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Review

Probiotics as Antibiotic Alternatives for *Salmonella* Control in Poultry Industry

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Abstract: *Salmonella* infection is one of major challenges to the poultry industry because of its pressing effects on health of poultry, food safety and human well-being that later may devastate economic losses to the poultry sector. The paper reviews public health implications and the use of antibiotics together with the risk of drug resistance. In recent years, the usage of probiotics in poultry industry has been growing to mitigate an increasing pressure to adopt sustainable farming practices. The mechanisms which probiotics may control *Salmonella* and important criteria for selecting effective probiotics in poultry are reported. Various studies highlighting the additional benefits of probiotics in poultry production in addition to *Salmonella* controls are also included. While probiotics offer promise in enhancing poultry health, challenges and limitations in their utilization must also be carefully considered.

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Introduction

In recent years, there has been a growing interest in the application of probiotics in poultry production because of the potential benefits of incorporating probiotics into poultry farming practices. As the world's demand for poultry products continues to rise, so does the need for sustainable and efficient methods of production.

Humans have a responsibility to uphold animal welfare by considering all aspects of animal well-being, such as proper husbandry, nutrition, disease prevention, and treatment. Maintaining a good health of poultry is crucial for poultry itself, humans, and the environment. Healthy poultry has an impact on production quality and quantity, which affects farmers directly and the economics of the industry. Furthermore, it is crucial to recognize the zoonotic potential of various poultry diseases, with transmission pathways primarily linked to food consumption and contact with infected birds.[1] The presence of uninfected birds can lower the risk of human infections and diseases outbreak. This directly influences public health system and country's economic status. Therefore, preventing *salmonella* infections, one of the most spread zoonosis diseases, can diminish the fatal occurrence.

Currently, antibiotics are utilized to prevent poultry pathogens in the poultry industry worldwide. This leads to many problems including development of antibiotic resistance in normal bacteria[2], drug residues in various tissues and organs of the birds, and dysfunctionality of beneficial gut microbiota.[3]

Probiotics have been recognized and proposed as a promising antibiotic alternatives in farm animal production.[4] Thus, the probiotic applications in animal feed could reduce the dependence of antibiotic usage which may cause the emergence of antibiotic resistance bacteria which later make humans and animals infections hard to treat, and reduce aforementioned common problems.[5]

This study will examine key factors driving the increased interest in utilization of probiotics in poultry, highlighting recent findings for their potential role to promote poultry health, reduce the dependence of antibiotic usage, and decrease the risk of *salmonella* infection. It will also review possible mechanisms of probiotics that help prevent and control *Salmonella* based on previous studies published in related journals.

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Economic and Public Health Implications from *Salmonella*

Salmonella, a genus of bacteria, imposes extremely harmful roles in farm animal production industry. *Salmonella* can cause harmful infections on many animals' species however it is very common in poultry. Poultry, including meat and eggs, stands as an important source of essential protein in the human consumption. Therefore, understanding these roles is crucial for poultry producers and consumers alike, as *Salmonella* can impact poultry health, food safety, and human health. Between 1985 and 2002 in the United States, the Center for Disease Control and Prevention (CDC) reported that egg contamination originated 53% of all reported cases of *Salmonella* in human.[6] Eggs contaminated with *Salmonella* Enteritidis caused salmonellosis outbreak in Europe between 2015 and 2018, resulting 838 confirmed and 371 probable cases in 16 countries.[7] Outbreaks of *Salmonella* infections have been reported in various countries both in developed and developing nations, involving a large number of infected and fatal individuals.[8] Although humans infections are commonly associated with food consumption, disease transmission is reported to be associated with direct and indirect contact.[1]

Salmonella Infections in Poultry

Salmonella is a genus consists of two species: *Salmonella bongori* and *Salmonella enterica* with over 2500 serotypes.[9] *Salmonella enterica*, according to the antigenic specificity, can subdivide into 6 species: *enterica, salamae*, *arizonae*, *diarizonae*, *houtenae*, and *indica*. *Salmonella enterica* subsp. *enterica* is mostly associated and causes infection in warm-blooded animals while *Salmonella bongori* and other 5 subspecies of *Salmonella enterica* were found in cold-blood animals and the environment.[10] The most important serovars in veterinary include *S. enterica* subsp. e*nterica* serovar Pullorum, *S. enterica* subsp. e*nterica* serovar Gallinarum, *S. enterica* subsp. *arizonae*, and other *salmonella* infection-causing serovars.[9]

First, *S. enterica* subsp. e*nterica* serovar Pullorum can infect almost all birds, primarily in chicks and poults. The disease is called Pollorum disease. In severe cases, newly hatched chicks die within a short time and may not show any gross lesion.[11] Second, *S. enterica* subsp. e*nterica* serovar Gallinarum has many similarities with *Salmonella* Pollorum in many ways; including history, clinical signs, epizootiology, pathological findings, as well as control and eradication methods. It affects primarily in chicks and poults as well. Third, *Salmonella enterica* subsp. *arizonae* affects most frequently in turkey but can infect other avian species, mammal including human, and reptile species as well.[11,12] *Salmonella arizonae* infections or Arizonosis in turkeys cause nonspecific symptoms.[11] Sato and Adler[13] noted that clinical sign was rarely seen in adult turkeys and no turkey died. The last two serovars have been reported to be the most common causes of human non-typhoidal salmonellosis. S*almonella enterica* subsp. *enterica* serovar Enteritidis infect mainly in poultry, and it can be transmitted to human through meat and egg. It can cause embryo mortality, exhibiting symptoms similar to other bacteria that cause acute septicemia. This pathogen can lead to morbidity and mortality in chicks and poults but no morbidity and mortality was found in mature poultry.[11,14] Depression, anorexia, reduced egg production, diarrhea, and mortality were found in orally inoculated laying hens.[15] Lastly, *Salmonella enterica* subsp. *enterica* serovar Typhimurium has a broad host range and can be linked to diseases in humans, livestock, rodents, and avian species[16,17] Report from OzFoodNet, *Salmonella* Typhimurium is a major cause of foodborne outbreaks linked to consumption of eggs.[18]

As *Salmonella* Enteritidis and *Salmonella* Typhimurium are responsible for non-typhoidal salmonellosis, one of the most widespread zoonotic diseases[19], this paper will mainly discuss *Salmonella* Typhimurium and *Salmonella* Enteritidis. Poultry referring to domesticated birds such as chickens, ducks, and turkeys is one of the most consumed meat and the reservoir for *Salmonella*.[20] While infected poultry does not have severe symptoms, it can produce contaminated eggs, and spread the infection to other susceptible hens which have not been exposed before .[21,22]

Mode of *Salmonella* Transmission

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Salmonella can be introduced into the flocks from many different sources.[23] Risk factors for contamination include feed[23], inadequate level of hygiene[24], farm structure[25], wet and cold season[25], and litter-beetle infestation.[26] In poultry, the *Salmonella* route of transmission can be both vertical and horizontal. Vertically transmission, *Salmonella* Enteritidis has a special affinity for the chicken reproductive system. It can migrate from the cloaca to the reproductive organs, infecting ovary and developing eggs.[27] A study by Gast and Beard[28] showed that freshly laid, contaminated eggs contain a small number of bacteria (5.50 CFU/ml). After storing eggs for 7 days at 25°C, the number of bacteria increases to a detectable level (15.59 CFU/ml). Moreover, when those eggs are incubated, the temperature, typically ranging between 37°C-39°C, is optimum for embryo, as well as bacteria to grow.[29] Horizontally transmission, *Salmonella* can transmit through contaminated manure, feed, water, rodents, insects, wild birds, transportation coops and vehicles, and farm environment.[30]

The Use of Antibiotics for Prevention and Treatment of Salmonellosis in Poultry

Antibiotics are favorably utilized in livestock mainly to prevent infections, treat infections, promote growth and improve production.[31,32] If needed, *Salmonella* infections in poultry are typically treated with antibiotics such as sulphonamides, neomycin, tetracyclines, amoxycillin, and fluoroquinolones in accordance with the sensitivity.[33] The administration of antibiotic doses can vary, but it is essential to follow local guideline and use it carefully. However, the indiscriminate use of antibiotics can accelerate the antibiotic resistance in pathogens which can result in treatment failures, economic losses, and gene pool for transmission to environment and humans through the residue in meat[3] and eggs.[34]

Recent study published in 2023 was done by collecting chicken meat and feces samples in Nakhon Ratchasima, Thailand from January 2021 to March 2022 and compared with samples from Japan. It showed that *Salmonella* prevalence at slaughterhouses in Thailand and Japan was not significantly different (41.2% and 40.7%, respectively). However, all the *Salmonella* isolates in Japan were not resistant to the nine antibiotics tested. On the contrary, the majority of Thai *Salmonella* isolates from chicken cloacal swabs and meat were resistant to doxycycline (78.3%) and colistin (63.5%).[35] These may suggest that there has been excessive antibiotic usage in Thailand, resulting in the development of drug resistance within the country.

The excessive use of antibiotics is contributing to a global public health crisis, resulting in as many as 3,500 human deaths daily due to antimicrobial-resistant infections (superbugs).[36] Consequently, many countries have banned the use of antibiotics and promote alternative substances.

Probiotics and Their Roles for Poultry Health

Probiotics, with their ability to promote health, improve performance, and reduce the reliance on antibiotics, have emerged as a promising solution to address the challenges faced by the poultry industry. The term 'probiotic' is derived from Greek language meaning "for life".[37] Lilly and Stillwell first coined the term "Probiotic" in 1965 and described it as substances secreted by one organism and have ability to stimulate the growth of another organism.[38] In 2002, Marteau et al.[39] defined it as "microbial preparations or elements derived from microbial cells that exert a positive influence on health and promote well-being". When consumed in sufficient quantities, probiotics positively affect humans or animals health by enhancing the properties of the existing gastrointestinal flora.[40]

There are many important properties of microorganisms to be considered as probiotics as shown in Table 1.

Table 1. Properties of microorganisms to be considered as probiotics [41].

Properties of microorganisms
Generally Recognized as Safe (GRAS)

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Bile, hydrochloric acid and pancreatic juice resistance
Anti-carcinogenic properties
Stimulate immune system
Intestinal permeability reduction
Lactic acid production
Resistance to acidic conditions of the stomach
Resistance to alkaline conditions of the duodenum

To be more specific for poultry applications, the selection of probiotics for poultry health demands careful consideration of several key factors to ensure their effectiveness in promoting the well-being of birds and enhancing overall production. Firstly, it is crucial that the chosen probiotics should be derived from specific poultry gut environment, as they are more likely to survive and thrive in the intestinal tract. In addition, practical considerations of probiotics include the ability to store and transport under typical storage conditions. The selection of probiotics must also include its suitability for large-scale industrial processes, with probiotics needing to be cost-effective for poultry farmers.[42] The criteria for selecting probiotics for poultry can be conveniently summarized in Table 2 and illustrated in Figure 1.

Table 2. Selection criteria of probiotics in the poultry industry [43–45].

Properties of microorganisms
Must be a normal inhabitant of the gut
Must be able to adhere to the intestinal epithelium
Can overcome the low pH of the stomach
Can overcome the presence of bile acids in the intestines
Can overcome competition against other micro-organisms in the gastro-intestinal tract
Must be viable under normal storage conditions and suitable for industrial processes
Must be cost effective to use for farm animals

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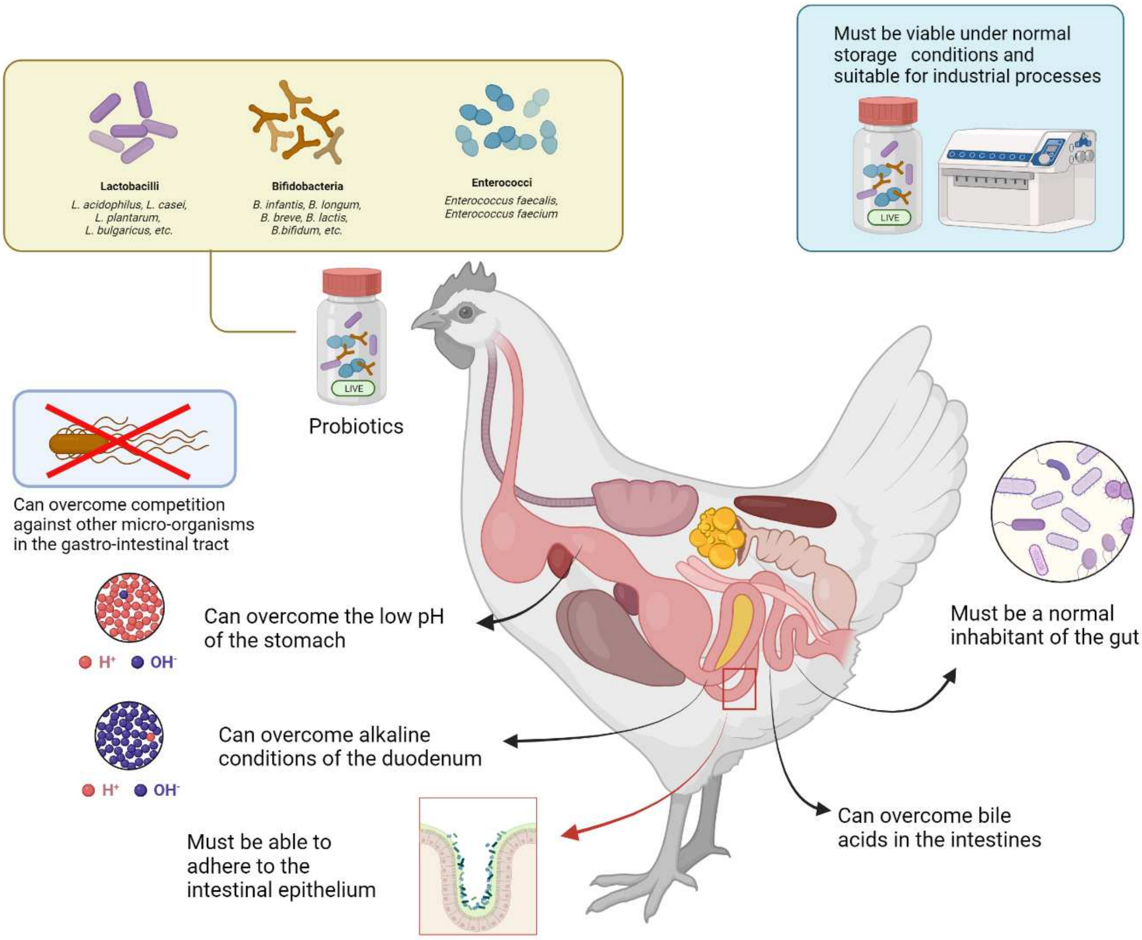

Figure 1. Illustration of selection criteria of probiotics in the poultry industry [43–45] (Created with BioRender.com).

In general, the application of diverse probiotics offers multiple advantages to the poultry industry, beyond focusing on *Salmonella* infections, which will be explored further in the subsequent discussion. Table 3 compiles various studies explaining benefits of probiotics in poultry industry.

Table 3. Examples of various studies explaining benefits of probiotics in poultry industry.

Probiotic strains	Categories of chicks	Starting age for administration	Administration	Benefits	Ref.
Lactobacillus acidophilus	Gnotobiotic chicks	2 days old	Inoculation 10^8-10^9 organism/ml.	Decreased mortality from 100% to 0% when challenged with pathogenic *Escherichia coli.*	46
Protexin® Boost *Lactobacillus plantarum, Lactobacillus bulgaricus, Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium bifidum, Streptococcus thermophilus, Enterococcus faecium,*	Broiler chicks	1 day old	Added to drinking water 2gm of Protexin® Boost/10 liters water	Significant live weight gain, high carcass yield, high breast and leg weight and high antibody production	47

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Aspergillus oryzae, and *Candida pintolopessi*					
Enterococcus faecalis UGRA10	Laying hens	16 weeks old	Fodder diet with the bacterium *E. faecalis* UGRA10 10^8 CFU/g of fodder	Maintained egg production levels	48
Lactobacillus acidophilus, *Bacillus subtilis*, *Streptococcus faecium*	Broilers	1 day old	Added to diet varied from 0-1 g probiotic²/kg feed for the first 3 wks and 0-0.5 g probiotic²/kg feed for wk 4 to wk 6	Reduced litter ammonia levels	49
Bacillus subtillus, *Streptomyces galilaeus*, and *Sphingobacteriaceae*	Broilers	1 day old	3.5×10^8 CFU/g of each strain	Improved growth performance and behavioral welfare	50

Probiotics Mechanisms to Prevent and Control Salmonellosis

The most important scientific question regarding the use of probiotics in medicine is to identify the mechanisms by which they impact health. Although several mechanisms have been proposed, most of them have not been experimentally proven.[51] Most of the main mechanisms of probiotics discussed is for human gastrointestinal but it is speculated that similar mechanisms occurring in poultry health.

The potential mechanisms through which probiotics aid in the prevention and control of *Salmonella* infections include:

(1) **Competition for nutrients**: Probiotics could sequester essential nutrients resulting in invading pathogens could not colonizing. Probiotics like *E. coli* Nissle 1917 can diminish *Salmonella* Typhimurium's colonization in intestines by competing for iron, a crucial but limited nutrient necessary for *Salmonella* Typhimurium's growth.[52]

(2) **Production of antimicrobial conditions and compounds**: Lactic acid bacteria can produce antimicrobial substances, e.g., lactic acid, hydrogen peroxide, and bacteriocins.[53] Production of organic acid may lower pH which cause an unfavorable environment for pathogen colonization.[51,54,55]

(3) **Blocking of adhesion sites**: When probiotics, for example Lactobacilli, are ingested, they adhere to intestinal mucosa, competing for binding sites. Therefore, less binding sites are available pathogens which make pathogens leave the body soon before they can colonize.[56]

(4) **Immunomodulation**: Probiotics can stimulate both adaptive (specific) and innate (nonspecific) immunity. When they colonized in the gut, they activate lymphocytes and mature the humoral immune mechanisms, especially the circulation of IgA and IgM secreting cells.[56]

As non-typhoid salmonellosis usually causes asymptomatic in poultry, it is crucial to prevent the proliferation to eggs, chicks, other poultry, humans, other animals, and the environment. The commonly used probiotics as a supplementation in poultry industry include several species of *Bifidobacterium*, *Lactobacillus*, and *Bacillus*, *Enterococcus* and *Pediococcus*.[57] In the following findings as shown in Table 4, some probiotics have been employed for *Salmonella* infections prevention and control in poultry.

Table 4. Some probiotics for Salmonella infections prevention and control in poultry.

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Categories of chicks	Age	Probiotics	Administration	Challenged pathogen	Results	Ref.
Broiler		*Lactobacillus salivarius* strain 3d (isolated from chicken feces)	Orally 10^8 CFU / 100fl of Phosphate Buffered Saline. one day before with selected pathogenic bacteria	*Salmonella Enteritidis*, *Clostridium perfringens* and *Campylobacter jejuni*.	Lower *salmonella* in caecal content after infection compared to control group and no *Salmonella* detection after 7 days.	58
Broiler chicks	1 day old	*Lactobacillus acidophilus*, *Enterococcus faecium*, *Lactobacillus plantarum* and *Lactobacillus casei*	Added in drinking water for 5 consecutive days in a dose of 1gm/4 liter of the drinking water	*Salmonella Enteritidis*	Significantly lower morbidity rates, fecal shedding rate of *Salmonella* Enteritidis, and re-isolation rate of *Salmonella* Enteritidis from different organs	59
Broiler chickens	newly hatched	*Lactobacillus crispatus*, *Lactobacillus salivarius*, *Lactobacillus gallinarum*, *Lactobacillus johnsonii*, *Enterococcus faecalis* and *Bacillus amyloliquefaciens*.	Added in feed 2.0×10^{10} to 8.9×10^{10} CFU per kg feed	*Salmonella* Enteritidis A9	Reduced *Salmonella* Enteritidis A9 in ceca: detected 95% of broilers on day 14 and 55% on day 28. Stimulated immune system	60
Broiler chicks	1 day old	*Lacticaseibacillus casei*, *Bifidobacterium breve*, *Bifidobacterium longum* and *Bifidobacterium infantis*.	Oral inoculation 2×10^9 CFU from each probiotic bacterium	*Salmonella* typhimurium	Prevention of the detrimental effects of acquired *Salmonella* infection by *B. breve*, *L. casei* and *B. infantis*, An ability to bind to intestinal cells in vitro, Reduction of *Salmonella* typhimurium recovery from the cecal tonsils in vivo.	61

Challenges and Limitations of the Usage of Probiotics in Poultry Production

In Thailand, Chaiyawan et al.[62] reported that the utilization of spore-based probiotics in poultry production remains limited due to inconsistent efficiency observed across various farms. It is worth mentioning that the spore probiotics available for use in commercial poultry farming are typically derived from laboratory strains or exogenous strains of *Bacillus* sp. Consequently, the widespread adoption of spore-based probiotic products in the Thai poultry farming industry has not been readily embraced. Moreover, Yaqoob et al.[63] explained in their recent review article about the inconsistency in results on effectiveness of probiotics. They found that both biotic and abiotic factors are crucial on

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the effectiveness. Thus, further studies are needed to investigate the specific mechanisms of those factor on how probiotic interacts with both biotic and abiotic factors of the host. The other important aspect is the mode of delivery, i.e., how the probiotic is administered, because different delivery methods may affect the efficacy of the probiotic. Krysiak et al.[64] explained that probiotic is usually prepared in single species in European market. However, non-European markets use multispecies probiotics of undefined composition. Consequently, the study of the occurrence of synergism between various bacterial strains is needed to be fully understood. Another challenge that is to be concerned is probiotic viability during feed manufacturing which involves high temperatures, high pressure and intensive mechanically sheer.[65,66] Further research is still needed to fully understand the potential of probiotics in poultry production and to ensure their safety and effectiveness.

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