Gram-negative bacteria. Fluorinated 4-quinolones were introduced to the market in the 1980s and were the top of the line antibiotics, offering a broad spectrum of activity and high efficacy in a wide range of infections both orally and parenterally (Piddock et al., 1990). Nevertheless, history has demonstrated that the extensive use of new antibiotics is eventually shadowed by the appearance of resistance to those chemicals which has become a major global problem. This was demonstrated by the higher incidence of salmonellae and *Campylobacter* infections worldwide, and several reports of fluoroquinolone resistance in clinical isolates for these and other enteric pathogens (Murray, 1986; Uwaydah et al., 1991; Griggs et al., 1994). Hence, the World Health Organization (WHO) published a list of antibiotics that should be reserved for human use only (Couper, 1997), and fluoroquinolones were among them, due to the alarming evidence of quinolone-resistant zoonotic pathogens. Soon after the publication of the WHO report, several countries banned the use of fluoroquinolones in animal production (Rodrigue et al., 1990; Randall et al., 2006). With growing consumer and scientific pressure, the European Union went one step further, creating new legislation banning the use of all antibiotics as growth promoters as of January 2006 (Castanon, 2007). However, in many countries, the indiscriminate use and misuse of antibiotics including fluoroquinolones are still a sad reality. Especially in countries where there is no legislation regulating the use of fluoroquinolones in animal agriculture and where there is an abundance of generic fluoroquinolones at a low cost. Typical management practices in those countries are to treat or dose healthy neonatal chickens and turkey poults with five times the recommended dose of enrofloxacin for five consecutive days in the drinking water (Tellez, 2015, pers. comm.). Interestingly, in those countries, the incidence of *Salmonella* spp. and *Campylobacter* spp. infection rates in both humans and agriculture are also high (Piddock et al., 1990; Acar and Goldstein, 1997; Piddock, 2002; Sierra-Arguello et al., 2016). In recent years, several investigators have shown that the use of certain antibiotics increases enteric colonization of antibiotic-resistant strains of selective enteric pathogens in domestic animals (Smith and Tucker, 1975; Manning et al., 1992), because some of these selective pathogens are extremely resistant to many antibiotics and are capable of rapidly developing resistance when exposed (Piddock and Wise, 1989; Acar and Goldstein, 1997). Antibiotic prophylaxis or treatment has been reported to actually increase the occurrence and severity of these infections in commercial poultry (Seuna and Nurmi, 1979; Niewold, 2007). In addition to the lack of effect of these antibiotics in resistant enteropathogens, some researchers have shown that antibiotics can actually cause disruption in the microbiome (Bartlett, 2002), accompanied with reduction of short-chain fatty acids (Van Der Wielen et al., 2000) and increased luminal pH in the distal GIT (Corrier et al., 1990). To evaluate the common management practice of using five times the recommended dose of

enrofloxacin, we recently published a study where neonatal chickens and turkey poult received different doses of this fluoroquinolone for five consecutive days after placement and looking at their susceptibility to salmonellae infections. It was remarkable to observe how this practice makes chickens and turkeys more susceptible to enteric infections (Morales-Barrera et al., 2016). In our study, microbiome analysis of the caecal content revealed that turkey poults treated with enrofloxacin had a lower proportion of *Firmicutes* and *Bacteroidetes* suggesting that the broad spectrum of enrofloxacin had a profound impact upon the microbiome. Interestingly, these poults had the highest proportion of *Proteobacteria*. Such a high dose of antibiotic also had a significant increase in Gammaproteobacteria. Changes in the proportion of phylum and class were associated with higher *Salmonella* Heidelberg intestinal colonization since *Salmonella* belongs to phylum *Proteobacteria*, class Gammaproteobacteria. Furthermore, poults treated with enrofloxacin had lower proportions of clostridia and bacilli when compared with control or probiotic experimental groups. In contrast, poults that received the probiotic had the highest proportion of *Firmicutes* and *Bacteroidetes*, but the lowest amount of *Proteobacteria*. These birds also showed a significant reduction in Gammaproteobacteria, and a higher proportion of clostridia and bacilli. These results suggest that five times the recommended dose of enrofloxacin, a broad-spectrum antibiotic, can have a negative effect on the microbiome that may be responsible for an enhancement of salmonellae colonization, which has been previously demonstrated with other selective enteropathogens (Uwaydah et al., 1991; Manning et al., 1992). Acquisition of resistance to fluoroquinolones has been reported to be a multifaceted process, which includes spontaneous point mutations that result in amino acid substitutions within the topoisomerase subunits GyrA, GyrB, ParC or ParE, as well as reduced expression of outer membrane porins, overexpression of multidrug efflux pumps and/or plasmid-mediated quinolone resistance (Angulo et al., 2000; Engberg et al., 2001). It is remarkable to contemplate that the alarming incidence of certain selective enteric pathogens is associated with the indiscriminate use of some antibiotics in animal agriculture in some countries (Hofer and Reis, 1994; Irino et al., 1996; Borsoi et al., 2009; De Moura et al., 2013). Since poultry products have been identified as important reservoirs of human infections, this is a growing public health concern. Given that fluoroquinolones and other antibiotics are overused in animal production, any effort to diminish the risk of resistance is crucial. The previous investigations involving antibiotics and other enteropathogens suggest that prophylactic utilization of some antibiotics in poultry increases the susceptibility to salmonellae colonization and organ invasion. Therefore, antibiotics should be limited to infections of specific bacteria with known antibiotic sensitivity. In addition, our findings also confirm previous studies suggesting that the use of alternatives such as probiotics can be an effective tool in controlling salmonellae infections.

4.1 *In ovo* strategies

Under commercial conditions, millions of chickens and turkeys hatch in a hostile environment and are exposed for several hours to heat stress and potentially pathogenic bacteria in the hatcheries. Increased stress along with the potential abundance of pathogens in the hatching cabinet leads to ideal conditions for pathogen colonization. It is generally accepted that the natural route of transmission of zoonotic pathogens, such as *Salmonella*, is faecal-oral (White et al., 1997; Galanis et al., 2006). However, published studies have also suggested that airborne transmission of *Salmonella* in poultry is possible (Wathes et al., 1988). Understanding the anatomical and immunological defences of the avian respiratory tract helps to clarify this issue. The architecture of the avian respiratory tract is an important component for susceptibility and resistance against infectious agents. In day-old chickens and turkeys, no or very few infiltrating lymphocytes are seen in the primary bronchi region (Fagerland and Arp, 1990; Śmiątek et al., 2011), and it is not until 3–4 weeks of age that the lymphoid nodules are developed at these locations (Fagerland and Arp, 1993; Drolet et al., 2010). During the following week, the number of IgG-, IgA- or IgM-producing cells continues to increase; however, the bronchial-associated lymphoid tissue (BALT) is not mature until chickens are 6–8 weeks old (Bienenstock, 1980; Bienenstock and McDermott, 2005; De Geus et al., 2012). Hence, commercial neonatal poultry are extremely susceptible to airborne pathogens, regardless of whether or not they are respiratory or enteric bacteria (Arshad et al., 1998). In support of these findings, our laboratory has recently shown that transmission by the faecal-respiratory route is a viable portal of entry for *Salmonella* (Kallapura et al., 2014a,b,c). This mode of infection could explain some clinical expression of relatively low-dose infectivity under field conditions in relation to the high oral dose challenge that is typically required for infection through the oral route in laboratory studies. This also supports previous studies demonstrating the fan-driven spread of *Salmonella* within the hatching cabinet and hatchery incubators (Hashemzadeh et al., 2010).

Recently, we evaluated the *in ovo* application as a practical and reliable way of delivering a probiotic mixed with the diluent of the Marek's disease (MD) vaccine (Teague et al., 2017). Although we previously reported the benefits of spray application of our LAB probiotic in the hatcheries (Wolfenden et al., 2007), this was the first report of *in ovo* application of this defined probiotic, mixed with MD vaccine. Interestingly, embryos, which received the probiotic before hatch, had a significant reduction in SE infection, lactose-positive Gram-negative bacteria without affecting the hatchability when compared with saline-treated chickens (Teague et al., 2017). Several studies conducted in our laboratory suggest that this particular probiotic is able to control salmonellae infections in poultry in both laboratory and commercial conditions (Farnell et al., 2006; Higgins et al., 2007; Vicente et al., 2007; Biloni et al., 2013;

Menconi et al., 2013). This current study further validated the probiotics efficacy via *in ovo* administration by reducing the recovery of SE when chickens were challenged on the day of hatch and cultured 24 h later. These results are in agreement with the work of De Oliveira et al. (2014) who demonstrated that *in ovo* colonization with probiotic could reduce *Salmonella* and other intestinal bacterial infections in poultry. In our study, we also observed that the higher body weight in the probiotic-treated group was due to the increase in villus height, leading to larger villus surface area, thus resulting in better nutrient absorption. These results are meaningful in context with the rapid early growth of broiler chickens. A newly hatched modern broiler chicken increases its body weight by 25% overnight and by 5000% in 5 weeks (Choct, 2009). Similarly, it is also important to consider the productive life of broiler chickens. The full genetic potential of modern chickens starts at conception and the first 21 days of embryo development. During this period, variables such as temperature and oxygen are important and any problem related to them could cause a big impact later in life. Hence, the 21 days of embryogenesis plus the first 7 days of the life of a chicken could potentially represent between 50% and 74% of the life of a commercial broiler chicken, depending on the time they are slaughtered (56 or 77 days) (Cherian, 2011). Therefore, earlier administration of probiotics to embryos can have a profound impact on the growth and overall health of the birds.

5 The use of direct-fed microbials

The use of selected strains from different beneficial microorganisms from the genus *Bacillus* and *Lactobacillus* have shown to be a suitable option for the poultry industry (Tellez et al., 2012). *Bacillus* spp. are a Gram-positive, facultative aerobe, endospore-forming, rod-shaped bacterium normally found in soil and water sources, as well as in the GIT of animals and humans (Hong et al., 2009). Its multiple flagella allow it to move quickly in liquids. *Bacillus* spp. are the most investigated Gram-positive bacteria and a model organism to study bacterial chromosome replication and cell differentiation, and together with other beneficial microbes have been extensively used as a source of industrial enzymes and antibiotics by biotechnology companies (Kunst et al., 1997). When environmental conditions are not favourable for growth and replication of bacteria from the genus *Bacillus*, dramatic metabolic changes occur, such as the induction of chemotaxis, cannibalism, production of macromolecular hydrolases (proteases and carbohydrases) as well as the formation of endospores (González-Pastor et al., 2003; Hong et al., 2005; López et al., 2009). Due to the capacity of bacterial spores to resist harsh environmental conditions and long storage periods, endospores from selected *Bacillus* strains have been used as reliable DFM in animal production (Tellez et al., 2013). Additionally,

Bacillus-DFM have previously been shown to prevent GIT disorders and impart numerous nutritional benefits for animals and humans (Hong et al., 2005; Duc et al., 2004; Cartman et al., 2007; Sen et al., 2012). Recent studies published by our laboratory have shown that approximately 90% of *Bacillus subtilis* spores germinate within 60 min in the presence of feed *in vitro* and *in vivo* in different segments of the GIT (Latorre et al., 2014a). After spore germination into vegetative cells, *Bacillus* spp. become metabolically active to produce chemical compounds that are beneficial to the host and the intestinal microflora (Jadamus et al., 2001; Leser et al., 2008).

In most of the United States and in other countries, including Brazil, broiler feed is based primarily on corn and soybean meal, which supplies the majority of energy and protein in the diet. Utilization of the nutrients contained in corn by broilers is generally considered to be high. Nevertheless, at times it is difficult to formulate least-cost diets using corn and unconventional grains with variable concentrations of anti-nutritional factors are used. Rye (*Secale cereale*) is a cereal member of the wheat tribe (*Triticeae*) and has been reported to contain 152 g of total non-starch polysaccharides (NSP) per kilogram of dry matter (Campbell et al., 1983). When chickens are fed alternative cereal grains such as rye that are high in soluble NSP, high digesta viscosity, poor nutrient digestibility and reduced bone mineralization have been reported before, resulting in decreased growth performance and reduced litter quality conditions caused by sticky droppings (Fengler and Marquardt, 1988). However, different studies have shown that the inclusion of carbohydrases such as xylanase in rye-based diets significantly improved all these negative factors reducing the impact of the anti-nutritional components present in the rye grain (Bedford and Classen, 1993; Silva and Smithard, 2002). Previously, we have evaluated the inclusion of selected *Bacillus*-DFM candidates that produce a different set of extracellular enzymes using different poultry diets *in vitro* (rye, wheat, barley and oat-based diets), resulting in a significant reduction in both digesta viscosity and *Clostridium perfringens* proliferation between control diets and *Bacillus*-DFM supplemented diets (Latorre et al., 2015). Rye has an elevated concentration of highly branched arabinoxylans in comparison to other cereals like wheat or corn (Bach Knudsen, 1997). The high concentration of soluble NSP in rye-based diets also have an impact on the intestinal bacterial population, probably as a consequence of the increased digesta viscosity and prolonged feed passage time (Choct et al., 2009; Bedford and Schulze, 1998). Furthermore, utilization of rye in poultry diets has also been related to malabsorption of lipids, deterioration of bone mineralization and reduced leg soundness (Kiarie et al., 2013). This negative effect on bone quality could also be related to an elevated digesta viscosity, therefore, enhancing the deconjugation of bile acids by the overgrowth intestinal microflora, resulting in a reduction of micelle formation, affecting fat solubilization and absorption of fat-soluble vitamins

and minerals (Esteve-Garcia et al., 1997). Since monogastric animals do not have endogenous enzymes capable of hydrolysing the β -linkages present in soluble NSP, exogenous carbohydrases (xylanase, β -glucanase, β -mannanase, α -galactosidase and pectinase) have been used in poultry diets as feed additives in an attempt to reduce the adverse impact of these anti-nutritional factors (Bedford and Schulze, 1998). It has been well documented that inclusion of xylanase in rye-based diets significantly improved viscosity of digesta supernatant, accelerated feed passage time through the GIT and enhanced digestibility of dietary protein and fat sources resulting in an improvement in growth performance (Langhout et al., 1997; Lázaro et al., 2004). The results of the *Bacillus*-DFM study from our laboratory support previous findings in turkey poult fed with rye-based diets (Latorre et al., 2014b). Further studies confirmed that our multiple enzyme-producing *Bacillus*-based DFM improved growth performance, digesta viscosity, bacterial translocation, microbiota composition and bone mineralization in broiler chickens fed with a rye-based diet (Latorre et al., 2015). In our studies with broilers and turkeys, the increase in digesta viscosity observed in the control group was also associated with elevated bacterial translocation to the liver and overgrowth of Gram-negative and anaerobic bacteria in the duodenal section when compared with animals that consumed the *Bacillus*-DFM diet. These differences could be due to fewer substrates available for bacterial growth, generating lower intestinal inflammation and translocation of bacteria when the intestinal viscosity was reduced by the inclusion of the DFM candidate, suggesting more absorption of nutrients by the intestinal brush border of supplemented groups. It has been previously reported that alterations in gut permeability are connected with bacterial translocation in the portal and/or systemic circulation during several types of 'leaky gut' syndromes leading to bacterial septicaemia (Ilan, 2012; Seki and Schnabl, 2012). Furthermore, the significant improvements in performance observed in animals consuming the *Bacillus*-DFM supplemented diet when compared to the unsupplemented control group suggests that the production of enzymes from the combined *Bacillus* spp. strains used as DFM could increase the absorption of nutrients promoting growth performance and a more efficient feed conversion ratio in addition to enhancing the physical and bacteriological conditions of the intestinal content. We also observed that the significant reduction in bone strength and mineralization generated by consumption of rye-based diets confirmed previous research from different authors that have shown that the inclusion of rye in poultry diets is associated with malabsorption of minerals and fat-soluble vitamins (Campbell et al., 1983; Wideman and Prisby, 2012). In our studies, the reduction of digesta viscosity together with the production of phytase by the *Bacillus*-DFM candidate could enhance the absorption of nutrients including minerals, hence improving bone strength and bone mineralization (Latorre et al., 2014a, 2015; Tellez et al.,

2014b). Our studies have shown that chickens and turkeys fed with rye-based diets have an increase in digesta viscosity and bacterial translocation associated with overgrowth of gut microflora, low performance and decreased bone mineralization. However, these adverse effects caused by the utilization of rye in poultry diets can be minimized by the inclusion of a selected *Bacillus*-DFM candidate, thereby enhancing intestinal integrity and absorption of nutrients resulting in an improvement of production performance.

6 Conclusion

Bacterial antimicrobial resistance in both the medical and agricultural fields has become a serious problem worldwide. During the last 15 years, our laboratories have worked towards the identification of probiotic candidates for poultry which can actually displace *Salmonella* and other enteric pathogens which have colonized the GIT of chickens and turkeys, indicating that selection of therapeutically efficacious probiotic cultures with marked performance benefits in poultry is possible, and that defined cultures can sometimes provide an attractive alternative to conventional antimicrobial therapy. Our studies have been focused on specific pathogen reduction, performance under commercial conditions and effects on both idiopathic and defined enteritis. We have also confirmed that selected heat-resistant spore-forming *Bacillus* species can markedly reduce *Salmonella* and *Clostridium* when administered in very high numbers, and we have developed a novel and simple technique for obtaining cultured *Bacillus* spore counts, providing a cost-effective, feed-stable inclusion in commercial poultry diets. In order to select even more effective isolates, we are still currently focused on the mechanistic action of the *Lactobacillus* probiotic previously developed as well as new *Bacillus* candidates. Current indications are that the mechanism of action involves rapid activation of innate host immune mechanisms, providing an exciting possibility for identification of vastly superior and more potent probiotics. In this chapter, we summarized the safety and efficacy of individual monocultures for prophylactic and/or therapeutic efficacy against *Salmonella* infections under both laboratory and field conditions as well as the development of a novel, cost-effective, feed-stable, direct-fed microbials (DFM) with potential for widespread utilization and improved production, delivery and clinical efficacy for animal use.

7 Where to look for further information

During the last decade, the increasing interest in renewable energy sources changed the distribution of corn utilization from human and animal consumption to biofuel production, leading to a continuous rise in feed costs of livestock diets. Therefore, alternative feed ingredients such as distillers dried

grains with solubles (DDGS), as well as cereals like wheat, barley and sorghum have become part of the feed matrix to maintain or reduce production costs. However, these raw materials often contain a higher concentration of anti-nutritional factors in comparison to corn, including non-starch polysaccharides which increase digesta viscosity and reduce nutrient absorption in monogastric animals. As a result, the addition of exogenous enzymes in poultry feed has steadily increased to maximize nutrient utilization and maintain performance parameters with diets containing less digestible ingredients. On the other hand, the poultry industry is also facing social concerns regarding the use of antibiotic growth promoters and the development of antibiotic-resistant microorganisms. One alternative among others is the *in ovo* utilization of probiotics candidates based on enzyme production profiles to improve nutrient absorption and intestinal integrity, as well as to maintain a healthy microflora balance in poultry-consuming commercial and alternative diets. Currently, our laboratories are also working on evaluating and selecting different *Bacillus* spp. strains for *in ovo* delivery.

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