Chapter

Use of *Bacillus Subtilis* Probiotics as Non-Antibiotic Gut Modulator and Growth Promoter in Broiler Chickens

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Abstract

Wide range of Antibiotics is being used as feed additives in Animal industry in order to get rid from pathogens and as growth promoters in developing world. But after the suggested prohibition on using antibiotics, products such as probiotics are getting substantial importance in nutrition because of their non-resistant and non-residual possessions. Basic aim of the chapter is to highlight fruitful effects of *Bacillus Subtilis* as non-antibiotic gut modulator and growth promoter in broiler chickens. Probiotics are the living culture of microorganisms. They flourish in the gut of the host and fortify the growth of valuable commensals in the digestive tract by minimizing the destruction triggered by pathogens, boost up the immune system, supporting the integrity of the gut mucosa and maintain a stability and balance of normal microflora. Probiotics can be used as best substitute to conventional antimicrobial therapy. In addition, it has been observed that probiotics plays a role in growth enhancement by augmenting useful enzymes in the body and promote the growth of other normal commensals such as Lactobacillus and having effect on gut luminal pH. Probiotics are quite active against intestinal pathogens in several ways, viz. including improved immune elimination, competing for mucosal attachment, striving for crucial nutrients, or producing antimicrobial complexes contrary to numerous enteropathogens. It can be concluded that *B. Subtilis* has the ability to modulate gut and immune system histophysiology and histomorphology and can be used as safe antimicrobial candidate in poultry nutrition. Knowledge of such possessions of the *B. Subtilis* as probiotics and the mechanisms of action may enable the researchers to manipulate the use of such alternatives for better growth production, and safe and healthy poultry industry.

Keywords: anatomy, commercial broiler, gut, microscope, physiology, probiotics

1. Introduction

Throughout the world, domestic animals are being kept producing high-quality meat. Chicken also contributes a major portion to the animal meat source and more than 45% of the world population is potential consumers of chicken meat [1]. A gape exists between supply and demand, and some sort of discriminations are observed among the availability of protein source to the people of technological

advanced countries and the underdeveloped countries. Annual meat availability per capita is 0.25 to 1.25 kg in Southern Asian Countries in comparison with 20–30 kg meat in some developed countries [2]. Current animal industry needs elevated production level and cost-effective feed conversion, which to a reliable level could be attained by the practice of precise additives. Sustained meat production can be hampered by various disease conditions especially those affecting digestive systems of the animals. Salmonellosis, colibacillosis, coccidiosis and some fungal toxins are the leading causative agents among those. Most of the diseases are transferable via food chain and have a zoonotic impact. Salmonellosisis among the foremost reasons of bacterial food poisoning in end-users (humans) in both the developing and technologically advanced countries [3]. Wide range of Antibiotics is being used in poultry sectorsin order to get rid from pathogens and as a growth promoter all over the world. The excessive use of dietary antimicrobials has caused a collective trouble, for instance the progress and growth of resistant microbes, disparity of gut commensals and drug residues in the bird's body [4]. But after the projected outlaw on practicing antibiotics in the feed, products like probiotics are getting substantial importance nutrition offered to the animals. The probiotics are well known for having non-resistant and non-residual properties [5]. Bacillus type of probiotics are being used against salmonellosis [6] and necrotic enteritis [7] and are enjoying the status of immune booster and safe growth promoter in commercial chickens.

To overcome the drawbacks of using antibiotics as feed additives, it is need of the day to find a substitute. Probiotics have the potential to be used for this purpose. Its supplementation in the feed markedly improves weight gain, carcass characteristics and amplifies the intestinal absorption area by increasing intestinal villi length and width. Microscopic modulation of mucosal morphology of the intestine is assumed to improve health indicators and production performance in broiler but the intermediate structural changes, which result into improved performance in this case, are not adequately documented. Basic aim of the chapter is to highlight fruitful effects of *Bacillus Subtilis* as non-antibiotic gut modulator and growth promoter in broiler chicken.

2. Probiotics and its properties in general

Probiotics are the living culture of microorganisms offered orally. These organisms flourish in the host gut and cause rapid activation of the intestinal functions, fortify the beneficial commensals growth in the gut by minimizing the destruction triggered by pathogens, boost up the immune system especially innate immunity, supporting the integrity of the gut mucosa and maintain a stability and balance of normal microflora and improvement of the growth performance [8, 9]. For getting determined benefit (as substitute to antibiotics and used against *Salmonella* spp), recently, de Oliveira et al. [10] recommend and advised the constant offering of probiotics (*B. subtilis*) spores or as vegetative cells. *B. Subtilis* are gram positive, chain/clumps forming rod-shaped endospore forming heterotrophic bacterium. They are facultative anaerobe and has flagella and a single chromosome that is circular in shape and positioned in cytoplasm at nucleoid region. A noticeable benefit of *B. subtilis* in feed is the permanency because it can stand with the hostile environment like low pH and intra luminal bile salt without dropping viability. *B. Subtilis* in animal nutrition is harmless probiotic. Being used extensively as a nutrition supplement in broilers due to its established immunomodulatory, enzymatic, anti-inflammatory and antioxidant activity (**Figure 1**) Bacillus can be used as best substitute to conventional antimicrobial therapy in broilers [11]. Additionally, various species of *Bacillus* organism have the ability to generate some useful

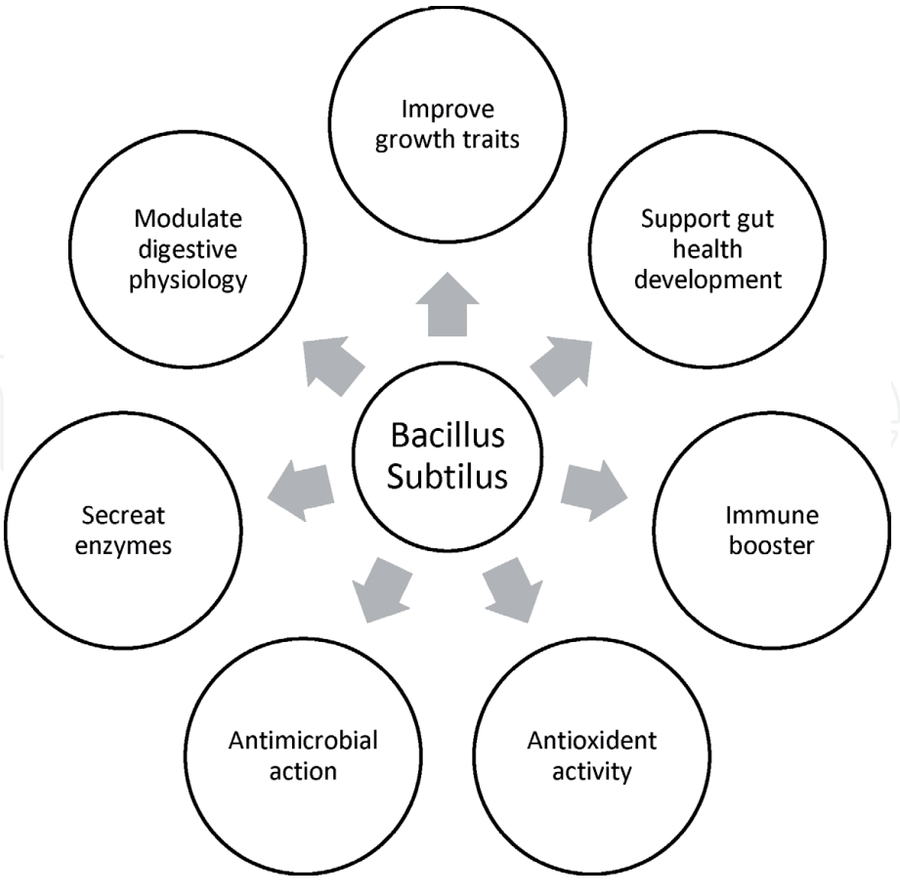

Figure 1.
Fruitful effects of B. Subtiliss *on chicken's body.*

exogenic enzymes like lipase, cellulase, protease, keratinase, xylanase and phytase [12]. These enzymes are expected to be involved in diminished gut luminal stickiness in starch less polysaccharide foods, decomposition of complex feed molecules in the host animal gut, improve nutrients absorption and decline the substrates accessible for pathogens growth. Furthermore, it is observed that *B. subtilis* plays a role in growth enhancement of other normal commensals such as *Lactobacillus* by decreasing pH of the gut luminal [13]. Probiotics are quite active against intestinal pathogens in several ways, viz. including improved immune elimination, striving for vital nutrients, opposing for attachment with mucosa, or producing antimicrobial complexes contrary to numerous pathogens in chickens [14]. The advantageous properties on improvement in digestive performance and also on immune boosting of probiotic supplements have been previously established in broilers [7].

3. Factors affecting animal performance and its possible solution

There are various exogenous and endogenous factors which weaken the performance of immune system in animals [15] and ultimately minimizing the growth production. Pathogens like bacteria, virus, parasites, fungi and harsh environmental stresses also contributing to creating adverse effects on the animal growth. To overcome these circumstances, and, to achieve maximum production, animal industry in the developing countries is using excessive amount of antibiotics as feed additives which is hazardous to human health. It is need of the day to formulate new safe ways and means in probiotics form to boost up the gut health and immune system of the animals in intensive farming systems to achieve maximum production potential and various researchers recommended the constant administration

of Bacillus spores or vegetative cells (a probiotics), along with acidifiers, enzymes, prebiotics and phytobiotics as a potential substitute to antibiotics [16]. These substances are focusing the gut luminal health. As good gut health reflects better growth performance in animals.

4. Association of gut histomorphology with immunity broiler chickens

With standing a good digestive track ecosystem is a criterion for obtaining well-organized animal health, immunity, and performance. Beside nutrients digestion and absorption, the alimentary tract is playing a decisive role as barrier against everlasting attack of disease-causing microorganisms. There is a bulk of pathogenic organisms in the luminal ecosystem of the gut within ingesta. The tight junction in the lining epithelial cells not allowing any pathogens to reach the lamina propria in which a rich blood vascular system exists. In addition to the tight junction, a thick mucus layer which is secreted by the lining goblet cells also complement to maintain the immunity. Disturbance in the immune system may leads to enteritis [17]. During pathogenic bacterial infection, lymphocytes will accumulate in the lamina propria to defend the tissue against the pathogens. Other mononuclear cells including macrophages, plasma cells accompanying the lymphocytes and may cause inflammation and causing thickness in the lamina propria [18]. If the pathogen persists in the lumen then there is high possibility to deteriorate the normal physiological functions of the gut. Normal integrity of the mucosa may be deteriorated. The lining epithelium may be sloughed-off and the mucosa will become shorter and also appear denuded [19]. Thickness will be descended till remaining portions of the mucosa and even submucosa. Fluid will accumulate in the lumen and its absorption will obviously minimizes. Such stressful conditions will leads to affect the systemic immune system of the animals. Important pathogen's effect and influencing that animal effortlessly.

Salmonellosis is one of the important pathogens of chickens which may leads to potential human foodborne infection also [3, 16]. Broiler chickens assist as haulers and express seldom signs of diarrhea not like human. Both living bacteria and its endotoxins displayed similar effects on the gut mucosal surface [19]. The broiler may be perceived as showing inversely synchronizing the physiology of gut rather than showing resistance to Salmonella infection [18]. Few moderate mucosal lesions have been observed by Porter and Holt [20] after *Salmonella Enteritidis* infection in chickens as compared with those in mammals. The later species are observed to be involved in diphtheritic typhlitis, inflammation in intestine, fibrinus filled ceca, penetration of plasma cells and macrophages in the mucosa, and sloughed lining epithelial cells [21]. *S. gallinarum* may be separated from spleen and liver of the chicken [6]. Kwag et al. [21] demonstrated numerous factors through which the pathogen may add to support the gut epithelium impairment. These includes lining mucosal infiltration, production of toxin, and causing enteritis, nevertheless, the precise means for this damage is still not clear [22]. So, we hypothesize that probiotics supplementations have favorable possessions on histology of small intestine and histomorphometric parameters of different organs of broiler chickens. Reviews of this chapters will also help us to understand the influence of *B. subtilis* on selected gut and immune systems parameters of broiler chickens.

Good gut health reflects safe meat production if best management and husbandry protocol is provided to the animal. The alimentary tract is playing a decisive role in nutrient digestion and absorption also act as a defensive obstacle alongside endless violence of disease-causing microorganisms. Numerous microanatomical features determine the total surface area for absorption of the GIT. The intestinal

villi and crypts microarchitecture are linked with the gut function which ultimately leads to the animal growth [23–25]. In order to assess the immune organ status under microscope in animals one needs to slaughter that animal. A prior approval from the ethical committee is utmost required to do this. Soon after humane sacrificing, the immune represented visceral organs including thymus and spleen may be grossly examined for any morphological changes and their representative samples may be collected and processed onward for histomorphometry. To identify the microstructural changes, around 2 cm segments is acquired from different segments of small and large intestines and fixed in some suitable fixatives. The same are then impregnating in paraffin to provide easy environment for micro-sectioning. The slices are afterward processed and stained with dyes namely hematoxylin and eosin along with using some other special staining materials. The prepared slides are then subjected to the process of cover slipping for everlastingly preservation [26]. Pictures obtained from the gut slides will be obtained from the suitable area under microscope fitted with digital imaging system and will be examined for determination of villus length, width, depth of crypt, surface area of villi. Villi and crypt microstructures are also required to study to determine the height of the villus: crypt depth ratio. The goblet cells numbers per villus and its variation on mucins basis viz. acidic, neutral, and mixed from and the histomorphometry (height, width and area of lymphatic tissues in one microscopic field of each section at 4X under light microscope) of immune organs (thymus and spleen) is also needs to be observed because these parameters are needed to provide some clue for immune indicator.

5. Effects of probiotics on growth performance

Probiotics are living microbes and after being supplementation to the feed, it maintains normal intestine commensal equilibrium. The probiotics has been reported to fortify the gut physiology including digestive and absorptive functions [8], which ultimately leads to obtain better growth and FCR in broilers [27, 28], turkeys [29], Pigs [30], Sheep [31], Rabbits [32], Fishes [33], Canine [34] Goats [35] and in Cattle [36]. It has been studied that developed egg weight, size and mass in layers are associated with offering Probiotics [35, 37], along with repressed cholesterol level in chickens [36, 38]. Such fruitful effects created in the organic protein source increases further its demands in the market. The end user love to approach for getting the safer organic meat from the supplier. Several findings have been revealed that direct feed microbials are possible substitutes to antibiotic growth promoters [39, 40].

6. *Bacillus Subtilis* as a probiotic in chicken industry

B. subtilis is gaining much attention towards animal nutrition in the area where animals are kept under stressful conditions such as harsh environment and food deprived locations. The stress may affect directly or indirectly the immune system of the animal body. The immune system comprising organs, tissues and cells those are responsible to maintaining integrity of the body. This system ensures the protective responses of the body to the external substance appropriately. It is observed that probiotics can defend the animals beside disease causing organisms [41] and has encouraging influence on cellular and humoral immunity [42]. Diet supplemented with *B. subtilis* showed better feed conversion ratio (FCR), statistically better mean live body weight, mean weight gains, relatively bigger breasts and reduced mortality [11, 43].

Various bacillus species produced spores that can germinate and became effective metabolically in the gastrointestinal tract, thus displaying achievement comparable to additional well-established probiotics [44]. Awad et al. [45] and Murugesan et al. [8] reported previously that probiotics strengthen the barrier task of the gut mucosa. In the past the Samanya and Yamaouchi [46] found that *B. subtilis* decreases the concentration of ammonia in blood, which ultimately results in motivation of intestinal function and reported to use *B. subtilis* in chicken's feed. In mucosa of the intestinal tract the villus height and its per field area numbers are associated to the maximum absorption capability of the gut lining cells. Enlarged gut mucosal villi is directly proportional to expand the absorptions activity of the nutrients in the GIT [24] and occurrence of small mucosal villi declines the nutrients absorption surface area which ultimately leads to lower the production performance [26]. It is recommended that in feed *B. subtilis* displayed significantly greater villus length of all the small intestinal segments in pigs [47]. Crypts are the areas where the epithelial cells of the villi originate, and deep crypt shows constant turnover of tissue and extraordinary need for fresh tissues [24, 46]. Molnar et al. [43] found that increased infiltration of lymphocytes in the gut mucosa is associated with the dietary inclusion of probiotics in broilers. Little knowledge is available in the literature to study the probiotics influence on animals [38] and there is also least information available regarding influence of various probiotics on histological alterations in gut mucosa of animals. *B. subtilis* has been reported to develop the immunity in weanling pigs [47] and the bacillus spores may cause propagation of follicular cells Peyer's patches in mice [48]. *B. subtilis* based direct feed microbial displayed the immunomodulatory effects on innate immunity in broiler chickens [49]. Molnar et al. [43] recommended the increase concentration of in fed *B. subtilis* significantly decreased the coliform bacterial population in the ileum or in the caecum of the commercial broiler chickens. *B. subtilis* displayed advantageous possessions via one or more of the following means: Reasonable exclusion and minimizing the pathogen colonization and consumption of O_2so that the anaerobic favorable bacteria may flourish [10, 50], production of useful enzymes [13], stimulation of gut physiology [45] and boosted the body immunity in broiler chickens [40].

7. Conclusion

The present work is planned to explore the importance of *Bacillus Subtilis* probiotics in broiler chicken as a feed supplementing agent. Information concerning the outcome of feed supplemented with probiotics on the growth performance, immune status and gut micro-architectural anatomy in health and disease is limited in literature. Knowledge of fruitful effects of the probiotics and the mechanisms may enable the researchers to manipulate the use of this alternatives for better growth production, and safe and healthy animal industry. Based on the latest scientific findings discussed in this chapter, the following main conclusions can be drawn that the *B. Subtilis* as probiotic:

1. Is a harmless and can be used an effective antimicrobial candidate in poultry nutrition.

2. Can withstand the harsh environment within the animal body.

3. Has the ability to modulate gut histomorphology and absorption process.

4. Fortify the enzymatic activity and digestibility of nutrients.

5. Can produce enzymes and release antioxidants.

6. Can modulate the immune system of the animal body and aid in maintenance of the body homeostasis.

7. Has better effect as growth promoter compared to antibiotic growth promoter.

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