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Probiotics Application in Poultry Industry: From Feed to Meat

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Probiotics as a potential substitute for antibiotics in animal farm especially poultry industry has become an area of great interest. The use of antibiotics in poultry farming results in presence of antibiotics residue in poultry product and may consequently affecting human health by developing pathogenic microbes to be drug-resistance. In addition to that, poultry are normally grown in dense population for economic efficiency which caused stress and disturbance in the intestinal microbiota of the poultry. This will lead to lower immune system of the poultry against diseases. Therefore, the use of probiotics is an alternative method to cater these challenges in poultry farming. This increase attention toward probiotic supplementation has generated an extensive body of research in the present day. However, there is still a lot of debate in scientific literature regarding the significant effect of probiotic on immune system against specific pathogens and growth performance in poultry. Taking into account the immune response and performance research work, this review provides a summary on the mode of actions and the potential application of probiotics in the growth performance of poultry, with critical evaluation of the recent published works.

Keywords: Probiotics, poultry industry, application, mechanism of action

INTRODUCTION

In 1928 with remarkable discovery of world's first antibiotic by Alexander Fleming, antimicrobial products are the most frequently prescribed medicine worldwide. In a decade time frame, antibiotic usage is predicted to increase more than two-folds than previous (Castanon, 2007; Awad et al. 2012). Widespread usage of antibiotics also had influenced livestock industries on extensive usage to control animal diseases. Poultry industry which is one of the high profit-driven agriculture subsectors, always prone to various infectious diseases (Zhou et al. 2020). There are hundreds of pathogen species that can potentially pose different levels of harm to the poultry. Antibiotics become critically important for preventing, controlling, and treatment of several diseases in the poultry industry (Zhou et al. 2020). Some antibiotics had been incorporated into the feed for increasing the growth rate, enhancing the feed efficiency, and improving the quality of the animal (Sarmidi & El Enshasy, 2012; Cheng et al. 2014;

Landoni & Albarellos, 2015).

Growing access to and use of antibiotics has been linked to increasing antibiotic resistance incidence in both pathogens and commensal bacteria. World Health Organization (WHO) had highlighted recently on the antimicrobial resistance problem which being a rising threat to human health in the 21st century (Sánchez-Salazar et al. 2020). Livestock has played an important role in the rising of antibiotic-resistance bacteria, leading to primary infection and intestinal dysbiosis among farming animals, and finally spreading to human (Peralta-Sánchez et al. 2019; Zhou et al. 2020).

Among numerous cases, antimicrobial resistance in *Salmonella* spp. being one of the principle zoonotic agent that threatens public health and animal production worldwide (Peralta-Sánchez et al. 2019). Poultry meat and eggs represented the principle sources of antibiotic resistance *Salmonella* infection in the food supply chain (Landoni & Albarellos, 2015; Zhou et al., 2020). About 15% of all antibiotics used in all over Europe went into animal feeds, in the year 1999. From this amounted to an estimated of 3.52 million kilograms of antibiotics predicted has entering human body via chicken and pork meat alone (Edens, 2003; Zhou et al. 2020). Rising antibiotic resistance incidence had driven European Union (EU) to officially ban the usage of all antibiotics for the sole purpose of growth promotion in poultry, in the year 2006 (Edens, 2003; Landoni & Albarellos, 2015).

Around the world, the EU decision has been extremely influential as it has caused unprecedented change in the way poultry production is practiced today. The EU decision has made the provision of a healthy food supply as the primary goal of the global poultry industry. This has drawn a great pressure upon the producer to make the product conform to the standards set by the consumers (Cheng et al., 2014; Landoni & Albarellos, 2015; Zhou et al., 2020). Thus, the need to find for effective alternatives to antibiotic growth promoters, has started to be excelled. This lead to the need to do significant change in animal feed formulation to add many functional ingredients to improve animal health and digestion without further addition of antibiotics. Among these additives, enzymes such as phytases and xylanases, vitamins, amino acids and natural immune-enhancing compounds (El Enshasy et al., 2016; Dailin et al., 2018; 2019a,b; Kandiyil et al., 2018). The most recent findings in the field of animal nutritional indicate that the constant use of probiotics enables the improvement of livestock health and production (Mookiah et al. 2014; Tayeri et al. 2018).

Effect of probiotics in human health is well established and extensively studied (Yadav & Shukla, 2017; El Sayed et al. 2014; El Baz et al. 2018; Dailin et al. 2019a). There were only 5 indexed publications under "probiotic" topic in the year 1990, meanwhile today it is exceedingly more than 1000 publications. These increased research outcomes have significantly improved understanding on fundamental mechanisms and beneficial effects by probiotics on the host (Yadav & Shukla, 2017; Kim et al. 2019). In addition to that, there are many previously reported researches involving probiotic cultivation process optimization for high cell mass production (Kepli et al. 2019; Selwamani et al. 2020; Eyahmalay et al. 2020). Benefits of probiotics microorganisms in the livestock industry, especially in poultry are being well characterized and utilized as promising poultry feed nowadays. Supplementation of single or mixture of probiotic bacterial or yeast strains prevent growth of pathogens in the animal and reduced antibiotic usage. Additionally, it is not only decreasing the farm diseases but also improved the overall performance of the birds (Khalique et al. 2020; Zhou et al. 2020). Therefore, this review will address the concept of probiotics for use in the poultry industry as an alternative to antibiotic growth promoters. The objectives of this review are to describe the principles, mechanisms of action and criteria for selection of probiotics, and to summarize their applications in the poultry industry.

Definition and Characteristics of Probiotics

Probiotics are known as viable microbial species, ingested for the purpose of promoting the gastrointestinal flora to improve guts health. It was first introduced by Elie Metchnikoff in 1907 who discovered the possibility to modify the gut microbiota by replacing the harmful microbes with useful ones. Probiotics have been defined in different terms over the past several years. The term probiotics which means "for life" in Latin (pro) and Greek (bios), could be directly translated as "promoting health". It was first used by scientists in 1953 as various organic and inorganic supplements that had the ability to restore health of malnourished patients (Kollath, 1953). Lilly and Stillwell (1965) introduced it as a substance produced by protozoa that could prolong the growth of other species.

Other scientists have proposed to include other substances that contributed to guts health besides microbial organisms (Parker, 1974). As the term progresses over the years, it was generally defined as “live microbial feed supplements which beneficially affects the host animal by improving its intestinal microbial balance” as proposed by Fuller (1989) and in 2001 the definition has been updated by FAO/WHO as “live microorganisms which, when administered in adequate amount, confer a health benefit to the host” (FAO, 2001).

Probiotics may contain various types of microorganisms; two most common are lactic acid bacteria from the genus *Lactobacillus* and *Bifidobacterium*. Besides these, *Bacillus, Streptococcus, Lactococcus and Saccharomyces cerevisiae* are among other bacteria that are used in poultry and animal probiotics (Mahfuz et al. 2017b). Medicinal fungi such as mushroom and yeast may also be used as probiotics in animal feed (Willis et al. 2011; Mahfuz et al. 2017a). It has been reported that *Lactibacillus* strains could promote growth performance, improve meat quality and egg production, enhance immune response and prevent diseases in poultry industry (Wang et al. 2017). Different strains may exhibit different properties and clinical effects from one another, even though they belong to the same bacterial species. The strains of bacteria are selected based on their characteristics for effectiveness use in the guts.

An important characteristic of a probiotic is the viability and survival ability on stress condition in digestive tract such as acidic condition, gastric juice and bile salt exposure (Damayanti et al., 2017). This includes its effectiveness to adhere to the intestinal epithelium of the hosts to demonstrate a hostile movement against pathogenic microbes (Ghadban, 2002). Noohi et al. (2014) revealed most of the lactic acid bacteria (LAB) isolated from poultry was of genus *Lactobacillus* with 78% from *Lactobacillus brevis* as major species and others from *L. reuteri* (16.6%), *L. plantarum* (3%), and *L. vaginalis* (2%). In their study, identification of the bacteria was performed by morphological, biochemical and molecular tests which includes Polymerase Chain Reaction and gene sequencing.Sanders (2008) has stated that it is difficult to characterize probiotic for instance in vitro study on the adherence of the probiotic to the intestinal epithelium of the hosts, which gives limited predictability to in vivo situation.

Limitation in animal feed especially for poultry industries includes the contamination of mold and mycotoxins in the feed. This issue could lead to fatal effect if the feed was contaminated with mycotoxins and accumulation of these contaminants causes immunosuppressive effect, unproductive livestock and even death (Topcu et al. 2010). One of the alternatives to overcome this issue is to use microorganisms that have binding ability and detoxification of the mycotoxins. The use of natural materials and microorganisms in feed for poultry is expected to be safe and ensure healthy and safe-to-eat livestock. Previous studies have investigated probiotic with antifungal properties isolated from *Lactobacillus* strains (Gomah et al. 2010; Damayanti et al. 2017). The ability of probiotic to inhibit aflatoxin is important to reduce contamination in the feed from growth of mycotoxin-producing fungal biomass.

Probiotic isolated from different strain such as *Enterococcus faecium* has been used to treat or prevent diarrhea, to facilitate immune stimulation, or to improve growth of broiler chicken (Wu et al., 2019a). This study has reported an improved growth performance and immune response of broiler chickens by diet supplemented with 5×10^7 CFU/kg *E. faecium* NCIMB 11181 under normal conditions. They have also reported a significant effect of probiotic from *E. faecium* on the improvement of gut injury of broiler chicken caused by necrotic enteritis (Wu et al., 2019b). Study on the effect of probiotics supplemented with vinegar has also been performed by Allahdo et al. (2018). They have reported significant increase in villus height, crypt depth and decreased in small intestine muscular thickness and abdominal fat of broiler chicken.

Probiotics Mechanism of Action

Mechanism of probiotics improving the health of organisms have been well reviewed previously (Sherman et al., 2009; Bermudez-Brito et al., 2012; Halloran and Underwood, 2019; Plaza-Diaz et al., 2019). However, Reid (2016) reported that actions of probiotics cannot be reviewed similar way as for drugs. Most of the prescription had been well studied on the complete mechanism of action. However, mechanism of probiotics must be ascribed with a shared mechanism of action (Figure 1) and it is to create more favourable gastrointestinal environment (Reid, 2016). This would have multiple and diverse influences on the host, directly. Figure 1 illustrating the three main mechanisms of action by probiotic microorganisms in poultry which provide maintenance of healthy gut microbiota, competitive exclusion of pathogens, and production of metabolic enzymes to breakdown complex carbohydrates in the feed.

One of the well understood action of probiotics would be establishment and modification of the intestinal microbiota of the host. Wang et al. (2017) had demonstrated that application of probiotic microorganism in the Qingjiaoma chickens had flourished the gut microbiota diversity. An increase in the abundance of *Bacteroidetes* and *Firmicutes* had been reported in the chicken faecal microflora. The supplementation of probiotics in chicken feed also proven to improve the chicken meat flavour. The study also reported an increase in acetate propionate, butyrate and isobutyrate contents in the chicken faecal positively correlated with the abundance of gut microbiota (Wang et al. 2017).

Probiotics antagonize pathogenic microorganisms by various mechanisms, after establishing a healthy gastrointestinal environment. These probiotics are naturally capable to produce various organic acids and short chain fatty acids (SCFAs) during fermentation of carbohydrates. In addition, other components of the probiotic metabolome were including organic acids, hydrogen peroxides, amines, volatile fatty acids and enzymes also have been reported to interact with multiple targets of cells. These probiotic components regulate cellular proliferations, differentiations, apoptosis, inflammations, angiogenesis and metastasis in the host (Plaza-Diaz et al. 2019). Probiotic strains of *L. reuteri* were proven to produce reuterin and biosurfactants that inhibit attachment of uropathogens in the broiler chickens (Langa et al. 2014; Greppi et al. 2019). Probiotics also produce numerous antimicrobial substances and defensins, which could alter luminal pH, inhabiting bacterial adherence and translocations in the gut (Ng et al. 2009; Bermudez-Brito et al. 2012). Some of the probiotics also can produce antimicrobial peptides known as bacteriocins that prevent the proliferations of pathogens.

Recent studies had demonstrated that application of probiotics in poultry industries has successfully reduced *Salmonella* sp. Carter et al. (2017) has used a combination of *L. salivarius* and *Enterococcus faecium* to treat *Salmonella* infection in poultry. Similarly, application of commercially available anti-*Salmonella* probiotic products such as FloraMax-B11 and FM-B11 which containing various *Lactobacillus* strains had reduced the infection caused by same pathogens (Prado-Rebolledo et al. 2017; Kowalska et al. 2020).Metabolic enzymes production by probiotics being another interesting and has wide application. Poultry is an industry consuming large quantities of grain ingredients. Probiotics enzymes are now being routinely used in the poultry feeds to improve digestibility of the feed ingredients.

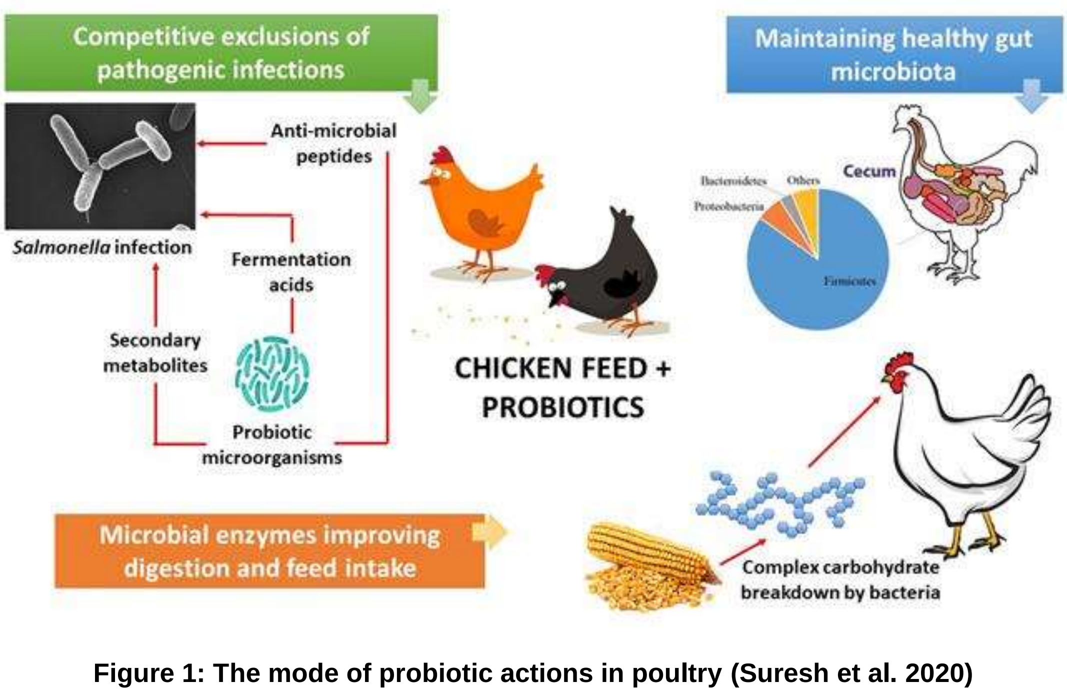

Figure 1: The mode of probiotic actions in poultry (Suresh et al. 2020)

Breaking down the shell of the plant cells in the feed increases access to their nutrients and increase feed consumption rate. Udeh et al. (2019) had demonstrated that broiler chicken served with dietary inclusion of probiotic *Saccharomyces cereviasae* and probiotic enzymes had increased final body weight. Various bioformulation of feeds incorporated with probiotics and their enzymes has been developed to enhance body weight gain with no significant impact on gut epithelium and reduced cholesterol levels in the broiler chickens (Suresh et al., 2020).

Factors Contributing In Probiotics Efficacy

The use of probiotics has rapidly grown in recent years, enhancing the performance of broilers and leading to the production of products free of any probiotic trace. Recent evidence suggests that the use of microbial probiotics can play a significant role in the future of the poultry industry (Aziz Mousavi, 2018). There are many factors that will contribute to the efficacy of using probiotics in poultry industries, such as the origin species and selection of microbial strain, probiotic preparation method, survival of colonizing micro-organisms in the gastrointestinal tract conditions, the environment where the birds are raised, nutritional, management (including probiotic application time and application route), physical and the immunologic state of the animals, the lineage of poultry evaluated, as well as age and lack of association with mother hens and concomitant use of antibiotics.

Nutritional Factor

There are many stress factors in the environment of newly hatched poultry species that could reduce the effectiveness of the maternal antibody defence mechanism and normal colonization of the gut by beneficial microorganisms allowing the colonization of pathogens during the early post-hatch stage. There are high probabilities that newly hatched chickens and turkeys will face a situation in commercial as well as in experimental settings that will alter the development of natural gut-associated beneficial microorganisms. The primary factor affecting this development can be the feed source and quality. Under-formulated diets result in nutritional stress and decrease the growth of beneficial organisms. Molds and mycotoxins further add to the problem of nutritional stress and may cause loss of essential nutrients for the gut microbes. However, nutrient degradation may be the most important factor to affect the gut microbes. This can be caused by numerous factors such as oxidized dietary fat and lipid peroxidation, vitamins, amino acids and proteins also influence the populations of beneficial organisms in the gut. Furthermore, in this era of concern about microbial contamination of feed, high pelleting temperatures in feed manufacturing causes destruction of not only pathogenic but beneficial organisms as well. The only probiotic organism that can tolerate relatively high temperatures associated with the pelleting of chicken and turkey feed are the spore-forming *Bacilli*. All other probiotic organisms will die as a result of pelleting. Therefore, most probiotics must be applied via drinking water or as a top dressing to pelleted feed (Edens, 2003).

Selection of Microbial Strain/ Origin Species

In addition to being non-pathogenic to animals, micro-organisms used as probiotics are selected on the basis of their survival in the gastro-intestinal environment and ability to withstand low pH and high concentrations of bile acids. In addition, the chosen strain should tolerate the manufacturing, transportation, storage and application processes, maintaining its viability and desirable characteristics (Collins et al., 1998). The capacity of potential probiotic micro-organisms to withstand the gastro-intestinal environment can be tested in vitro by challenging with low pH (Hood and Zoitola, 1988; Collado and Sanz, 2006). The capacity to tolerate an acidic environment and bile varies among strains (Mishra and Prasad, 2005). Another desirable characteristic is the ability to adhere to the intestinal epithelium, enabling the probiotic strain(s) to colonize the intestine (Guarner and Schaafsma, 1998). In addition, ability to grow rapidly on inexpensive media is a requisite (Collins, Thornton and Sullivan, 1998) for economically viable production. Spore forming bacteria, particularly from the genus Bacillus, are increasingly being used as probiotics. Bacillus spores are resistant to physical and environmental factors, such as heat, desiccation and UV radiation (Mason and Setlow, 1986; Nicholson et al., 2000; Setlow, 2006; Cutting, 2011) enabling them to maintain their viability during feed pelleting, storage and handling. *Bacillus lavolacticus* DSM 6475, and two species (total four strains) of Sporolactobacillus (*Inulinus* Sp. and *Laevus* Sp.) were resistant to pH 3, and *B. racemilacticus* and *B. coagulans* were tolerant of bile (Hyronimus et al. 2000).

Probiotic Preparation Method

Growth in inexpensive media is important for commercial production but the ideal growth medium that maximizes microbial growth can be very complex and expensive (Muller et al. 2009). Different probiotic strains generally require different media. Growth conditions temperature and pH affect fermentation growth rates, which are species and strain dependent. Probiotics can be produced by either batch or continuous fermentation. In batch fermentation, all of the substrate (sterilized) and the inoculum are mixed together in the fermenter at the beginning and kept at the optimum temperature for the growth of the probiotic. In fed-batch fermentation, limiting nutrients can be added during the fermentation. The reduction of pH in the fermentation medium, to the level where it inhibits the rate of microbial growth, is one of the challenges with batch fermentation and is generally managed by adding a base or a buffer to the medium to maintain pH (Muller et al. 2009). After completion of the fermentation process, which is generally determined by measuring the concentration of probiotic in the fermenter, cells are recovered by centrifugation or filtration (Champagne et al. 2007). Obtaining a high cellular concentration while maintaining low viscosity is an important objective in optimizing the batch fermentation process, as high viscosity hinders the recovery of cells from the growth medium (Champagne et al., 2007). For spore-forming bacteria, vegetative cells are induced to sporulation, generally by limiting nutrient availability, before harvesting. Reduction of pH is another method of triggering sporulation. With continuous fermentation, fresh growth medium is continuously added to the culture while bacterial cells and any inhibitory substances produced during fermentation continuously removed so that continuous production of the probiotic can be maintained (Muller et al. 2009).

After fermentation the bacterial and yeast cells are usually dried for ease of transport and storage thus avoiding any need for specialized facilities for storage and transport of liquid inoculants or frozen cells. Probiotic micro-organisms are generally dried by freeze drying or spray drying (Muller et al. 2009), but vacuum drying and fluidized bed drying are also used. Maintaining cell viability during drying is critical for successful probiotic production (Meng et al. 2008). A two-step process of freezing and drying is used. Although this is the best method to dry bacteria, in terms of maintaining viability, the high cost associated with the process often hinders its application (Chávez and Ledeboer, 2007). Desmond et al. (2001) reported that in order to increase the viability of probiotic strains of *Lactobacillus paracasei* NFBC 338 during spray-drying, a pre-stressing of the culture would be useful. This was carried out by exposure to temperature of 52ºC for 15 minutes increased in 700 fold the survival of the strain (in reconstituted skimmed milk) during caloric stress and 18 fold during spray drying when compared to non-adapted cells. Thus the outcomes demonstrating that the probiotic preparation method can aid for a larger survival time and consequent results obtained.

Environmental Factor

Spore forming bacteria, particularly from the genus Bacillus, are increasingly being used as probiotics. Bacillus spores are resistant to physical and environmental factors, such as heat, desiccation and UV radiation (Setlow, 2006; Cutting, 2011) enabling them to maintain their viability during feed pelleting, storage and handling. It also can tolerate relatively high temperatures associated with the pelleting of chicken and turkey feed. Environmental factors during the preparation of probiotics (pH, water activity, salts and preservative content) influence in the resistance of *Lactobacillus* to caloric stress and spray drying (Casadei et al., 2001; Desmond et al., 2001).

Also, for a microorganism to be selected to be used as probiotic, it is necessary that it can be able to overcome some barriers that would be harmful to its survival in the gastrointestinal tract. Mills et al. (2011) reported before probiotic bacteria can start to perform its physiological role in the intestine, they should support a number of tensions to ensure it reaches the target site in sufficient number to elucidate its effect.They stated that first the bacterium must be processed in an appropriate manner to allow oral consumption and be able to resist the inhospitable conditions imposed during its passage through the gastrointestinal tract. In order to be in a highly viable state during processing, storage and intestinal transit, bacteria go through adverse conditions including temperature, acidity, bile, exposure to osmotic and oxidative stress both in the production matrix and during intestinal transit (Corcoran et al. 2008). According to Weinack et al. (1985), the physiological stress induced by high or low environmental temperatures or withdrawal of food and water interfere either with the colonization of protective micro-organisms or reduces the protection provided by the probiotic. Exposure of chickens and turkeys to extreme conditions in the environment can induce nonspecific stress responses leading to depressed immuno-responsiveness that will influence gut microbial populations.

Meanwhile, Fuller (1986) reported that the stressor agent must be present before any effect of the probiotic supplement can be observed and that there will only be stimulus to growth if the depressor agent is present. The author emphasized that for the evidence of improvement on the performance of animals, the breeding environment must not be free from challenges. In experimental conditions, the absence of beneficial results can be justified by this statement.

Montes & Pugh (1993) reported showed that in birds, the best results with the use of probiotics happened when the birds were submitted to stress conditions, being by the increase or decrease of temperature, transportation, vaccination and overcrowding. In these conditions, an imbalance in the intestinal microbiota is created and the body defense mechanisms are decreased (Jin et al., 1997), which by the supplementation of probiotics, such problems would be minimized, evidencing differences in the performance results.

Use of the Antibiotics

Over use of antibiotics can have very negative effects in the young bird. In some commercial operations, it is common practice to add high levels of antibiotics to the first feed given to chickens and turkeys. Usually, in the USA, this medicated feed can be available for as long as 10 days after placement. This medicated feed is replaced then with feed that does not contain antibiotics. Within a few days after the new feed has been provided, the chickens and turkey poults may begin to refuse feed and to develop signs of an enteritis that is now frequently called "off-feed enteritis". The end result of prolonged use of antibiotics is antibiotic resistant bacteria and inhibition of growth of beneficial bacteria in the intestinal tract of poultry and other livestock (Edens, 2003). Nevertheless, to overcome this problem, the poultry industries can reduce antibiotic use on a prophylactic basis, and can develop a managerial plan that incorporates the use of probiotics into flock management programs.

Physical and Immunological

Modern broilers and turkeys present a depressed systemic innate immune response to allow fast growth, once the deviation of nutrients to the development of systemic inflammatory response is minimum, and despite presenting better immunity mediated by cells, there is evidence of increase in the mortality among fast-growth poultry when compared with slow-growth ones, which might justify differences in the effects between the different bird lineages.

Regarding age, Mohan et al. (1996) found that beneficial effects of probiotics were seen during the initial growth phase, happening before 28 days and not after 49 days of age. Meanwhile, Siriken et al. (2003) reported that the existence of an intestinal microbiota at the time of administration and the health of the host must be considered when a probiotic is supplemented for the suppression of pathogenic bacteria. It should also be noticed that some micro-organisms that can act as probiotics do not resist the action of some antibiotics or anticoccidial used in the feed of birds (Jin et al., 1997, 1998a; Tournut, 1998).

Exposure of chickens and turkeys to extreme conditions in the environment can induce nonspecific stress responses leading to depressed immuno-responsiveness that will influence gut microbial populations. Unfortunately, the depression in the production of immunoglobulins, specifically IgA, tends to influence pathogen growth more than beneficial microbes. Many managerial stressors such as beak and claw trimming and other hatchery processes such as vaccinations and handling for sexing and high population densities after placement contribute to immuno-suppression in poultry (Edens, 2003).

Stress Factors Affecting Probiotic Performance

Use of probiotics for poultry production is not without certain risks and limitations. Table 1 lists the stress factor and causes of the stress that could reduce the effectiveness of maternal antibody defence mechanism and normal colonization of the gut by beneficial microorganisms effectively allowing the colonization of pathogens during the early post-hatch stage.

Table 1: Factors that limit efficacy of probiotics in poultry (Adapted from Edens, 2003)

Stress factors affecting probiotics performance	Causes of stress
Nutritional	Improper formulation of diets, poor quality proteins and other nutrients, poor water quality, nutrition degradation, mold and mycotoxins, other toxic substances.
Environmental	Excessive cold, excessive heat, high level of chlorine or fluoride in drinking water, excessive humidity, ammonia, poor ventilation, wet litter, excessively dry litter, lack of maintenance of water supply lines and waterers, pathogenic microbes in overwhelming numbers.
Physical and Immunological	Poor chick quality, immunological diseases (infection bursal diseases, mareks disease, all leukosis diseases including J-virus infections.
Managerial	Setting of dirty eggs, hatching to early, late removal from hatcher, poor beak trimming, toe trimming, over-crowding, poor disinfection and sanitation programs, poor litter managements, interrupted feed and water supply, lack of removal of moribund and dead birds.
Uses of antibiotics	Uncontrolled antibiotic uses, antibiotic destruction of normal intestinal microbes, non-specific enteritis of viral origin (antibiotics are not indicate for use).
Lack of association with mothers hen	Hatchery-supplied chicks that have never been on the ground with the mother hen require longer time for development of normal intestinal microbial populations. Lack of association with the healthy adult chickens in a flock Hatchery associated services of the chicks (under managerial).

The probiotic bacteria for poultry industry must fulfil the following conditions: it must be a normal inhabitant of the gut, and it must be able to adhere to the intestinal epithelium to overcome potential hurdles, such as the low pH of the stomach, the presence of bile acids in the intestines, and the competition against other micro-organisms in the gastro-intestinal tract. In addition, potential probiotics must exert their beneficial effects (e.g., enhanced nutrition and increased immune response) in the host. Finally, the probiotics must be viable under normal storage conditions and technologically suitable for industrial processes (e.g., lyophilized). Besides, probiotics for animal nutrition need to maintain their viability during manufacturing, storage and handling, and quality control assurance is necessary.

Delivery Techniques of Probiotics

The health benefits of probiotics are dependent on the viability and sufficient number of probiotics in the target intestine. Due to probiotics' vulnerability to several environmental factors such as temperature and pH, maintaining the viability of probiotics has long been a hurdle to develop successful probiotic delivery system. Therefore, an ideal probiotic delivery system should protect probiotics from adverse conditions during manufacturing and storage and in the acidic gastric environment so that the sufficient amount of probiotics is available at the action site (Figure 2).

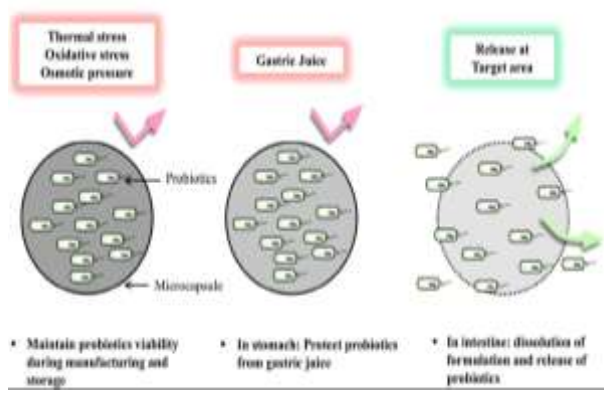

Figure 2: Proposed ideal probiotic delivery (Jihyu et al. 2016)

Factors That Affect Viability of Probiotics

Although the viability of probiotics is essential for functioning, it is a difficult task to maintain the viability from fabrication/storage to the target site in the GI tract. For this reason, a majority of probiotic delivery studies focus on improving the probiotic viability. This section discusses factors that affect probiotic viability during manufacturing, storage and delivery through the GI tract. Thermal stress could damage the integrity of probiotics during a long-term storage as well as commonly applied manufacturing processes such as drying and pasteurization (Burns et al. 2008).

Since many of probiotic strains are anaerobes or microaerophiles, the viability of probiotics can be deteriorated by the existence of oxygen or so-called oxidative stress. Reactive oxygen species are generated under oxidative condition and they interact with probiotic components such as proteins, lipid or nucleic acid (Santivarangkna et al. 2008).

Osmotic shock also impairs the viability of probiotics during a drying process. Dehydration that happens during a drying process leads to efflux of water from a probiotic cell, which causes the osmotic shock by increased intracellular molarity in probiotic cells, resulting in damaged cell functions (Poolman 2002).

Gastric juice, after intake of probiotics, the first and biggest barrier for maintaining the viability of probiotics is the harsh environment in the stomach, more specifically the gastric juice, which is extremely acidic. Probiotics cannot survive under the acidic conditions for 2 h owing to disruption in metabolic and cytoplasmic activities (Hutkins and Nannen 1993).

Materials for Encapsulating Probiotics

The objective of probiotic encapsulation, is not only to protect the cells against adverse environments, but also to liberate probiotics to the target intestine in a viable and functional state (Picot and Lacroix 2004). The viability of encapsulated probiotics depends on the physicochemical properties of the encapsulating material (Chen and Chen 2007). Alginate is commonly used materials for microencapsulation of probiotics. It is a natural polysaccharide derived from brown algae or bacteria, has been widely used as an encapsulating material for probiotics due to biocompatibility and an easy gelling process by an ionic gelation with Ca2 (Krasaekoopt et al. 2003).

Another material is Xanthan Gums, an exopolysaccharide derived from *Xanthomonas campestris*, is the most commonly used gum which composed of glucose, mannose and glucuronic acid (Garcia-Ochoa et al. 2000). Xanthan gum is known to possess resistance to a wide range of pH and thermal stress (Leela and Sharma 2000).

Proteins also can be used as a protective material for probiotics and become a popular choice in recent years. Probiotics are encapsulated into proteins by an enzymatic or chemical cross-linking or temperature-dependent gelation (Cook et al. 2012). Amphiphilic nature of the proteins provides unique property for the probiotic delivery system.

Synthetic polymer such as poly (D, L-lactic-co-glycolic acid) (PLGA), polyvinyl alcohol (PVA) and polyacrylamide has been employed as an encapsulating material. PLGA is a FDA-approved biocompatible material which used for time-dependent release (Della Porta 2012). However, use of synthetic polymers as an encapsulating material is still challenging due to involvement of organic solvents during fabrication, which causes cell damages.

Recent Trends of Probiotic Delivery System

As described previously, encapsulating probiotics into carrier materials had been a common strategy for probiotic delivery until recently. However, challenges still exist for effective protection of probiotics from tough conditions during a manufacturing process, a long-term storage and a transit in the GI tract in order to obtain a sufficient number of viable bacteria in the target site. This has propelled development of new strategies in probiotic delivery.

Alginate has been the most extensively studied encapsulating material; however, a protective effect of bare alginate is not enough to obtain a sufficient number of viable probiotics in target sites due to a porous nature and an uncontrollable swelling behavior, which could allow hydrogen ion penetration and make the alginate system susceptible to acids. In addition, cell leakage by low mechanical durability in storage is a potential problem of alginate (Kim et al. 2014). Recent studies have employed various coating technologies to overcome the limitation by providing an additional protection to the surface of alginate microparticles or beads. Chitosan coating on alginate beads has been used to provide probiotics for protection from acids by reducing pore size of alginate beads (Cook et al. 2013b).

Polydopamine coating on alginate beads was used to encapsulate *Saccharomyces cerevisiae* (Kim et al. 2014). It was found that polydopamine coating enhanced mechanical durability of alginate beads. For enhanced target delivery of encapsulated probiotics to the target intestinal area, protamine was formulated with alginate (Mei et al. 2014). Enteric-coating materials have been used for targeted delivery of probiotics. Eudragit L 100 55 was used with ethylcellulose to protect Bifidobacterium breve from gastric juice (de Barros et al. 2014).

To maximize probiotic-conferred health benefits, prebiotics such as galactooligosacchride and chicory have been added to probiotic delivery systems. Prebiotics is a non-dietary fiber that can selectively boost probiotic strains and confer synergistic effects (Kolida and Gibson 2011). When galactooligosaccharide-loaded PLGA particles were encapsulated in alginate beads with Bifidobacterium breve, the viability of Bifidobacterium breve increased up to 8 log log CFU/mL (Cook et al. 2014). Manufacturing processes can influence viability of probiotic bacteria (Grzes´kowiak et al. 2011). Since many of probiotics can be exposed to high temperatures for pasteurization and spray-drying process and low temperatures for a freeze-drying process, maintaining viability during the manufacturing processes is also of importance (Tripathi and Giri 2014).

Recently, non-microencapsulation-based probiotic delivery systems have been attempted. For examples, tablet-based systems have been investigated as a probiotic delivery system (Govender et al. 2015). The tablets also protected cells during storage at 4 °C for over 6 months (Villena et al. 2015a, b). Cell surface engineering has also emerged as a non- microencapsulation-based technology to protect probiotics from gastric conditions. Cell permeability can be modulated by adhesion of polymer molecules on the cell surface (Fakhrullin et al. 2012). Recently, an array of novel technologies, such as coating systems, prebiotics and microencapsulation with newly developed materials, have been developed to enhance the viability. Another novel aspect of probiotic delivery is a controlled release of probiotics at the target site. Despite all the efforts, however, most delivery systems still suffer from loss of viable probiotics and a need for an ideal probiotic delivery system.

Effect of Probiotics Usage on the Immune System

Perhaps the greatest motivation behind the use of probiotics in animal feed is its potential to replace or reduce antibiotics in poultry production. Conventionally, antibiotic is used to increase animal resistance to diseases, for better growth and improved immune system. However, the extensive and prolonged use of antibiotics has triggered antibiotic resistance and drug residue problems, not only in the animals themselves but could also be passed to humans that consume these animals. The positive impacts of probiotics in improving the immune system has been studied primarily in poultry animals. In studies mostly done on chickens, probiotics has been shown to improve gastrointestinal tract (GIT) colonization of beneficial microbes and lower infection various microbes. Probiotics supplementation can provide protective effects against many pathogens including *Salmonella* (Qin et al., 1995; Stern et al., 2001), *E. coli* (Chateau et al., 1993), *Clostridium* (La Ragione et al., 2004) and *Campylobacter* (Hakkinen and Schneitz, 1999; Morishita et al., 1997; Stern et al., 2001). More recently, the positive impact of *Lactobacillus*-based probiotics against *Eimeria acervulina* infection in broiler chickens was also reported (Dalloul et al. 2005).

The effects of probiotic supplementation on immune response in broilers raised under hot climate were studied by Fathi et al. (2017).They found that at 6 weeks, dietary supplementation of probiotic *Bacillus subtilis* positively impacted serum immunoglobulin (Ig) M and cell-mediated immunity, while IgY and IgA were improved albeit not significant (Fathi et al. 2017). Similarly, in a separate study, the authors observed increased IgM immunoglobulin concentration in chickens fed with probiotic *Bacillus subtilis* supplemented at 200ppm and 400ppm, compared with untreated hens (Fathi et al. 2018). When the probiotics was added in drinking water, similar trend in immunoglobulin response was also observed although slower at day 42 as reported (Jamshidparvar et al. 2017). Immunoglobulin IgY, IgA and IgM levels in serum are typically used as the indicator of the humoral immunity of chickens (Mountzouris et al., 2010). IgM and its isotype are the mostly established natural antibody in mammals with various mechanisms in the immune system, while IgA and IgG have been reported as well (Boes, 2000; Parmentier et al. 2004).

In addition, the influence of probiotics on the intestinal microbiota and the development of natural antibodies in the intestines and sera of chickens was examined by Haghighi et al. (2006). When 1-day old chicks were treated with probiotics (a mixture of *Lactobacillus acidophilus*, *Bifidobacterium bifidum*, and *Streptococcus faecalis*), serum and intestinal antibodies reactive to tetanus toxoid (TT) and *Clostridium perfringens* alpha-toxin were boosted. Furthermore, the authors also investigated the mechanisms of the action of probiotics against colonization of the chicken intestine by *Salmonella enterica* subsp. *enterica* serovar Typhimurium (Haghighi et al. 2008). The chickens received oral gavage of probiotics on day 1 and subsequently *Salmonella* on day 2. Interleukin IL-12 expression was similar to that observed in uninfected control chickens when chickens were treated with probiotics prior to experimental infection with *Salmonella*. Significant decrease in interferon IFN-γ gene expression in cecal tonsils was also found in chickens pre-treated with probiotics, suggesting that the expression of IL-12 and IFN-γ is correlated with probiotic-mediated decrease in *Salmonella* intestinal colonization (Haghighi et al. 2008).

Despite these successful examples of implementation of probiotics in poultry production, there are also reports that suggested no effects of probiotics feeds. Midilli et al. (2008) found no significant differences in the serum IgG levels of broilers treated with probiotics mixture. Another report revealed that while probiotic *Bacillus subtilis* injection into the amniotic fluid of live chicken embryos significantly decreased *E. coli* and increased lactic acid bacteria population during the first week post-hatch, it has no effect on the cell-mediated immune response (Majidi-Mosleh et al. 2017). The sensitivity of probiotic microbes such as *Lactobacillus* to environmental conditions has been proposed as one of the factors that limit the efficacy of probiotics. Thus, several researchers attempted microencapsulation of probiotics as a measure for better effects of probiotic feed. Wang et al. (2018) fed broiler chickens with microencapsulated probiotics and prebiotics, and measured the level of immunoglobulins afterwards. Their results showed that at day 21, the serum IgA and IgM concentrations in the probiotics and prebiotics supplemented group were higher than that of the basal diet and aureomycin-supplemented groups . Overall, one can conclude that the success of probiotics supplementation depends on many factors, including treatment mode, method of delivery, type of probiotic strains and environmental aspects.

Effect of Probiotics Usage on Lipid Composition and Oxidation of the Meat

Lipid oxidation is one of the main causes of meat product quality deterioration through negative modifications in flavor, color, texture and nutritional value. The thiobarbituric acid reactive substance (TBARS) test is the most commonly used technique in meat and meat products to quantify the level of lipid oxidation. In a previous study, supplementation of *Alisma canaliculatum* (a type of herbal plant) with probiotics (ACP) in broiler chickens was shown to increase the crude protein content in breast meat and decrease crude fat content in thigh meat. ACP-supplemented broiler groups also showed lower TBARS value after second week of feeding, indicating lower level of lipid oxidation (Hossain et al. 2012).

In terms of fatty acid composition, the breast meat from ACP groups had lower arachidonic acid, total n6 fatty acid, docosahexaenoic acid and polyunsaturated fatty acid (PUFA) contents compared to the control groups. Similarly, in thigh meat, linoleic acid, PUFA, PUFA/SFA, and n6 fatty acid were lower when ACP was added to the basal diet (Hossain et al. 2012). The fatty acid composition is one of the indicators for the quality of meat products as it affects tenderness, juiciness, color and flavor, as well as contributes to the nutritional value of meat.

In another study by Ghasemi et al. (2016), the addition of synbiotic (probiotic and prebiotic) to broiler chicken feed decreased serum cholesterol and low-density lipoprotein cholesterol concentrations. Consistently, TBARS value decreased after 30 days of storage at 4 °C, proportion of monounsaturated fatty acids decreased and n-6 PUFA increased in thigh meat, although no such alterations of fatty acid profile were observed in breast meat (Ghasemi et al. 2016). They also discovered that synbiotic is able to increase the capacity of canola oil for enhancing PUFA/SFA ratio in chicken breast meat.

Similarly, lower lipid oxidation was found in broiler chickens supplemented with probiotic *Lactobacillus fermentum* (Bobko et al. 2015). The thiobarbiturates numbers were measured in terms of the concentration of malondialdehyde (MDA). Overall, they observed reduced MDA and thus lower oxidation in the breast and thigh meats during chilling storage when probiotics were added to the feed mixture for broiler chickens.

Wang et al. (2017) reported that chickens supplemented with *Pediococcus pentosaceus* had the average higher short chain fatty acids (SCFAs) contents. In addition, the chicken breast meat displayed more characteristic flavour compounds such as higher concentrations of (E)-2-heptenal, (E,E)-2,4-nonadienal, and certain C6-C9 unsaturated fatty acids, which correlates with stronger chicken fatty odour. The results suggest that probiotics can alter lipid profile and thus meat flavour of chicken (Wang et al. 2017).

In contrast, Chang et al. (2018) found no significant effects of dietary probiotics *L. plantarum* on TBARS values in Landrace×Yorkshire×Duroc (LYD) 3-way crossbred pigs. However, they discovered higher

PUFA and omega-3 and omega-6 fatty acids in probiotics treated pigs, while monounsaturated fatty acid (MUFA) was decreased in probiotic-treated group. The linoleic acid (C18:2 n-6) and linolenic acid (C18:3 n-3) content were also much higher than control group, even higher than the recommended values for human health.

Effect of Probiotics Usage in Eggs Productivity and Quality

The eggs productivity in poultry industry is affected by several factors such as the discovery of new pathogen and also bacterial resistance (Lutful Kabir, 2009). Antimicrobial agents had been used as a solution to this matter. However, there is a lot of questions arise among consumer and manufacturer concerning the side effect of antimicrobial agent for poultry. This situation put probiotics as an alternative in preference to antibiotics (Griggs and Jacob, 2005). Previous research had successfully proven the increased of egg productivity with addition of probiotics in the diet (Chung et al. 2015

Moreover, inclusion of probiotics in laying hens.' diets shows improvement to eggshell quality and breaking strength of the eggs produced (Mikulski et al. 2012, Fathi et al. 2018). Low egg shell quality is a one of major problem in the poultry industry. It resulted in decrease of egg production, hatchability and at the same time increased embryonic mortality (Harikrishnan and Mohan, 2018). Positive effect of probiotics on eggshell quality of laying hens (64 weeks aged) were observed by Abdelqader et al. (2013) which reported increased in eggshell weight and thickness, along with reduced number of damaged eggs.

Table 2 listed common strains used as probiotics for poultry and its effect towards eggs productivity and quality. Different strain of bacteria showed various outcomes either positively or no changes in eggs productivity and quality of laying hens, however without any reported adverse effect.

Table 2: Probiotics strains proved to enhance eggs productivity and/or quality

Strains	Finding	References
Lactobacillus salivarius and *Bacillus subtilis*	Combinations of probiotics increased performance and egg quality of laying hens.	Zhang et al. (2012)
Pediococcus acidilactici	-Addition of whey protein with *Pediococcus acidilactici* during the late stage of production did not improve productivity -Increase on egg production was observed when *Pediococcus acidilactici* was not added into the whey protein	Pineda-Quiroga et al. (2017)
Bacillus subtilis and *Bacillus amyloliquefaciens*	The use of probiotic prepared with soybean positively affects the egg production and egg quality.	Mazanko et al. (2018)
Bacillus subtilis	-Inclusion of probiotics did not affect egg production traits -Inclusion of probiotics increased shell thickness; improve eggshell quality and breaking strength.	Fathi et al. (2018)
Lactobacillus casei, Lactobacillus acidophilus, Bifidobacterium bifidum, and *Enterococcus faecium*	Using *Artemisia annua* leaves with probiotics demonstrate no adverse effect on the egg productive traits.	Baghban-Kanani et al (2019)

CONCLUSION

The concept of using probiotics as a potential substitute for antibiotics in poultry industry has become an area of great interest.Due to their many beneficial effects, scientists are now working hard to establish a platform for used of this beneficial microbe in larger scopes. Industrialising of this concept for applied biotechnological solution is crucial as a method for safe diseases prevention, increase poultry quality and productivity, and safe for human consumption. In addition to that, this is also important factor for the sustainability of poultry industry.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

ZH, DJD, DNAZ, AHMH, ES, YMS, NP and SS involved in data collection and writing the manuscript. IIM, TH and HAE reviewed the manuscript. All authors read and approved the final version.

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