Animal Review
2019 Vol. 6, No. 1, pp. 5-16
ISSN(e): 2409-6490
ISSN(p): 2412-3382
DOI: 10.18488/journal.ar.2019.61.5.16

PROBIOTIC SUPPLEMENTATION IN POULTRY PRODUCTION AS AN ALTERNATIVE TO ANTIBIOTIC FEED ADDITIVE

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Article History
Received: 6 March 2019
Revised: 8 April 2019
Accepted: 14 May 2019
Published: 2 July 2019

Keywords
Antibiotics resistant
Growth promoters
Probiotics
Poultry production.

ABSTRACT

Provision of safe and healthy poultry products to the human being is the prime objective of the poultry industry, but one should keep in mind the welfare of animals and reverence for the environment. Meat and eggs demand also increasing with the increase in world human population. To meet this increasing demand for meat and eggs, antibiotics growth promoters were used world widely. After the ban on antibiotics growth promoters due to its residual effects on human health, nutritionists are focusing on novel growth promoters those without causing productivity loss, or product quality has augmented. Therefore, probiotics are a safe alternative to antibiotics and its beneficial effects proven from many decades. Live microorganisms are used to deliver health benefits to chicken, usually by reestablishing or improving the intestinal microbiota. Probiotics have a beneficial effect on weight gain, immunity, meat traits and somewhat impact on gut health of poultry. The subject of review paper will be worthwhile meant for researchers and poultry nutritionists to advance their understanding of alternatives to antibiotics in poultry production without affecting performance and the welfare of chicken.

Contribution/Originality: This study is important in regards to antibiotic resistance in human being coming from animal products. Antibiotic resistance is one of the biggest public health challenges of our time. Each year so many people get an antibiotic-resistant infection, and many people die. Fighting this threat is a public health priority that requires a collaborative global approach across sectors. So, this study will give direction to researchers and food producers to avoid from antibiotics harmful effects to animals and as well as human being.

1. INTRODUCTION

Poultry business recognized as one of the rapidly growing section among different agriculture sectors. Consumption of meat and eggs is increasing due to easy availability, comparatively reasonable price and also rich in entire important nutrients which could cover the deficiencies of essential food amino acids, minerals, and vitamins Dhama, et al. [1]; Ajit, et al. [2]. Rinttilä and Apajalahti [3] reported that poultry intestinal health has been investigated and studied extensively for the higher production performance of chicken, as the intestine is an important site where nutrient absorption takes place. While the gut is the main organ which exposed more to pathogens microorganisms after skin [4]. For this purpose, since 1940 antibiotics were used in animal feed as

growth promoters and for gut health maintenance [5-8]. Antibiotics can improve growth rate [9] but on the other hand, resistance to antimicrobial agents is increasing between foodborne microbes that can make difficulties in public health remedies [10, 11]. Also, antibiotics also destroy some beneficial microflora of gut which may help in feed digestion. Furthermore, antibiotics reduced the growth rate by escalating dysbacteriosis and subclinical necrotic enteritis [12]. Started from Sweden in the year 1986, antibiotics were taken out from the diet of poultry, and pig world widely [6]. Since 2006, antibiotics usage as an animal feed additive is banned by the European Union. Therefore, a need for the development of novel interventions that only target pathogenic microorganisms whereas safe for beneficial organisms [13]. Recently nutritionists put their efforts on given that a unique and different growth promoter and therapeutic agents for disease prevention as well as for the enhancement of chicken immune status to avoid antibiotics harmful residual influences on meat and eggs products [14, 15]. Manufacturing probiotic feed and food is a crucial progress area for future practical feed and food industry. Currently, the use of probiotics is generating a great deal of interest for improvement in poultry production [16].

Probiotics have been used for health benefits in animal feed as well as in human diet for several years as antibiotics alternative [17]. Both FAO, as well as WHO in 2002, has well accepted that probiotics are viable microorganisms those must be safe and sufficient for body function that gives benefit to the health status of both animals and humans [18]. Moreover, Hill, et al. [19] also gave statement same as FAO and WHO for probiotics definition, but he opined simple grammatical correction that probiotics are living microorganisms when consumed by animals in sufficient quantities, will improve animals health and productivity. Probiotics when added into feed the gut microflora can also be balanced. Probiotics microorganisms provide benefits to the animals through improving intestinal microbial balance and as a result enhancing absorption of nutrients, feed efficiency, the growth rate of chicken and economic output of birds [20]. There are so many beneficial and protective effects of probiotics such as removing pathogenic bacteria by competitive exclusion.

Furthermore, the positive use of live microorganisms helps in increasing growth and productive performance, egg quality, and composition in layers chicken, improve nutrients digestion and absorption and also maintain the chicken health [21-23]. The ultimate noteworthy advantage of probiotics is that in animal products, which consumed by humans have no residues and it does not exert any resistance in the human while consuming animals products. Also, they have a beneficial impact on the production performance of chicken [24, 25]. Many studies told us that probiotics have immunomodulatory effects [26, 27]. Probiotics as an alternative to antibiotics growth promoters have become prevalent in the poultry industry because consumers demand meat from animals without antibiotics residues [28]. Probiotic species used in chicken were *Streptococcus*, *Bifidobacterium*, *Lactobacillus*, *Bacillus*, *Candida*, *Enterococcus*, *Saccharomyces*, *and Aspergillus* have a beneficial influence on the weight gain of broilers [29, 30]. The principal objective of our review paper is to describe the positive effects of probiotics in poultry production as an alternative to antimicrobial growth promoters, their mechanism of action, and impact on immunity, meat traits, and their selection criteria and also to review their uses in the poultry production.

2. WHY WE NEED AN ALTERNATIVE TO ANTIBIOTICS?

When the ban on antibiotics growth promoter agents in animals in 2006 by the European Union due to the following possible risks to animals as well as to human.

1. Antimicrobial resistance develops and threatens the actual prevention and treatment of an ever-increasing range of infections produced by microorganisms.
2. Antimicrobial resistance is a serious increasing risk for global public health that necessitates action across all societies worldwide.
3. If antibiotics lose their effectiveness due to resistance come from animal meat consumption, the success in the treatment of major diseases would be compromised.

4. Treatment cost of the antimicrobial resistant patient may increase to longer illness duration, extra tests and more expensive drugs use [31].

Due to the above possible threats to human health from antibiotics, we need an alternative growth promoter for poultry production.

3. WHAT IS PROBIOTIC?

Probiotic is single or multi-cultured alive microorganisms which improve the beneficial indigenous microflora of host [32]. Probiotics were first used in 1954 to indicate substances that were required for a healthy life. Rather, et al. [33] included the bacterial metabolites and dead bacterial culture in the probiotics definition. They are applied in farm animal nutrition to increase weight gains and for the improvement of feed conversion. Microorganisms such as mix cultures of different microbes, fungi especially mushroom and yeast and bacteria have been previously used as probiotics. Willis, et al. [34] constantly used fungi myceliated grains colonized as probiotic for broiler chicken by the eatable mushrooms shiitake (*Lentinula edodes).* For the treatment of *Eimeria tenella* infection in chickens, wild mushroom *Ganoderma lucidum* used Ogbe, et al. [35]. Lee, et al. [36] reported that *Saccharomyces boulardii* yeast also used as a treatment of *Eimeria* infection in poultry. In poultry for the control of pathogenic bacterial infection, yeast *(Saccharomyces cerevisiae)* and *Aspergillus oryzae* (fungi) also were used as probiotics [37]. While bacterial species are more frequently used as probiotic than fungal probiotic species. Probiotics also have the ability to eliminate many pathogenic bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Clostridium perfringens*, and so on Iannitti and Palmieri [38]. Kabir [39] reported, commonly used probiotics microbes are intestinal strains mostly, *E.coli*, *Lactobacillus casei*, *L.helveticus*, *L.plantarum*, *L.acidophilus*, *L.lactis*, *L.salivarius*, *Enterococcus faecium*, *E.faecalis*, and *Bifidobacterium* species.

4. SELECTION CRITERIA OF PROBIOTICS

The probiotics strains selection is based mainly on its potential to confer a health benefit for poultry. For achieving beneficial results from the use of probiotics must fulfill the following requirements. Should be nonpathogenic and nontoxic to poultry. Exact taxonomic identification of probiotic species must consider. A typical inhabitant of the directed species that can survive, colonization and must be resistant to bile, enzymes and other gastric juice of host and metabolically active in the targeted site [40]. Bile tolerance in the small intestine is important for the survival of lactic acid producing bacteria and also for the functionally active in the intestine [41, 42]. Can compete with pathogenic microflora of gut for adhesion to the intestinal epithelium. Genetically stable that can produce antimicrobial substances to antagonize the pathogenic flora and to regulate the host immune response. When added in the poultry feed it must show there required organoleptic and technological properties. A concerned strain of probiotic must have stability during manufacturing, storage, and during delivery. It should have maximum sustainability at higher populations [40].

5. FORMS OF PROBIOTICS

Probiotics are presented in different forms for example in liquid, powder, sachets, paste, tablets, gel, and granules or capsules and many other different forms [38]. Dry forms of probiotics showed enhanced tolerance power to gut environment and higher shelf life during storage. Good viability of probiotic in poultry is available with the use of hydroxypropyl methylcellulose phthalate-55 as a tablet matrix [38].

6. MECHANISMS OF ACTION OF PROBIOTICS

Probiotics have a number of the mode of actions based on the competitive exclusion of pathogenic bacteria, production of antibacterial substances and organic acids production for inhibition of all pathogens Mazmanian, et al. [43]; Tiwari, et al. [44]. Roselli, et al. [45] proposed that probiotics can moreover govern the anti-inflammatory

cytokines production. Probiotics have the ability to regulate and maintain the intestinal barrier functions and gut microbiota homeostasis, Salminen, et al. [46] regulate the gut enzymatic activity and absorption of essential nutrients Hooper, et al. [47]; Timmerman, et al. [48] also Gill [49] studied that it prevents procarcinogenic enzymes activity and also interfere with pathogens to inhabit and infect the gut mucosa. Furthermore, probiotics have the ability to improve the protein as well as some minerals like calcium, iron, manganese, and copper absorption from the gut by making acidic pH of the intestine; regulate the production of mucous, regulate epithelial functions and increase intestinal motility [50]. Also act as an immunomodulator [51] as probiotics exert effects on host antigen presenting cells, regulatory T cells, effector B and T cells, and enterocytes as well [52]. Probiotic can activate various immune cells and exert immunomodulatory effect by affecting the T-helper cells in a specific manner [53]. Probiotic bacteria can also lessen the cardiovascular diseases risk by lowering blood cholesterol levels of the host animal [54]. Unraveled antiviral potential of probiotic bacteria (*Lactobacillus Plantarum* YML009) against H1N1 influenza virus was demonstrated by Rather, et al. [33]. Hydrogen peroxide and lactic acid production increased in the gut when fed probiotics, as probiotics have the ability to enhance the growth of beneficial gram-positive bacteria, lower the number of intestinal pathogenic microbes [14].

7. ADVANTAGES OF PROBIOTIC FOR POULTRY

There are so many beneficial properties of probiotics for poultry proposed by Piva [55]; Jenkins, et al. [56]; Simmering and Blaut [57].

1. Have the ability to modify intestinal microbiota of chicken
2. The stimulatory effect on the immune system of chicken
3. Reduce inflammatory reactions in the host
4. Prevent pathogen colonization in the intestine
5. Enhance the performance of animal
6. Have the ability to lessening the carcass contamination
7. Lower the blood cholesterol level observed beneficial for chicken
8. It decreases the ammonia and urea excretion from the chicken

8. EFFECT OF PROBIOTICS ON WEIGHT GAIN

Many studies revealed that probiotics supplementation to poultry has a constructive influence on chicken health and performance. Improved performance of broiler and reduced level of blood cholesterol were seen with the supplementation of the probiotic (*Pediococcus* acidilactici). Significant improvement in daily weight gain and in carcass yield was observed in experimental birds than control during all study periods of 6 weeks in both vaccinated and non-vaccinated birds [30, 58, 59]. The addition of milk kefir about 2% as prebiotics in meat-type chickens drinking water would increase growth performance of broilers, and it may also be supplemented in the chicken feed. For achieving the best performance of poultry, only optimum probiotics level was better than an increase in doses (quantity) of probiotics [60]. Reduction in the requirements of protein as well as essential amino acids was observed when spores of Bacillus subtilis used as probiotics feed additive and as a result, the cost of production for per kilogram weight gain can be decreased Mojtaba, et al. [61]. Rajput, et al. [62] reported that the addition of *Saccharomyces boulardii* and *Bacillus subtilis* as probiotics bring improvement in chicken to live body weight, increases bursa of fabricius, as well as thymus weight. Also, it was reported that a significant improvement observed in weight gain and the weight of thymus and bursa of fabricius in probiotics fed groups [60].

9. EFFECT OF PROBIOTICS IN IMMUNOMODULATION

In addition on the way to the improvement in production performance, probiotics also enhance immunity against Salmonella challenge without adverse effects on kidney functions in broiler chicks observed by Dina, et al.

[27]. Newcastle antibody titers were significantly higher in broiler chicken fed probiotics as a supplement in the diet as compared to control group Rowghani, et al. [63]. Alkhalf, et al. [60] investigate that at 28 and 42 days of age the thymus relative weight was greater in all supplemented groups than the control group which leads to an increase in lymphocytes numbers in the primary lymphoid organs of the chicken. Spleen weight was observed greater for the probiotics fed chickens than the control group [64]. Moreover, the relative weight of bursa was improved and increased number of medullar follicles found in the probiotics fed groups as compared to control groups of animals [65]. Increase in the bursal relative weight could be a primary positive indication for immunity improvement in meat-type chickens [66]. *Clostredium butyricum* when given as a supplement in the feed as probiotics produce large amounts of short-chain fatty acids, such as butyrate and acetate [67] which are an energy resource for animals and exert proliferative effects on colonocytes [68]. In broiler chickens improved immunity and balance in intestinal microbiota was observed when chicken fed with *C. butyricum* as a probiotic Yang, et al. [69]. Midilli, et al. [70] investigated that the antibody-mediated immune response was higher in probiotic-fed animals group. Moreover, presentation of immune-related T helper cells was higher due to probiotic supplementation in diet [71]. These above findings were confirmed and elaborated by Koenen, et al. [24] when they use probiotics and investigated that these special effects were mediated by cytokines which secreted by immune cells and this process were stimulated by probiotics.

10. EFFECT OF PROBIOTICS ON GUT HEALTH

We find after a brief review of the literature that probiotics have very limited effects on the core physiological functions (digestion, absorption, and propulsion) of the digestive tract of poultry. The strengthening of the intestinal mucosal barrier against harmful agents is the major function of probiotics. However, the increase in jejunal goblet cells, jejunal and ileal villus height and width were improved when giving *Saccharomyces boulardii* and *Bacillus subtilis* in the diet. The findings of Rajput, et al. [62] revealed that, an increase in jejunal immunoglobulins (IgA-positive) cell numbers in probiotic fed animal groups. It has been stated that probiotics species those have more influence on the modulation of gut microbes and the inhibition of pathogenic microbiome are *Saccharomyces*, *Aspergillus*, *Bacillus*, *Candida*, *Streptococcus*, *Bifidobacterium*, *Enterococcus*, and *Lactobacillus* [25, 72]. The pathogenic microbes inhibition could be somewhat because of the acidic pH of the gut. The decrease in pH is due to the breakdown of carbohydrates by probiotics bacteria in the gut which consequently produces volatile fatty acids as well as lactic acid and succinic acid some time, and these acids production in gut ultimately reduces the pH of the intestine [73]. It is recommended that probiotics supplementation in poultry make a valuable contribution as they do not require a withdrawal period.

11. PROBIOTICS EFFECTS ON CHICKEN MEAT TRAITS

For meat processing and storage physicochemical properties (pH, color, texture, etc.) are much important. As these physicochemical properties are interconnected and could affect the meat quality. For processing and storage of meat, meat color and pH of meat is an important trait of meat quality, and these both traits together should be considered for meat quality evaluation [74]. Above mention traits are closely linked to water holding capacity of meat, it is another significant characteristic of meat. When *Bacillus subtilis* fed to broiler chickens and meat from these chickens was stored for 7 days, during storage the significant decreased in pH was observed and this lower pH is beneficial for meat storage [75]. Significantly increase in the breast meat redness was observed when *Lactobacillus fermentum* was added in broiler drinking water as probiotics [76]. And the redness in meat is most favored by consumers [77]. However, the other experiment of Haščík, et al. [78] revealed the significant increases in the lightness of thigh and breast meat cuts and also increased in yellowness and redness in thighs cuts when supplementation of probiotic used in mixture with bee pollen in the broiler. Overall meat sensory attributes were evaluated and described that supplementation of probiotics in broiler chicken diet enhanced the meat quality and

microbiological values through the pre-freezing as well as post freezing meat storage [79]. The total viable bacterial count was lower for meat obtained from probiotic fed chickens as compared to the meat of control birds. Also, quality traits of the meatballs appearance, juiciness, texture, and wholesome acceptability were significantly higher in probiotic fed birds while for flavor were lower in probiotics fed birds [80]. On the other hand, improved tenderness and overall quality of meat reported when fed cell components of *Saccharomyces cerevisiae* as probiotics by [81]. Tenderness of poultry, as well as other livestock meat, might be made better by supplementing the whole yeast or yeast extract from *Saccharomyces cerevisiae*.

12. EFFECT OF PROBIOTICS ON THE OXIDATION OF LIPID IN MEAT

Lipid oxidation is a very important deteriorating factor of meat quality and also for other food quality. Lipid oxidation commonly complemented via the development of negative changes in odors and flavors. Fatty acids composition of meat, linked with the nutritional value of meat is a key component of meat quality. Different studies revealed that the fatty acids profile of meat positively affected by probiotics feeding, a major positive effect was the reduction of saturated fatty acids contents and an increase in polyunsaturated fatty acids in meat. When *Aspergillus awamori* and *Saccharomyces cerevisiae* used separately or in a combination in broiler diet, the significant reduction in saturated fatty acids profile and increase in polyunsaturated fatty acid in meat was observed [82]. The thiobarbituric acid reactive substances (TBARS-test) profile is usually a measure of oxidation in food and also in meat. Significantly decreased content of TBARS in breast broiler was observed those fed probiotic (*Aspergillus awamori* and *Aspergillus niger*). Also increased tocopherol level in muscle was observed and led to low lipid oxidation in the experimental groups Saleh, et al. [83]. Abdurrahman, et al. [84] studied that probiotics showed significant antioxidant activity observed in both in-vitro as well as in-vivo trials.

13. CONCLUSION

The demand for antibiotics free residues meat and eggs are probably increasing with the increase in world population and advancement in technology. Therefore, to cope with the increasing meat and food demands we need more proficient and fast methods. Even though still probiotics are not well established in animal diet, but probiotics are a much better alternative to antibiotics in poultry production as probiotics have ability to improving poultry intestinal microflora, nutrients utilization, immune response and so on. With the advancement in research and technology, we have a list of probiotics species that have a beneficial impact on the animal as well as on a human. While the implementation of probiotics as an alternative growth promoter is not well developed in poultry production, with research advancement, probiotics will grow into an effective means for the antibiotics free residues meat and eggs production. Further research on the beneficial impact of probiotics as antibiotics alternative will definitely advantage for all poultry producers countries.

Funding: This study received no specific financial support.
Competing Interests: The authors declare that they have no competing interests.
Contributors/Acknowledgement: All authors contributed equally to the conception and design of the study.

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