TITLE PAGE

- Food Science of Animal Resources -

Upload this completed form to website with submission

ARTICLE INFORMATION	Fill in information in each box below
Article Type	Review article
Article Title	Effect of Spore-forming Probiotics on the Poultry Production: A Review
Running Title (within 10 words)	Spore-forming Probiotics in Poultry Feed
Author	Anam Khalid[1], Fatima Khalid[1], Nida Mahreen[2], Syed Makhdoom Hussain[3], Muhammad Mudassar Shahzad[4], Salman Khan[1], Zaigui Wang[1*]
Affiliation	[1]College of Life Science, Anhui Agricultural University, 230036, No. 130, Changjiang Road, Hefei, Anhui, the People's Republic of China. [2]Department of Horticulture, Ayyub Research Institute, Faisalabad, Pakistan. [3]Department of Zoology, Government College University, Faisalabad, Pakistan. [4]Department of Zoology, Division of Science and Technology, University of Education, Lahore, Pakistan. [*]Corresponding author: Zaigui Wang, E-mail: wangzaigui2013@163.com, Tel: +86-551-6578-6232, Fax: +86-551-6578-6232, College of Life Science, Anhui Agricultural

	University, 230036, No. 130, Changjiang Road, Hefei, Anhui, the People's Republic of China.
Special remarks – if authors have additional information to inform the editorial office	N/A
ORCID (All authors must have ORCID) https://orcid.org	Anam Khalid: 0000-0001-8936-9979 Fatima Khalid: 0000-0001-7868-5730 Nida Mahreen: 0000-0003-1395-7947 Syed Makhdoom Hussain: 0000-0001-5087-3887 Muhammad Mudassar Shahzad: 0000-0002-3987-4093 Salman Khan: 0000-0003-0454-3785 Zaigui Wang: 0000-0001-7354-4314
Conflicts of interest List any present or potential conflict s of interest for all authors. (This field may be published.)	The authors declare no potential conflict of interest.
Acknowledgements State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available. (This field may be published.)	This study is supported by the Major Science and Technology Program of Anhui Province, China (Grant No 201903a06020020), Funds of the College of Natural Science from Anhui Province, China (Grant No KJ2018A0141), Anhui Industry and Technology System of Poultry Science (Grant No AHCYTX-10), and the Fund of State Key Laboratory of Animal Nutrition (Grant No 2004DA125184F1725)
Author contributions (This field may be published.)	**Conceptualization:** Zaigui Wang, Syed Makhdoom Hussain, Anam Khalid **Data curation:** Anam Khalid, Fatima Khalid, Nida Mahreen **Software and Graphics:** Muhammad Mudassar Shahzad, Salman Khan **Writing, Reviewing, Editing:** Anam Khalid, Fatima Khalid, Nida Mahreen, Syed Makhdoom Hussain, Muhammad Mudassar Shahzad, Salman Khan
Ethics approval (IRB/IACUC) (This field may be published.)	This article does not require IRB/IACUC approval because there are no human and animal participants.

CORRESPONDING AUTHOR CONTACT INFORMATION

For the <u>corresponding</u> author (responsible for correspondence, proofreading, and reprints)	**Fill in information in each box below**
First name, middle initial, last name	Zaigui, Wang
Email address – this is where your proofs will be sent	wangzaigui2013@163.com
Secondary Email address	anamkhalid166@hotmail.com
Postal address	College of Life Science, Anhui Agricultural University, 230036, No. 130, Changjiang Road, Hefei, Anhui, the People's Republic of China.
Cell phone number	+86-551-6578-6232
Office phone number	+86-551-6578-6232
Fax number	+86-551-6578-6232

Effect of Spore-forming Probiotics on the Poultry Production: A Review

Running Title: Spore-forming Probiotics in Poultry Feed

Abstract

Due to the bad aspects associated with the use of antibiotics, the pressure on poultry production prompted the efforts to find out suitable growth-promoting and disease-preventing alternatives. Although, many cost-effective alternatives have been developed. Currently, one of the most auspicious alternatives for poultry feed is spore-forming probiotics, which can exert more beneficial effects as compared to normal probiotics, because of their ability to withstand the harsh external and internal conditions which result in increased viability. Many studies have already used spore-forming probiotics to improve different parameters of poultry production. Our lab has recently isolated a spore-forming bacterial strain, which has the potential to be used as a probiotic. So, to provide a detailed understanding, the current review aimed to collect valuable references to describe the mechanism of action of spore-forming probiotics and their effect on all the key aspects of poultry production.

Key Words: Spore-forming Probiotics, Poultry Feed, Growth performance, Health, Antibiotics

Introduction

The excessive use of antibiotics produced resistance in animals' microbiota, with the ability to transfer antibiotics resistant genes into human microbiota (Mingmongkolchai and Panbangred, 2018). As more and more cases of antibiotic resistance emerged from all over the world, the controversy on their use became a global issue (Cogliani et al., 2011). It was reported that the use of antibiotics in poultry feed caused diseases in humans, due to the development of drug-resistant strains of *C. jejuni* (Iovine and Blaser, 2004). Health issues related to the use of antibiotics have been systematically reviewed (Muhammad et al., 2020). Many studies have now identified the presence of antibiotics in water and soil (Yang et al., 2011; Jiang et al., 2013; Kim et al., 2017) , and the excessive use of antibiotics enrich the environment with antibiotic-resistant bacteria and genes (Franklin et al., 2016). Due to all the problems associated with their use, antibiotics have been prohibited for the animal feed industry, and Sweden was the first country to ban them in 1986 (Castanon, 2007). However, prohibiting the use of antibiotics caused a decrease in production, which suggested the urgent need to find a potential alternative to antibiotics.

In recent years many researchers focused to find out some novel and beneficial replacements of antibiotics with the potential of promoting growth and preventing birds from pathogens (Yadav et al., 2016). Due to their useful applications for animals and humans, probiotics were considered as a potential alternative to antibiotics (Zorriehzahra et al., 2016). Probiotics include different species, such as bacteria, fungi, and yeast (Iannitti and Palmieri, 2010). The use of probiotics has been increased during the last few years, as they can improve the production performance and enhance the immune system (Mingmongkolchai and Panbangred, 2018).

However, the researchers now believe that spore-forming probiotics (SFP's) are the best alternative among the available options (Popov et al., 2021). The mechanism of antibiotics as growth promoters is based on their interaction with intestinal microbiota (Niewold, 2007). Due to their encapsulation ability, the SFP's can reach the specific part of the gastrointestinal (GI) tract easily. During the last few decades, the SFP's showed significant improvement in prophylactic activities, growth promotion, and feed conversion rate in broiler as well as laying hens (Popov et al., 2021).

Many studies reported the use of different strains of SFP's in poultry feed. So, to help the researchers for a better insight into the effect of SFP's on poultry, we summarized the valuable literature.

Why Spore-forming Probiotics

Despite of no clear boundaries, the microbiota found in different parts of the GI tract varies in quantity, characteristics and composition (Oakley et al., 2014). Due to differences in the environment, all microorganism are not able to survive in different types of environment so, it became one of the most important parameter, while choosing the probiotic species. Because the probiotic should be able to reside in the area of interest in GI tract. The diversity and dynamic nature of the GI environment make gut microbiology a quite complicated area of research (Cartman et al., 2007). Hence, it is necessary to find potential probiotics, which can colonize in the area of interest.

SFP's have a greater ability to survive and pass through the GI tract and also to proliferate and colonize the digestive tract of animals (Popov et al., 2021). The stability of spores during their passage through the upper GI tract was better as compared to vegetative cells, and the spores were able to proliferate in lower parts of the GI tract and exhibit their beneficial properties

(Mingmongkolchai and Panbangred, 2018). This ability makes SFP's a best feed additive for livestock, especially in the poultry industry (Popov et al., 2021).

Furthermore, the veterinary sector has now acknowledged that the application of spores has several benefits over the vegetative cells. As compared to vegetative cells, spores are more easily stored, manipulated, and delivered to farm animals (Todorov et al., 2021).

Mechanism of Action of Spore-forming Probiotics in Poultry

Poultry production in a commercial environment faces continuous exposure to the various microbes usually through their mucosa (Broom, 2019). Depending upon the interaction of host and microbe diseases may occur (Garcia et al., 2010).

The mechanism of action of SFP's is still not very clear. However, it is reported that the mechanism of action of SFP's is the same as the normal probiotic micro-organisms (Cartman et al., 2007).

The mode of action of probiotics in poultry was reported in many different ways (Fig. 1). The first strategy was known as the competitive exclusion, competition for the adhesion sites prevents the colonization of pathogenic bacteria (Chichlowski et al., 2007; O'dea et al., 2006). This competitive exclusion was achieved through the adhesion of beneficial bacteria (Chichlowski et al., 2007). Maintenance of this activity depends upon the ability of the SFP's strain to adhere to the intestinal wall (AFRC, 1989). It was reported that the *Bacillus* spores can not only germinate and grow in the intestine (Nakano and Zuber, 1998) but also re-sporulate in the lower part of the small intestine (Tam et al., 2006). Furthermore, the spore's adhesion to the intestinal wall can help to retain the spores in the intestine (Popov et al., 2021) and thus can contribute to competitive exclusion. The adhesion of probiotics to the gut mucosa is important for colonization and interaction with the host (Bermudez-Brito et al., 2012).

The gut bacteria produce metabolites that control the growth of pathogenic compounds and compete for adhesion in the intestinal epithelium. These metabolites are short-chain organic acids, bacteriocins as well as hydrogen peroxides (Dankowiakowska et al., 2013), help in attachment to the intestinal mucosal layer (Buck et al., 2005). The newborn has a sterile digestive system and before its organs produce antibodies, microbes from the environment start to colonize in the gut. Therefore the use of probiotics due to their adhesion ability with the intestinal mucosa creates a natural blockade against disease pathogens (Dankowiakowska et al., 2013).

The second mode of action of probiotics was reported through balancing the dysbiosis. The enteric dysbiosis may cause alteration in host-microbial interaction, leading to disease conditions (Byndloss and Bäumler, 2018; Plaza-Díaz et al., 2018). Recently, it was suggested that probiotics can treat dysbiosis and restore the imbalanced or disturbed microbiota, (Mendes et al., 2018; Vieira et al., 2016). Another study suggested the same findings that probiotics can maintain the colonization of disturbed gut microbiota (Plaza-Diaz et al., 2019). This mechanism suggests that the balanced gut microbiota also helps to reduce the emission of ammonia through feces.

The third mechanism was achieved through the antagonistic activity of probiotics. Probiotics produce a class of small antimicrobial molecules (bacteriocins, mucin, defensins) which obstruct the pathogens, and their colonization (Khan and Naz, 2013). One study reported that bactericidal substances produced by bacteria can lyse the pathogens, and receptors on the surface of probiotics can mimic the intestinal wall, and antagonize the pathogens, and then they neutralize them (Sleator, 2010). Similarly, it was suggested that the mucin secreted by the intestinal epithelial cells, reduces the adhesion of pathogenic bacteria (Preedy, 2010).

The fourth mechanism of probiotic action is known to modulate the GI immunity, which is also considered as the most important benefit of probiotics. Different studies have shown that probiotics induce anti-inflammatory mediators, such as interleukin-10 (IL-10), thymic stromal lymphopoietin (TSLP), and transforming growth factor beta (TGF-β). And simultaneously decrease the production of pro-inflammatory cytokines such as interleukin 8 (IL-8) and tumor necrosis factor-alpha (TNF- α) (Georgieva et al., 2015).

Effect of Spore-forming probiotics on Different Key Aspects of Poultry

Effect on the GI Tract of poultry

Among all spore formers, bacterial spores are particularly well studied for use as a probiotic because they are dormant, metabolically inactive, and highly resistant to environmental stresses (Elisashvili et al., 2019). These distinctive properties are beneficial for the commercial perspective which means that they have a long shelf life and can maintain viability during distribution and storage. Despite all this knowledge, how the bacterial spores may function as probiotics are limited at present.

Spore germination is requisite to function as a probiotic. Jadamus et al. (2001) first time experimented to check the germination of bacterial spores in the GI tract of poultry and concluded that *B. cereus var. toyoi* germinates rapidly. Later, a similar study was performed by Cartman et al. (2008) suggested that the spores must be viable to function as a probiotic and to produce the antimicrobial compounds, and the results reported that germination of *Bacillus subtilis* spores occurs in the GI tract of poultry. Another study reported that the spore germination in the GI tract of poultry occurs at such a faster rate that, 90% of spores germinated within one hour (Latorre et al., 2014).

Bacillus spp. are generally known as aerobic bacteria, and it is important to know that how they survive in the anoxic environment of the GI tract if germinates. So, the studies concluded reported that these bacteria can use nitrate or nitrate as an electron acceptor instead of oxygen for their growth and survival (Nakano and Zuber, 1998; Shivaramaiah et al., 2011).

Effect on Ammonia Reduction through feces

Currently, the excess emission of ammonia is a major environmental problem related to the poultry industry. The SFP's and the environment of the GI tract can contribute to overcoming this serious issue (Fig. 2). It was reported that the uric acid produced in the chicken's liver is excreted into the intestine and microbial urease converts it into ammonia by hydrolysis (Karasawa et al., 1988). Many intestinal microorganisms (*Clostridia*, *Bacteroides*, and *Proteus*) possess urease activity (Ahmed et al., 2014), through which uric acid is hydrolyzed into ammonia. Gram-positive, SFP's have been reported to inhibit the production of urease-producing bacteria, either by inhibiting the growth of pathogenic bacteria, producing antimicrobial compounds, or by lowering the pH, which in turn reduce ammonia production in ceca (Goudarzi et al., 2017). Similar studies reported that *B. subtilus* reduced the emission of ammonia and promoted the growth performance by inhibiting the pathogenic bacteria in broiler chicken (Chen et al., 2012; Teo and Tan, 2007). Thus the stabilized gut microbiota maintains the intestinal health of poultry (Hong et al., 2005). Zhang and Kim, (2013) reported that the dietary supplementation of *B. subtilus* can improve the enzymatic activity of intestinal microbiota and improved microbial balance in the GI tract was the main reason for the reduction in ammonia production. According to Han et al. (2001), several studies reported that infeed probiotics have reduced ammonia by improving the feed efficiency and microbial ecology of GI tract. Similarly Jeong and Kim (2014) observed a significant decrease in ammonia excretion when they used *Bacillus* strains in poultry feed. Ahmed et al. (2014) suggested that *B. amyloliquefaciens* is also an effective microorganism for suppressing ammonia production by improving the environment of GI tract.

Effect on the Egg Quality

Poultry farmers aimed to produce eggs with normal shape, eggshell thickness, and eggshell strength to maintain the freshness of eggs according to the consumer's demand (Fouad et al., 2016). Studies suggested that diets supplemented with *B. subtilis;* a spore-forming bacterium can improve the production performance and eggs quality for economic benefits (Xu et al., 2006).

It was very important to note that the stress conditions affect the eggshell thickness, and thus increase the number of cracked or broken eggs. SFP’s are reported to heal the birds suffering several stressors (Fathi et al., 2018). It was reported that the increase in the availability of intestinal calcium produced a positive effect of probiotics on the eggshell strength (Świątkiewicz et al., 2010). An increase in egg shall thickness and reduction in damaged eggs was recorded in the hens, fed diet fortified with SFP’s *B. subtilus*, which may be related to the calcium retention (Fathi et al., 2018).

Another study reported that compared to the control group significant increase in the egg production rate was observed, when hens were fed with *Lactobacillus sporogenes*, as well the persistency was better in the probiotic group (Panda et al., 2008). But some studies reported contradictory results, which might be pertinent to the bacterial strain, form, and concentration used (Nahashon, 1992; Tortuero and Fernandez, 1995).

Effect on the Growth Performance Parameters

The stabilized gut microbiota can help to improve the feed conversion ratio and consequently enhance the digestion as well as absorption of nutrients (Pan and Yu, 2014). Panda et al. (2003) revealed that the *L. sporogens* supplemented diet improved weight gain and FCR in broilers. The inclusion of *B. subtilis* C-1302 at the level of 300 and 600 mg/kg of diet showed positive effects on the growth rate (increased Average daily gain) and decrease the FCR in broilers (Jeong and Kim, 2014). Similarly, Fathi et al. (2018) concluded that dietary supplementation of *B. subtillus* had no significant effect on the FCR of laying hens. According to Lei et al. (2013) adding *Bacillus licheniformis* to the diet showed no significant effect on the feed consumption and FCR of the laying hens. Furthermore, probiotics have been reported to give inconsistent results for average daily feed intake (ADFI) (Otutumi et al., 2012) sometimes showing no effect at all. A significant increase in (ADFI) was observed when Hy-Line Brown laying hens fed a diet supplemented with *Bacillus velezensis* (Ye et al., 2020). Ross 308 broiler chicks fed a diet supplemented with *C. butyricum* also showed significant increase in ADFI (Zhao et al., 2013). However, many studies reported no significant effect increase in ADFI, such as Broiler Cobb 500 fed a *B. subtilis* supplemented diet (Oladokun et al., 2021), Hy-Line Brown laying hens fed a diet supplemented with *B. amyloliquefaciens* (Zhou et al., 2020) and Hy-Line Brown laying hens fed a diet supplemented *B. licheniformis* (Yang et al., 2020). Zhu et al. (2009) explained that the benefits associated with probiotics depend upon many factors, such as, probiotic species, strain, application method, age of flocks, over all hygiene and housing conditions on farm, and external environmental factors.

Effects on the Immune System

The basic and most important organs of the immune system consisted of the thymus, bursa of Fabricius, and spleen, which are involved in the production and differentiation of immune cells and production of antibodies (Fouad et al., 2016). Stress leaves damaging effects on the natural defense system and intestinal epithelial cells of hens (Soderholm and Perdue, 2001; Taché et al., 2001). The response to the immune system as well as intestinal epithelial cells is directly controlled by the neuroendocrine system (Levite, 2001; Petrovsky, 2001).

The commensal bacteria present in the gut, influence the development of the immune system by interacting with the probiotics (Tannock, 1999) because these commensal bacteria have close contact with the cells of the immune system (Haghighi et al., 2005). Colonization of microbes in the chicken gut begins early after hatching and becomes stabilized within the first fifteen days of their life (Rehman et al., 2007). Another study suggested that some immunomodulatory effects of probiotics involve changes in signaling pathways and activation of transcription factors and thus enhance the expression of messenger RNA genes, related to innate immunity (Al-Khalaifah, 2018). RNA of the immune system, for example, the maturation process of the intestinal TCRαβ T cell (Mwangi et al., 2010) and the immunoglobulin repertoire (den Hartog et al., 2013) is dependent, on the enteric microbiota.

Effect on the Meat Quality

The storage time and processing depend upon the physicochemical properties of the meat (Popova, 2017). *Bacillus*-based probiotics were reported to improve the pH, stiffness, and color of the meat (Pelicano et al., 2003). Color is an important quality parameter because more than 80% of the consumers focus on the color of meat when they tend to buy it (Bai et al., 2017; Węglarz, 2010). Recently, a spore-forming bacteria, *B. amyloliquefaciens* B-1895 was reported to improve the meat mass in broilers. Similarly, Li u et al. (2012) showed that hens drinking *B. licheniformis* supplemented water showed significantly improved meat color, juiciness, and also high protein content. Studies showed that SFP's can improve meat quality, but the type of probiotic and the approach of administration can influence the performance (Bai et al., 2017). As observed by Hossain et al. (2012) the higher efficiency ratio of protein may help to promote meat yield.

Effect on the Enzyme Production

Bacillus-based SFP's can produce beneficial enzymes, which help to regulate the function of the digestive system (Ramlucken et al., 2020). Enzymes such as protease and carbohydrate help to lower the non-digestible proteins and carbohydrates, which act as a nutrient source for pathogenic bacteria (Kiarie et al., 2013). Ramlucken et al. (2020) reported that poultry is not known to produce enzymes for the digestion of non-starch polysaccharides, which if remain un-hydrolyzed results in low feed conversion. So, the addition of enzymes through the feed is necessary, and enzyme-producing probiotics are the best alternative. The study reported that *Bacillus* spp. can produce several exogenous enzymes, (protease, β-glucanase, amylase, xylanase, phytase, lipase, and cellulase) which are crucial for the digestion in poultry (Latorre et al., 2015). These probiotic enzymes improve the nutrient availability to the microflora in the GI tract (Ramlucken et al., 2020). Wang and Gu (2010) reported that *Bacillus coagulans* increased the activity of protease and amylase, which in turn improved the broiler growth. Study performed in our lab has isolated protease and β-glucanase producing *B. velezensis* Y1 strain from the manure of piglets (Khalid et al., 2021). Several other studies have already reported the enzyme production ability of *Bacillus* species (Adeola and Cowieson, 2011; Latorre et al., 2014). All these enzymes (protease, lipases, glucanase, cellulase, xylanase, and phytase can help to balance the anti-nutritional factors in the feed (Popov et al., 2021).

Future Implications

The review concluded that several SFP's have been studied until now, but a few of them were used commercially such as *B. subtilus*, *B licheniformis B. cer*eus (Larsen et al., 2014). However, another recent study used *B. amyloliquefaciens* B-1895 in poultry feed and reported beneficial results (Farhat‐Khemakhem et al., 2018).

Recently, our lab has isolated a strain of spore-forming *B. velezensis* from the manure of piglets (Khalid et al., 2021), described its characteristics (Ye et al., 2018), and used it in poultry feed. The results showed significant improvements in different growth parameters, blood biochemistry, and egg quality indices of laying hens (Ye et al., 2020). Hence, we suggest that *B. velezensis* being a spore-forming species can also be used as a probiotic in poultry feed.

Competing Interests

The authors declare that no competing interests exist between them.

Acknowledgments

This study is supported by the Major Science and Technology Program of Anhui Province, China (Grant No 201903a06020020), Funds of the College of Natural Science from Anhui Province, China (Grant No KJ2018A0141), Anhui Industry and Technology System of Poultry Science (Grant No AHCYTX-10), and the Fund of State Key Laboratory of Animal Nutrition (Grant No 2004DA125184F1725)

References

Adeola O, Cowieson A. 2011; Board-invited review: Opportunities and challenges in using exogenous enzymes to improve nonruminant animal production. J Anim Sci. 89:3189-3218.

Afrc RF. 1989; Probiotics in man and animals. J Appl Bacteriol. 66:365-378.

Ahmed ST, Islam MM, Mun H-S, Sim H-J, Kim Y-J, Yang C-J. 2014; Effects ofbacillus amyloliquefaciens as a probiotic strain on growth performance, cecal microflora, and fecal noxious gas emissions of broiler chickens. Poult Sci. 93:1963-1971.

Al-Khalaifah H. 2018; Benefits of probiotics and/or prebiotics for antibiotic-reduced poultry. Poult Sci. 97:3807-3815.

Bai K, Huang Q, Zhang J, He J, Zhang L, Wang T. 2017; Supplemental effects of probiotic bacillus subtilis fmbj on growth performance, antioxidant capacity, and meat quality of broiler chickens. Poult Sci. 96:74-82.

Bermudez-Brito M, Plaza-Díaz J, Muñoz-Quezada S, Gómez-Llorente C, Gil A. 2012; Probiotic mechanisms of action. Ann. Nutr. Metab. 61:160-174.

Broom LJ. 2019; Host–microbe interactions and gut health in poultry—focus on innate responses. Microorganisms. 7:139.

Buck BL, Altermann E, Svingerud T, Klaenhammer TR. 2005; Functional analysis of putative adhesion factors in lactobacillus acidophilus ncfm. Appl. Environ. Microbiol. 71:8344-8351.

Byndloss MX, Bäumler AJ. 2018; The germ-organ theory of non-communicable diseases. Nat. Rev. Microbiol. 16:103.

Cartman ST, La Ragione RM, Woodward MJ. 2007; Bacterial spore formers as probiotics for poultry. Food Science and Technology Bulletin: Funct. Foods. 4:30.

Cartman ST, La Ragione RM, Woodward MJ. 2008; Bacillus subtilis spores germinate in the chicken gastrointestinal tract. Appl. Environ. Microbiol. 74:5254-5258.

Castanon J. 2007; History of the use of antibiotic as growth promoters in european poultry feeds. Poult Sci. 86:2466-2471.

Chen K, Gao J, Li J, Huang Y, Luo X, Zhang T. 2012; Effects of probiotics and antibiotics on diversity and structure of intestinal microflora in broiler chickens. Afr. J. Microbiol. Res. 6:6612-6617.

Chichlowski M, Croom J, Mcbride B, Havenstein G, Koci M. 2007; Metabolic and physiological impact of probiotics or direct-fed-microbials on poultry: A brief review of current knowledge. Int. J. Poult. Sci. 6:694-704.

Cogliani C, Goossens H, Greko C. 2011; Restricting antimicrobial use in food animals: Lessons from europe. Microbe. 6:274.

Dankowiakowska A, Kozłowska I, Bednarczyk M. 2013; Probiotics, prebiotics and snybiotics in poultry–mode of action, limitation, and achievements. J Cent Eur Agric. 14:467-478.

Den Hartog G, Crooijmans RP, Parmentier HK, Savelkoul HF, Bos NA, Lammers A. 2013; Ontogeny of the avian intestinal immunoglobulin repertoire: Modification in cdr3 length and conserved vh-pseudogene usage. Mol Immunol. 56:811-818.

Elisashvili V, Kachlishvili E, Chikindas ML. 2019; Recent advances in the physiology of spore formation for bacillus probiotic production. Probiotics Antimicrob. Proteins. 11:731-747.

Farhat‐Khemakhem A, Blibech M, Boukhris I, Makni M, Chouayekh H. 2018; Assessment of the potential of the multi‐enzyme producer bacillus amyloliquefaciens us573 as alternative feed additive. J Sci Food Agric. 98:1208-1215.

Fathi M, Al-Homidan I, Al-Dokhail A, Ebeid T, Abou-Emera O, Alsagan A. 2018; Effects of dietary probiotic (bacillus subtilis) supplementation on productive performance, immune response and egg quality characteristics in laying hens under high ambient temperature. Ital. J. Anim. Sci. 17:804-814.

Fouad A, Chen W, Ruan D, Wang S, Xia W, Zheng C. 2016; Impact of heat stress on meat, egg quality, immunity and fertility in poultry and nutritional factors that overcome these effects: A review. Int. J. Poult. Sci. 15:81.

Franklin AM, Aga DS, Cytryn E, Durso LM, Mclain JE, Pruden A, Roberts MC, Rothrock Jr MJ, Snow DD, Watson JE. 2016; Antibiotics in agroecosystems: Introduction to the special section. J Environ Qual. 45:377-393.

Garcia CC, Guabiraba R, Soriani FM, Teixeira MM. 2010; The development of anti-inflammatory drugs for infectious diseases. Discov. Med. 10:479-488.

Georgieva M, Georgiev K, Dobromirov P. 2015. Probiotics and immunity. In Immunopathology and immunomodulation. IntechOpen.

Goudarzi L, Kermanshahi RK, Moosavi-Nejad Z, Dalla MMS. 2017; Evaluation of antimicrobial activity of probiotic lactobacillus strains against growth and urease activity of proteus spp. J. Med. Bact. 6:31-43.

Haghighi HR, Gong J, Gyles CL, Hayes MA, Sanei B, Parvizi P, Gisavi H, Chambers JR, Sharif S. 2005; Modulation of antibody-mediated immune response by probiotics in chickens. Clin. Diagn. Lab. Immunol. 12:1387-1392.

Hong HA, Duc LH, Cutting SM. 2005; The use of bacterial spore formers as probiotics. FEMS Microbiol. Rev. 29:813-835.

Hossain ME, Kim GM, Lee SK, Yang CJ. 2012; Growth performance, meat yield, oxidative stability, and fatty acid composition of meat from broilers fed diets supplemented with a medicinal plant and probiotics. Asian-Australas J Anim Sci. 25:1159.

Iannitti T, Palmieri B. 2010; Therapeutical use of probiotic formulations in clinical practice. Clin. Nutr. 29:701-725.

Iovine NM, Blaser MJ. 2004; Antibiotics in animal feed and spread of resistant campylobacter from poultry to humans. Emerging Infect. Dis. 10:1158.

Jadamus A, Vahjen W, Simon O. 2001; Growth behaviour of a spore forming probiotic strain in the gastrointestinal tract of broiler chicken and piglets. Archives of Animal Nutrition. 54:1-17.

Jeong J, Kim I. 2014; Effect of bacillus subtilis c-3102 spores as a probiotic feed supplement on growth performance, noxious gas emission, and intestinal microflora in broilers. Poult Sci. 93:3097-3103.

Jiang J-Q, Zhou Z, Sharma V. 2013; Occurrence, transportation, monitoring and treatment of emerging micro-pollutants in waste water—a review from global views. Microchem. J. 110:292-300.

Karasawa Y, Kawai H, Hosono A. 1988; Ammonia production from amino acids and urea in the caecal contents of the chicken. Comparative biochemistry and physiology. B, Comp. Biochem. 90:205-207.

Khalid A, Ye M, Wei C, Dai B, Yang R, Huang S, Wang Z. 2021; Production of β-glucanase and protease from bacillus velezensis strain isolated from the manure of piglets. Prep Biochem Biotechnol. 51:497-510.

Khan R, Naz S. 2013; The applications of probiotics in poultry production. Worlds Poult. Sci. J. 69:621-632.

Kiarie E, Romero LF, Nyachoti CM. 2013; The role of added feed enzymes in promoting gut health in swine and poultry. Nutri. Res. Rev. 26:71-88.

Kim JH, Kuppusamy S, Kim SY, Kim SC, Kim HT, Lee YB. 2017; Occurrence of sulfonamide class of antibiotics resistance in korean paddy soils under long-term fertilization practices. J Soils Sediments. 17:1618-1625.

Larsen N, Thorsen L, Kpikpi EN, Stuer-Lauridsen B, Cantor MD, Nielsen B, Brockmann E, Derkx PM, Jespersen L. 2014; Characterization of bacillus spp. Strains for use as probiotic additives in pig feed. Appl. Microbiol. Biotechnol. 98:1105-1118.

Latorre J, Hernandez-Velasco X, Kallapura G, Menconi A, Pumford N, Morgan M, Layton S, Bielke L, Hargis B, Téllez G. 2014; Evaluation of germination, distribution, and persistence of bacillus subtilis spores through the gastrointestinal tract of chickens. Poult Sci. 93:1793-1800.

Latorre JD, Hernandez-Velasco X, Kuttappan VA, Wolfenden RE, Vicente JL, Wolfenden AD, Bielke LR, Prado-Rebolledo OF, Morales E, Hargis BM. 2015; Selection of bacillus spp. For cellulase and xylanase production as direct-fed microbials to reduce digesta viscosity and clostridium perfringens proliferation using an in vitro digestive model in different poultry diets. Front. Vet. Sci. 2:25.

Lei K, Li Y, Yu D, Rajput I, Li W. 2013; Influence of dietary inclusion of bacillus licheniformis on laying performance, egg quality, antioxidant enzyme activities, and intestinal barrier function of laying hens. Poult Sci. 92:2389-2395.

Levite M. 2001; Nervous immunity: Neurotransmitters, extracellular k+ and t-cell function. Trends Immunol. 22:2-5.

Liu X, Yan H, Le Lv QX, Yin C, Zhang K, Wang P, Hu J. 2012; Growth performance and meat quality of broiler chickens supplemented with bacillus licheniformis in drinking water. Asian-Australas J Anim Sci. 25:682.

Mendes MCS, Paulino DS, Brambilla SR, Camargo JA, Persinoti GF, Carvalheira JBC. 2018; Microbiota modification by probiotic supplementation reduces colitis associated colon cancer in mice. World J. Gastroenterol. 24:1995.

Mingmongkolchai S, Panbangred W. 2018; Bacillus probiotics: An alternative to antibiotics for livestock production. J Appl Microbiol. 124:1334-1346.

Muhammad J, Khan S, Su JQ, Hesham AE-L, Ditta A, Nawab J, Ali A. 2020; Antibiotics in poultry manure and their associated health issues: A systematic review. J Soils Sediments. 20:486-497.

Mwangi WN, Beal RK, Powers C, Wu X, Humphrey T, Watson M, Bailey M, Friedman A, Smith AL. 2010; Regional and global changes in tcrαβ t cell repertoires in the gut are dependent upon the complexity of the enteric microflora. Dev. Comp. Immunol. 34:406-417.

Nahashon S. 1992; Effect of direct-fed microbials on nutrient retention production parameters of laying pullets. Poult Sci. 71:111.

Nakano MM, Zuber P. 1998; Anaerobic growth of a “strict aerobe”(bacillus subtilis). Annu. Rev. Microbiol. 52:165-190.

Niewold T. 2007; The nonantibiotic anti-inflammatory effect of antimicrobial growth promoters, the real mode of action? A hypothesis. Poult Sci. 86:605-609.

O’dea E, Fasenko G, Allison G, Korver D, Tannock G, Guan LL. 2006; Investigating the effects of commercial probiotics on broiler chick quality and production efficiency. Poult Sci. 85:1855-1863.

Oakley BB, Lillehoj HS, Kogut MH, Kim WK, Maurer JJ, Pedroso A, Lee MD, Collett SR, Johnson TJ, Cox NA. 2014; The chicken gastrointestinal microbiome. FEMS Microbiol. Lett. 360:100-112.

Oakley BB, Lillehoj HS, Kogut MH, Kim WK, Maurer JJ, Pedroso A, Lee MD, Collett SR, Johnson TJ, Cox NA. 2014; The chicken gastrointestinal microbiome. FEMS Microbiol. Lett. 360:100-112.

Oladokun S, Koehler A, Macisaac J, Ibeagha-Awemu EM, Adewole DI. 2021; Bacillus subtilis delivery route: Effect on growth performance, intestinal morphology, cecal short-chain fatty acid concentration, and cecal microbiota in broiler chickens. Poult Sci. 100:100809.

Otutumi LK, Góis MB, De Moraes Garcia ER, Loddi MM. 2012; Variations on the efficacy of probiotics in poultry. Probiot. Anim. EC Rigobelo, Ed. InTech, Rijeka, Croatia.203-220.

Pan D, Yu Z. 2014; Intestinal microbiome of poultry and its interaction with host and diet. Gut microbes. 5:108-119.

Panda A, Reddy M, Rao SR, Praharaj N. 2003; Production performance, serum/yolk cholesterol and immune competence of white leghorn layers as influenced by dietary supplementation with probiotic. Trop Anim Health Prod. 35:85-94.

Panda AK, Rama Rao SS, Raju MV, Sharma SS. 2008; Effect of probiotic (lactobacillus sporogenes) feeding on egg production and quality, yolk cholesterol and humoral immune response of white leghorn layer breeders. J Sci Food Agric. 88:43-47.

Pelicano ERL, De Souza P, De Souza H, Oba A, Norkus E, Kodawara L, De Lima T. 2003; Effect of different probiotics on broiler carcass and meat quality. Brazilian Journal of Poultry Science. 5:207-214.

Petrovsky N. 2001; Towards a unified model of neuroendocrine–immune interaction. Immunol Cell Biol. 79:350-357.

Plaza-Díaz J, Ruiz-Ojeda FJ, Gil-Campos M, Gil A. 2018; Immune-mediated mechanisms of action of probiotics and synbiotics in treating pediatric intestinal diseases. Nutrients. 10:42.

Plaza-Diaz J, Ruiz-Ojeda FJ, Gil-Campos M, Gil A. 2019; Mechanisms of action of probiotics. Advan. Nutri. 10:S49-S66.

Popov IV, Algburi A, Prazdnova EV, Mazanko MS, Elisashvili V, Bren AB, Chistyakov VA, Tkacheva EV, Trukhachev VI, Donnik IM. 2021; A review of the effects and production of spore-forming probiotics for poultry. Animals. 11:1941.

Popova T. 2017; Effect of probiotics in poultry for improving meat quality. Curr. Opin. Food Sci. 14:72-77.

Preedy VR. 2010. Bioactive foods in promoting health: Probiotics and prebiotics. Acad. Press.

Ramlucken U, Lalloo R, Roets Y, Moonsamy G, Van Rensburg CJ, Thantsha M. 2020; Advantages of bacillus based probiotics in poultry production. Livest. Sci.104215.

Rehman HU, Vahjen W, Awad WA, Zentek J. 2007; Indigenous bacteria and bacterial metabolic products in the gastrointestinal tract of broiler chickens. Arch. Anim. Nutr.61:319-335.

Shivaramaiah S, Pumford N, Morgan M, Wolfenden R, Wolfenden A, Torres-Rodriguez A, Hargis B, Téllez G. 2011; Evaluation of bacillus species as potential candidates for direct-fed microbials in commercial poultry. Poult Sci. 90:1574-1580.

Sleator RD. 2010; Probiotic therapy-recruiting old friends to fight new foes. Gut Pathog. 2:5.

Soderholm JD, Perdue MH. 2001; Ii. Stress and intestinal barrier function. Am. J. Physiol. Gastrointest. Liver Physiol. 280:G7-G13.

Świątkiewicz S, Koreleski J, Arczewska A. 2010; Laying performance and eggshell quality in laying hens fed diets supplemented with prebiotics and organic acids. Czech J Anim Sci. 55:294-306.

Taché Y, Martinez V, Million M, Wang L. 2001; Iii. Stress-related alterations of gut motor function: Role of brain corticotropin-releasing factor receptors. Am. J. Physiol. Gastrointest. Liver Physiol. 280:G173-G177.

Tam NK, Uyen NQ, Hong HA, Duc LH, Hoa TT, Serra CR, Henriques AO, Cutting SM. 2006; The intestinal life cycle of bacillus subtilis and close relatives. J Bacteriol. 188:2692-2700.

Tannock G. 1999; Probiotics: A critical review. J. Antimicrob. Chemother. 43:849.

Teo A, 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). J. Appl. Poult. Res. 16:296-303.

Todorov SD, Ivanova IV, Popov I, Weeks R, Chikindas ML. 2021; Bacillus spore-forming probiotics: Benefits with concerns? Crit. Rev. Microbiol.:1-18.

Tortuero F, Fernandez E. 1995; Effects of inclusion of microbial cultures in barley-based diets fed to laying hens. Anim. Feed Sci. Technol. 53:255-265.

Vieira AT, Fukumori C, Ferreira CM. 2016; New insights into therapeutic strategies for gut microbiota modulation in inflammatory diseases. Clinic. trans. immunol. 5:e87.

Wang Y, Gu Q. 2010; Effect of probiotic on growth performance and digestive enzyme activity of arbor acres broilers. Res. Vet. Sci. 89:163-167.

Węglarz A. 2010; Meat quality defined based on ph and colour depending on cattle category and slaughter season. Czech J Anim Sci. 55:548-556.

Xu C-L, Ji C, Ma Q, Hao K, Jin Z-Y, Li K. 2006; Effects of a dried bacillus subtilis culture on egg quality. Poult Sci. 85:364-368.

Yadav AS, Kolluri G, Gopi M, Karthik K, Singh Y. 2016; Exploring alternatives to antibiotics as health promoting agents in poultry-a review. J Exp Biol. 4:3S.

Yang J, Zhan K, Zhang M. 2020; Effects of the use of a combination of two bacillus species on performance, egg quality, small intestinal mucosal morphology, and cecal microbiota profile in aging laying hens. Probiotics Antimicrob. Proteins. 12:204-213.

Yang Y, Fu J, Peng H, Hou L, Liu M, Zhou J. 2011; Occurrence and phase distribution of selected pharmaceuticals in the yangtze estuary and its coastal zone. J. Hazard. Mater. 190:588-596.

Ye M, Tang X, Yang R, Zhang H, Li F, Tao F, Li F, Wang Z. 2018; Characteristics and application of a novel species of bacillus: Bacillus velezensis. ACS Chem. Biol. 13:500-505.

Ye M, Wei C, Khalid A, Hu Q, Yang R, Dai B, Cheng H, Wang Z. 2020b; Effect of bacillus velezensis to substitute in-feed antibiotics on the production, blood biochemistry and egg quality indices of laying hens. BMC Vet. Res. 16:1-8.

Zhao X, Guo Y, Guo S, Tan J. 2013; Effects of clostridium butyricum and enterococcus faecium on growth performance, lipid metabolism, and cecal microbiota of broiler chickens. Appl. Microbiol. Biotechnol. 97:6477-6488.

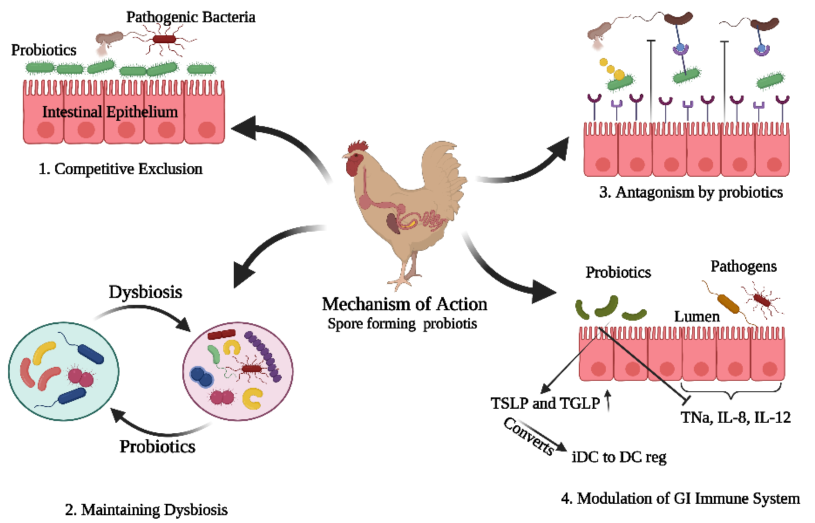
Zhou Y, Li S, Pang Q, Miao Z. 2020; Bacillus amyloliquefaciens blcc1-0238 can effectively improve laying performance and egg quality via enhancing immunity and regulating reproductive hormones of laying hens. Probiotics Antimicrob. Proteins. 12:246-252.

Zhu N, Zhang R, Wu H, Zhang B. 2009; Effects of lactobacillus cultures on growth performance, xanthophyll deposition, and color of the meat and skin of broilers. J. Appl. Poult. Res. 18:570-578.

Zorriehzahra MJ, Delshad ST, Adel M, Tiwari R, Karthik K, Dhama K, Lazado CC. 2016; Probiotics as beneficial microbes in aquaculture: An update on their multiple modes of action: A review. Vet Q. 36:228-241.

Figure 1.

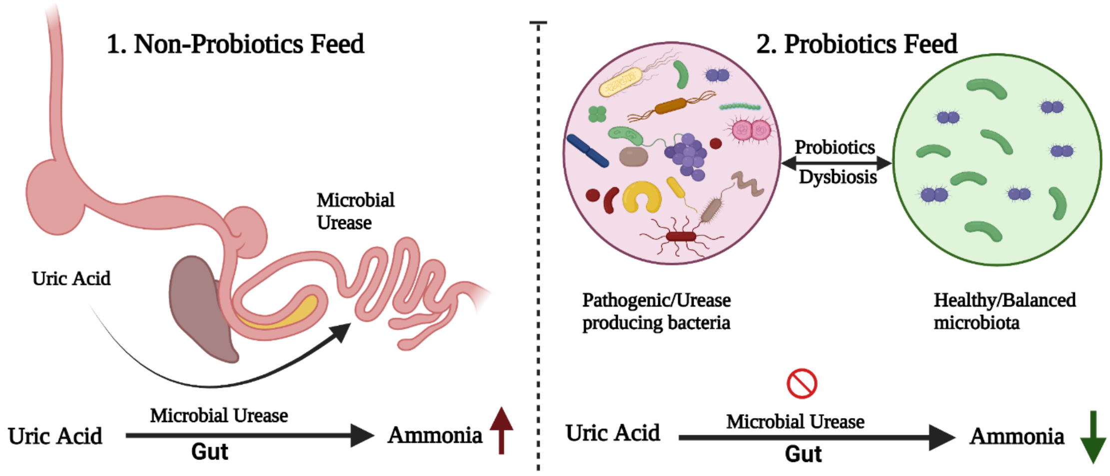
Title: Mechanism of probiotics action



(1) Competitive Exclusion by probiotics. **(2)** Dysbiosis caused by any reason restored by probiotics. **(3)** Bactericidal substances produced by probiotics lyse the pathogen, the receptors on the probiotic surface antagonize the pathogen and neutralize the pathogens. **(4)** Gastrointestinal cells exposed to pathogens produce pro-inflammatory compounds (TNFa, IL-6, and IL-12). Probiotics decrease the production of pro-inflammatory compounds and increase the production of the anti-inflammatory mediator (TSLP and TGLP), which then converts the immature dendritic cells to regulatory dendritic cells.

Figure 2.

Title: Effect of spore-forming probiotics on Ammonia reduction through feces



(1) Uric acid produced in the liver moved to the gut, pathogenic bacteria in the gut produce urease, which converts uric acid into ammonia. **(2)** Probiotics stabilized the gut environment by inhibiting the production of urease-producing bacteria, and ammonia production decreased.