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Sarayu Bhogoju
University of Kentucky, sarayubhogoju@gmail.com

Samuel Nahashon
Tennessee State University

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Review

Recent Advances in Probiotic Application in Animal Health and Nutrition: A Review

Sarayu Bhogoju¹ and Samuel Nahashon^{2,*}

¹ College of Medicine, University of Kentucky, Lexington, KY 40506, USA; sarayubhogoju@gmail.com

² Department of Agricultural and Environmental Sciences, College of Agriculture, Tennessee State University, Nashville, TN 37209, USA

* Correspondence: snahashon@tnstate.edu; Tel.: +1-615-963-5829

Abstract: Biotechnological advances in animal health and nutrition continue to play a significant role in the improvement of animal health, growth, and production performance. These biotechnological advancements, especially the use of direct-fed microbials, also termed probiotics, those genetically modified and otherwise, have minimized many challenges facing livestock production around the world. Such advancements result in healthy animals and animal products, such as meat, for a growing population worldwide. Increasing demand for productivity, healthy animals, and consumer food safety concerns, especially those emanating from excessive use of antibiotics or growth promoters, are a driving force for investing in safer alternatives, such as probiotics. The advent of vastly diverse pathogens and bacterial organisms, some of which have acquired antimicrobial resistance due to therapeutic use of these antibiotics, has had a negative impact on the animal and food industries. Probiotics have been chosen as substitutes to counter this excessive use of antibiotics and antibiotic resistance. Over the last decade, probiotics have gained recognition, increased in importance, and stimulated growing interest in the animal health and nutrition industry. Probiotics are considered to be favorable live microorganisms by the host organism by maintaining microbial homeostasis and healthy gut, and can be a viable alternative to antibiotics in addition to providing other growth-promoting properties. Even though various studies describe the modes of action of probiotics, more research is needed to illuminate the exact mechanism of action of probiotics and how they benefit the host. This review describes the importance of probiotics in animal health, nutrition, and in growth and production performance. It also provides a thorough review of recent advances in probiotics research and application in animal health and nutrition and future directions on probiotic research to enhance animal performance.

Keywords: probiotics; animal nutrition; animal health; pathogens; antimicrobial resistance; growth performance



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1. Introduction

In 1965, the term “probiotic” was first introduced by Lilly and Stillwell [1] to describe growth-promoting factors produced by microorganisms. Probiotics are live microorganisms mainly used to balance the gastrointestinal tract microflora of organisms which in turn have beneficial effects on their hosts such as promoting health and growth [2]. Previous reports documented how probiotics secrete anti-toxins and antimicrobial substances, which suppress pathogen activity, and they help to regulate immune system and induce antibody production [3,4]. The Food and Agriculture Organization of the United Nations/World Health Organization and the International Scientific Association for Probiotics and Prebiotics defined probiotics as ‘living microorganisms which, when administered in acceptable amounts to employ health benefits to the host’. Probiotics are, generally recognized as safe (GRAS) and they are a natural approach that aids in preventing unwanted bacteria that can cause microbial infections [5]. Even though probiotics are generally regarded

as safe, they must be verified as to whether or not they are beneficial to the host with a practical approach.

In the 20th century, antibiotics were used for the prevention, control, and treatment of diseases and infections. Antibiotics enhance growth and feed efficiency as well as reduce mortality [6]. However, studies showed that therapeutic use of these antimicrobials in animals has contributed to the emergence of antimicrobial resistance. This allows antibiotic resistance to be transferred to humans, therefore reducing the effectiveness of antimicrobial drugs for treating human diseases [7]. Animal contamination of food and bacteria can cause infections in humans [8]. Mead et al. [9] reported that food borne pathogens lead to five million illnesses, 46,000 hospitalizations, and 1458 deaths in the United States each year. In livestock, the continuous increase in antimicrobial resistance is seen as a probable source of antimicrobial resistant genes that may eventually spread to human pathogenic bacteria [10]. Probiotics are therefore considered to be an excellent replacement choice for antimicrobial agents or antibiotics in animal health and in livestock production.

Lactic acid bacteria (LAB) are mostly used in animal health improvement as commercial probiotics. According to American Food and Drug administration (FDA), LAB abundantly available in the gut of healthy animals [11,12]. LAB are Gram-positive facultative aerobic or anaerobic cocci or rod shaped bacteria. They produce Lactic acid as the major metabolic end product of carbohydrate fermentation. They also help in the breakdown of foods, therefore producing hydrogen peroxide, lactic acid, and other substances which create an acidic, unfavorable environment for harmful or pathogenic organisms. LAB are generally recognized as safe due to their ubiquitous appearance in food and their contribution to the healthy microbiota of animal and human mucosal surfaces. Due to their beneficial and nonpathogenic effects, LAB are considered to be potential probiotics.

The most widely used bacteria as probiotics are *lactobacilli* and *bifidobacteria*. *Lactobacillus reuteri* is one of the well-documented probiotic species in LAB and is mainly found in Gram-positive bacterium in the gut flora of animals and birds [12]. Other than LAB, several other bacteria also can be used as probiotics. In aquaculture, *Streptomyces* is mainly used as a probiotic because of its unique ability to produce several antimicrobial agents as secondary metabolites. Das et al. and Augustine et al. revealed several promising results of genus *Streptomyces* as probiotics in aquaculture [13,14].

2. Modes of Action of Probiotics

There are numerous anticipated modes of action of probiotics. Some of these mechanisms are related to the enteric pathogenic microbe inhibition, while others are responsible for animal performance improvement. However, the exact mechanisms of actions by which probiotics exert biological functions is not fully understood, but nonspecifically competitive exclusion or colonization resistance are the terms used to describe their mode of action (Figure 1). According to Oelschlaeger, the way probiotics work in the host system is described in three ways [15]:

- i. Probiotics might be effective in modifying both the innate and acquired immune system of the host. This will be effective in preventing infectious diseases and ameliorate the inflammation of the host's digestive tract;
- ii. The action of probiotics directly on other microorganisms will prevent and serve to control infections and restore the microbial equilibrium in the gut;
- iii. Microbial products such as toxins, antimicrobials and host metabolites may be the key components for probiotic actions. The probiotics help to inactivate the toxins and bile salts detoxification and enhance digestion of food ingredients and absorption of nutrients in the gut.

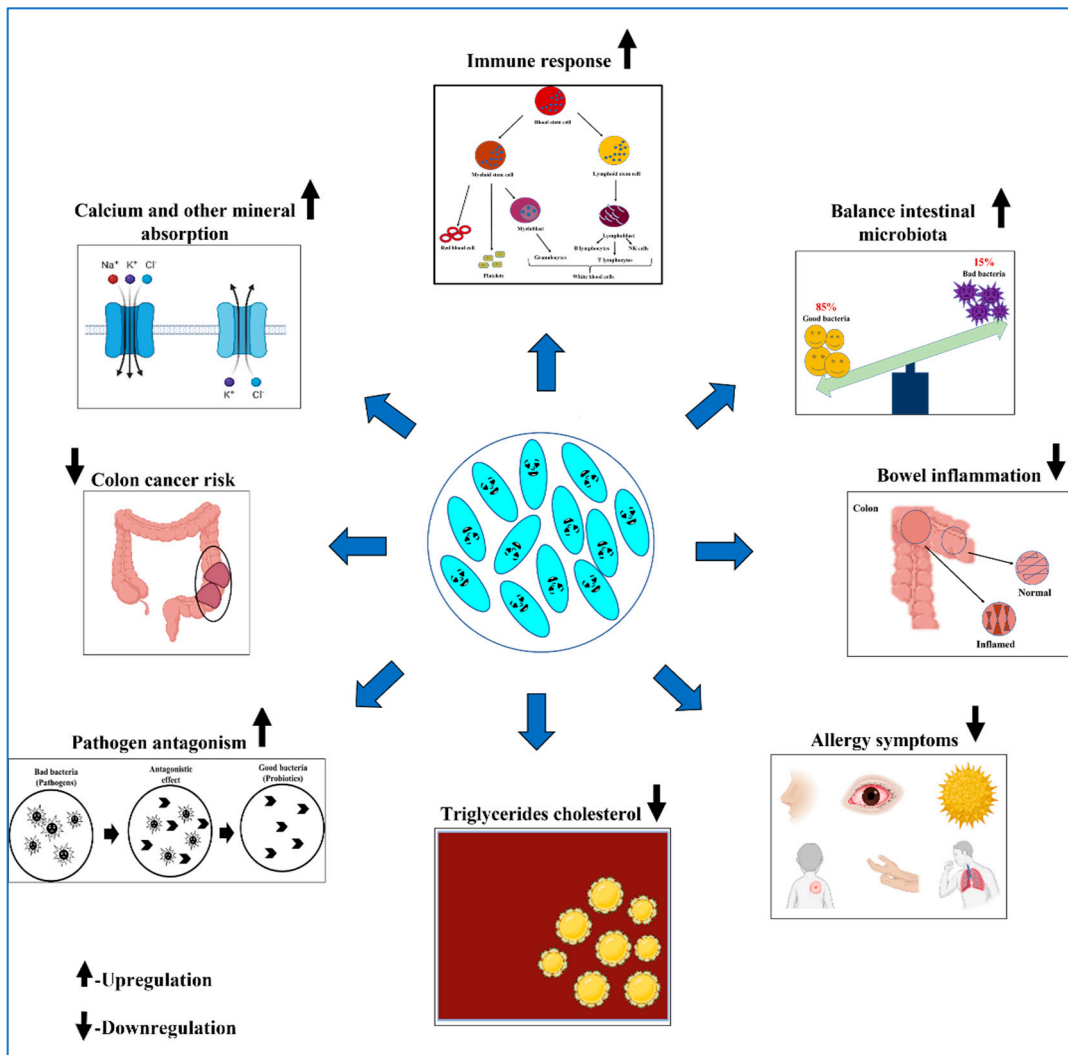


Figure 1. Proposed modes of action of probiotics.

In studies by Galdeano et al., probiotics contend with the pathogenic bacteria to attach to mucus, as these can attach to the mucosal wall and help to adjust to immune responses by the host [16]. Probiotics can also stimulate synthesis of vitamins in the host by supplying an extra source of nutrients and digestive enzymes [5,17]. These can avert the progress of harmful bacteria and can produce inhibitory substances such as volatile fatty acids and hydrogen peroxide to enhance the host’s resistance to pathogens [18].

Research indicates that in ruminants, the number of cellulolytic bacteria increased due to yeast-based probiotics, which shows their effect on microbial fermentation, leading high cellulose degradation and improved microbial protein production [19]. Both *Lactobacilli* and *Bifidobacteria* enhance the growth of closely related bacterial species by producing proteins or polypeptide bacteriocins, this will help to lessen the number of unsafe microbes in the Gut. Probiotic species, including LAB, *bifidobacteria* and *bacillus* can also produce a few kinds of thermostable bacteriocins that exert antimicrobial action in contrast to a variety of pathogens such as *Bacillus*, *Staphylococcus*, *Enterococcus*, *Listeria*, and *Salmonella* species [20].

Depending on various properties of a beneficial microorganism, Havenaar et al. [21], proposed some specific characteristics of probiotics, which include showing favorable effect on the host, being nonpathogenic and nontoxic in nature, with the ability to survive for a long time with high cell counts, their ability to survive through the digestive system passage and colonize the gastrointestinal tract (GIT) by attaching to the intestinal epithelium, the

potential to yield antimicrobial substances against pathogens, and the capacity to stabilize gut microflora, use the nutrients in a normal diet, and confer health benefits to the host.

It was reported that a few probiotic strains have anti-inflammatory properties which promote the balance between pro- and anti-inflammatory cytokines [22,23], along with the production of antimicrobial substances, such as bacteriocins, hydrogen peroxide, and volatile fatty acids [24]. Studies showed that organic compounds produced by probiotic bacteria have proven to exhibit inhibitory effects against pathogenic bacteria such as *H. pylori* [25]. According to the studies of Dai et. al., probiotics can reinforce intestinal barrier integrity by improving tight junction protein expression by activating the p38 and ERK signaling pathways [26]. Along with the anti-inflammatory responses, probiotics also show anti-viral properties in animals [27].

In cancer research, it was speculated that probiotic cultures might have the potency to reduce chemical carcinogens exposure. This can be done by: (i) detoxifying consumed carcinogens; (ii) modifying the intestine atmosphere which helps to reduce carcinogenic producing bacteria populations or metabolic activities; (iii) generating metabolic products (e.g., butyrate) that induce apoptosis; (iv) producing inhibitory compounds to prevent tumor cell growth; or (v) motivating the immune system in contradiction of cancer cell propagation for a better defense mechanism [11].

Probiotics can also protect birds from the negative impact of enzyme activity and increase the activity or production of digestive enzymes. In addition, probiotics may also produce enzymes that hydrolyze or release nutrients in the gastrointestinal tract of the host. Nahashon et al. [28] reported an increase in phytase activity in the crop but not in the gastrointestinal tract of layers fed diets containing *L. acidophilus*. Additionally, increased phytase activity in the lactobacillus fed birds was associated with improved P retention in layers.

To have a clear understanding of probiotic mode of action, all methodologies need to be studied on a case-by-case basis. The consequences of the interaction between host and probiotic microorganisms leads to the effects of probiotics. Therefore, there is a need for additional studies on host-microbes interaction, which could clarify the modes of action of probiotics. Previously, research expenses and undeveloped molecular techniques could be the reason to not study in-depth actions of probiotics. However, currently, several amazing molecular techniques are available, which can be used as tools to understand the effects or modes of actions of probiotics. Rapid developments in molecular methods and DNA sequencing can greatly facilitate in understanding microbial ecology and the way probiotics work.

3. Health Benefits of Probiotics in Animal Growth Performance

The gut micro flora of animals has a vital role in maintaining their health and processing normal digestive procedures. Traditionally, probiotics have been used in human nutrition; but now their use has been expanded to animal feeding. Animal feed containing intestinal microbiota have been developed to improve nutrient use and gut health and ultimately to profit the animal production industry. Reports have shown that probiotics can be beneficial to the host animal by enhancing intestinal microbial symmetry [2].

Administration of probiotics has shown increased effect in the levels of immunoglobulins such as M and A along with the increased levels of total antioxidant capacity in serum [29]. Probiotic microbes also have the ability to balance the immune response in the host by increasing the number of anti-inflammatory agents such as IL-10 and TGF- β [30]. Literature also shows that the administration of *Lactobacillus rhamnosus* improves epidermal growth in the intestine leading to the intestinal epithelial apoptosis reduction, which in turn helps to fight against GI tract diseases [31].

3.1. Probiotics in Ruminant Nutrition and Health

Rumen Microbial ecology is very complex; in this, microorganisms degrade polysaccharides and protein ingested by the host, resulting in the synthesis of short chain fatty acids

(SCFAs) and microbial protein, which can be energy and protein sources of the host. In ruminants, the commonly used probiotic is yeast (*Saccharomyces cerevisiae*); mainly it has an effect on the microbial population and in the breakdown of nutrients [32]. According to reports of Nocek and Kautz [33], cow's milk produce was enhanced by 2.3 L per cow per day with a dietary supplementation of 5×10^9 cfu of *E. faecium* and 2×10^9 yeast cells (*S. cerevisiae*) per cow per day. In ruminants, probiotics can also enhance their weight gain. On average body weight, a 9% improvement was observed in goats fed a probiotic containing a mixture of *E. faecium* DDE 39, *L. alimentarius* DDL 48, *L. reuteri* DDL 19 and *B. bifidum* DDBA, which are isolated from a healthy goat [34]. An enhanced growth rate was observed in growing dairy heifers with administration of *S. cerevisiae* [35]. When it comes to the livestock feeding and medicine, besides lactic acid bacteria and other non-pathogenic microorganisms with health-promoting characteristics, certain strains of yeast, *Saccharomyces boulardii* and *Escherichia coli* such as *E. coli* Nissle 1917, have also been employed [36].

According to the studies of Ma et al. [37], probiotic microbes such as *Bacillus subtilis*, *Saccharomyces cerevisiae*, and *Enterococcus faecalis* can enrich milk secretion in cows and an inhibition of the milk allergy reaction was noted by the administration of *Bifidobacterium bifidum* [38]. Studies also showed that by stimulating GH/IGF-1 hormone, both *Bacillus subtilis* and *Bacillus amyloliquefaciens* can upgrade intestinal maturation and growth competency [39]. Improved mammary gland condition along with the improved functions of the teat sphincter were observed by the effect of Lactobacillus base teat spray [40]. Chen et al. [41,42] showed improved microbial fermentation and high rumen microbial growth performance by the addition of *Rhodopseudomonas palustris*.

3.2. Probiotics in Monogastric Animals Feeding

Extensive effects of probiotics were described in poultry and humans when compared to other monogastric animals. Some probiotic bacteria are presented in Table 1, which are commonly used in monogastric animals. In broiler chickens, probiotics can enhance growth performance and control enteric diseases, like; Salmonellosis, coccidiosis, and necrotic enteritis [43]. An increase in egg production with the reduction in yolk cholesterol by lactic acid bacteria, bacillus spores and yeast, was reported in poultry [44,45].

According to Guo et al., *B. subtilis* strain MA139 was more effective in significantly improving feed conversion ratio (FCR) ($p < 0.05$) in poultry [46]. In primiparous sows, by supplementing *E. faecium* at 5×10^8 cfu/kg feed, increase in feed consumption and enhanced reproductive performance were detected [47]. An intense reduction in the level of *E. coli* was demonstrated according to Le Bon et al. after four weeks of treatment of weaned piglets with *P. acidilactici* and *S. boulardii* [48]. Administration of *S. cerevisiae* to mature horses increased apparent nutrient digestion rate according to reports of Agazzi et al. [49].

In atopic children, due to the action of probiotics, up-regulation of anti-inflammatory cytokines, such as interleukin-10, was reported in the studies of Pessi et al [50]. Pneumonia severity was reduced in children with cystic fibrosis with the administration of *Lactobacillus* GG in a placebo-controlled trial [51]. In rats, due to the dietary supplementation of *L. Acidophilus* of colon cancer, cell number decreased significantly in a dose-dependent manner [52].

3.3. Significance of Probiotics in Poultry Feeding and Health

Poultry is a major protein source worldwide and makes a major contribution to the economy of the United States and in other parts of the world. Poultry corporate has developed as a significant cost-effective organization in many nations. Poultry are the cheapest animal source of protein and the consumption and demand for poultry products is rapidly increasing with the increasing human population. The attractiveness of poultry as a primary source of protein stems from low input cost in production which is in part due to their efficiency of feed use, conversion and overall production efficiency. Because feeding is a major factor in the cost of poultry production, constituting 65–75% of the total

cost of production, there is great need to continue to improve the efficiency of feed use. The use of probiotics has contributed immensely in this effort by creating microbial homeostasis and a healthy gut and efficient absorptive surface.

Table 1. Some common probiotic bacteria and their benefits in monogastric animals.

| Microorganisms | Animals | Common Benefits |
|--|---------|---|
| | Pig | |
| <i>E. faecalis</i> <i>E. faecium</i> <i>Bacillus cereus</i> <i>B. subtilis</i> <i>B. licheniformis</i> <i>L. reuteri</i> <i>L. acidophilus</i> <i>S. cerevisiae</i> | | Colostrum quality improvement, increase in milk quality and quantity Size of litter and vitality improvement weight gain in piglets Reduction of diarrhea Feed efficiency improvement, increase in diet digestibility and meat quality Control of constipation and Decrease in stress |
| | Poultry | |
| <i>L. animalis</i> <i>L. fermentum</i> <i>L. salivarius</i> <i>L. acidophilus</i> <i>S. faecium</i> <i>L. reuteri</i> <i>E. faecium</i> <i>S. cerevisiae</i> <i>Bacillus sps</i> | | Body weight gain improvement Mortality reduction Carcass quality improvement and decreasing contamination Increase in bone quality Increase in egg production Increased immune response Increase enzymatic activity in digestion and absorption of nutrients |
| | Horse | |
| <i>Lactobacillus pentosus</i> <i>L. rhamnosus</i> <i>L. acidophilus</i> <i>L. plantarum</i> <i>L. casei</i> <i>S. boulardii</i> <i>S. cerevisiae</i> | | Improvement in diet digestibility, milk quality and quantity Reduction in diarrhea Avoid hindgut disorders (acidosis, colic) Reduce stress (Transportation, race etc.) |

The primary functions of the GIT include the absorption of nutrients from the diet and the excretion of waste products. The microbial ecosystem of the gut is influenced by the flow of diet nutrients, host derivative substrates such as mucin and bile acids, and host and gut anatomy immunological responses [10,53]. Currently, in commercial poultry production, feed enzymes are excessively used to boost poultry performance by altering the gut environment and its connected microbiota [54].

To further sustain efficiency of nutrient use, even under stressful environmental conditions such as those prevalent in commercial poultry production systems, traditionally antibiotics have been used as antimicrobials and growth promotants. Consequently, the emergence of antimicrobial drug resistance emanating from therapeutic use of the antibiotics is forcing the industry to rethink use of these antibiotics in poultry production. Probiotics are live or viable microbial feed supplements or viable, defined microorganisms in enough numbers which beneficially affect the host animal by improving its intestinal microbial balance [55,56]. They significantly affect the health of the host by manipulating digestion and nutrient absorption, intestinal morphology, and defense of the host against infection [57]. Some of these microorganisms have been characterized while others have not, yet they might bear beneficial effects for bird performance.

According to Walker [58], bacteria that interact with the gastrointestinal mucosa, such as probiotics, can communicate with the underlying epithelial and mucosal lymphoid elements, an interaction that stimulates host defenses in the gut. Probiotics, the beneficial

microbes, also play an important and significant role in maintaining a balance among the intestinal microbiota, and in turn with the ideal balance, enhance nutrient use, and exclude pathogenic microorganisms, hence minimizing the use of antibiotics in poultry production. The gastrointestinal microbiota of monogastric animals is composed primarily of anaerobic bacteria, especially the Gram-positive bacteria, whose densities increase from the proximal to the distal gut. It is among these microorganisms we often find candidate probiotics which maintain homeostasis of the gut flora and sustained gut health.

Much effort has been directed toward identification and characterization of these microorganisms, and the recent adoption of Next Generation Sequencing technologies instead of traditional culture methods has hastened the discovery and characterization of beneficial bacteria or candidate probiotics and their diversity in the gastrointestinal tract of chickens. The goal is to arrive at a normal and stable microbiota, which has a symbiotic equilibrium between the intestinal tract functions and the microbiota. Such an equilibrium is fundamental in maintaining the welfare and performance of poultry by enhancing digestion and absorption, functional immune status, integrity of gut mucosa, and neuroendocrine and motor function of the gut [59]. Oral administration of ducks with lactic acid bacteria (*Lactobacillus casei* 1.2435, *Lactobacillus rhamnosus* 621 and *Lactobacillus rhamnosus* A4) improved performance and the interaction pattern within their cecal microbiota and induced intestinal flora dysbiosis [60].

Previous reports demonstrate that the addition of probiotics enhances digestibility and absorption of nutrients in poultry [61]. Birds fed with *B. subtilis* showed significantly higher digestibility of crude fiber and crude protein when compared non treated birds [62]. Addition of *Pediococcus acidilactici* with or without a combination of oligosaccharides and butyric acid revealed the ability to restore the amylase activity in *Salmonella typhimurium*-challenged broilers [63]. Similarly, in the studies of Jin et al., a mixture of 12 *Lactobacillus* strains (2 strains of *L. acidophilus*, 3 strains of *L. fermentum*, 1 strain of *L. crispatus*, and 6 strains of *L. brevis*) increased the levels of amylase in the small intestine and reduced the intestinal and fecal β -glucuronidase and fecal β -glucosidase activities at 40 days of feeding in day-old Arbor Acres chicks [64]. Recent studies showed that the addition of *L. reuteri*, *B. licheniformis* and *B. subtilis* improved Body weight gain (BWG), feed conversion ratio (FCR) in broilers when compared to non-treated controls [65,66]. Along with this, studies from Zhen et al. also observed increased BWG and FCR on day 15 to day 21 compared to non-treated birds when *Bacillus coagulans* supplemented in Cobb broilers challenged with *Salmonella enteritidis* [67].

Poultry are exposed to stressful conditions in large-scale rearing facilities; this leads to the problems related to diseases and different environmental circumstances and result in serious economic loss. In recent decades, control and prevention of diseases was carried out by extensive use of antibiotics and veterinary medicines. The therapeutic use of antibiotics has led to acquisition of antimicrobial resistance causing a negative impact in the animal and food industries because such antimicrobial resistance is transferrable to humans. Therefore, in the poultry industry, alternatives to the antibiotics such as probiotics have been sought.

Lack of a healthy diet and dietary changes can impact the equilibrium of gut microflora leading to digestion upsets. Recently, the appropriate use of nutrients and probiotics for poultry growth promotion has received great attention from nutritionists and veterinary experts. Enteric diseases such as salmonella, campylobacter, etc., loss of productivity and even mortality are of great concern to the poultry industry and consumers. Dietary supplementation of *Bacillus subtilis* DSM 32315 was beneficial in attenuating the negative effects of *Clostridium Perfringens* challenge on the performance and intestinal microbiota of broiler chickens [68].

Whelan et al. [69] reported positive alterations of microbial populations in the gut of broilers when *B. subtilis* DSM 32315 was administered to the birds. This improved performance of broilers challenged with necrotic enteritis (NE), a sign that the probiotic *B. Subtilis* was associated with some mechanism that reduces pathology of the NE. To ensure the beneficial effect of probiotics in poultry, the timing of the administration of prebiotics

may be critical as well. Early administration of probiotics to chicks or at early age was shown to dictate the microflora profile of the GIT of the adult bird. There is evidence that the gastrointestinal tract microbiota develops relatively early in the young chick's life cycle, perhaps even in vivo [70].

In newly hatched chicks, the oral administration of probiotics was reported to rapidly establish an adult-type intestinal microflora, producing almost immediate resistance to GIT colonization by pathogenic microorganisms such as salmonella and campylobacter. According to the report of Mead [57], the exploitation of the 'competitive exclusion' (CE) effect is now an accepted part of the overall strategy by which poultry-associated salmonellas are being controlled in some countries.

3.4. Probiotics in Poultry Production

La Ragione stated that *Bacillus subtilis* spores would reduce *E. coli* colonization in chickens through oral consumption [71]. Salmonellosis, Campylobacteriosis (zoonotic disease), necrotic enteritis, and coccidiosis are enteric diseases that can cause massive economic damage to the poultry industry [72]. Especially in poultry, the main food safety issue is due to Salmonellosis as the pathogen results in a major foodborne illness in humans as well. Probiotics are considered to be an alternative approach to *Salmonella* control and to address the demanding concern about antibiotic resistant strains of *Salmonella*. Haghghi et al. revealed that depending on probiotic dose, reduction in caecal colonization occurs by several fold (1.2 to 3.0 log₁₀) by *Salmonella* [73]. From studies of Biloni et al. with administration of probiotics such as *L. salivarius* and *Pediococcus parvulus*, transmission of *Salmonella* infection was slowed down within the flock [74]. Giannenas et al. discovered a decline in coccidiosis through *E. faecium*, *B. animalis*, *L. reuteri*, and *B. subtilis* probiotics either individually or in combination [75]. According to Gaggia et al., while probiotics are supplied to the host through the initial stages of life change in gene expression in the intestinal epithelial cells occurs by the activity of probiotic bacteria, thus generating a favorable habitat for themselves and significant reduction in death rate. Improved body weight (BW), and reduced FCR were observed using *Lactobacillus*-based probiotic products in broilers [76].

Probiotics also have an effect on egg production in poultry along with beneficial effects to growth rate, feed conversion, and feed efficiency. In laying hens, mixed probiotic culture of *L. acidophilus*, *L. casei*, *B. thermophilus*, and *Enterococcus faecium* enhance size of egg and dropped feed cost were observed [77]. Improvements in egg quality and production were recorded using *Bifidobacterium thermophilus* and *Enterococcus faecium* [78]. Feeding *E. faecium* as probiotics in chickens for prolonged time, improved egg laying intensity, and feed conversion efficiency [44].

Earlier reports (Nahashon et al., 1994 [79]) demonstrated that feeding 1100 ppm *L. acidophilus* in diets to Single Comb White Leghorn layers stimulated appetite and improved egg production, egg mass, egg weight, egg size, and feed conversion ratios. The addition of fat to the diets containing lactobacillus reduced daily feed consumption as expected without adversely affecting bird performance and provided better feed conversion, egg masses, egg sizes, and body weight gains. In this study, the retention of nitrogen and phosphorus were also improved significantly ($p < 0.05$).

Feeding *L. Acidophilus* to Single Comb White Leghorn pullets had a long-term and positive effect on the bird's performance during the laying period [80]. Positive correlations were observed between Lactobacillus diets and nitrogen and calcium retention, and egg mass and between fat, nitrogen, calcium and phosphorus retention, and body weight gain, calcium and phosphorus retention, and egg mass, respectively. Intestinal length and dry weight were lower ($p < 0.05$) in layers fed the Lactobacillus diets compared to the control diet. Daily feed consumption, egg size, nitrogen, and calcium retentions increased whereas intestinal length decreased in layers fed Lactobacillus diets and observed to 59 weeks of age.

The effect on layer performance of *Lactobacillus* supplementation in diets varying in ingredient compositions was reported by Nahashon et al. [79]. Corn-soy-based diets were reported to improve egg weight, egg mass, egg size, and body weight gains, whereas the addition of barley in the corn-soy diets only improved body weight gains. In this study, regardless of diet type, the passage rates of digesta through the gastrointestinal tract was significantly increased. *Lactobacillus* supplementation also increased significantly the retention of fat, calcium, phosphorus, copper, and manganese in laying hens. It was also noted that feeding *Lactobacillus* increased cellularity of Peyer's patches in the ileum suggesting a stimulation of the mucosal immune system that responds to antigenic stimuli by secreting immunoglobulin (IgA).

It was proposed that probiotics play a key role in regulating the immune response by stimulating various subclasses of immune cells for the production of cytokines. In particular, *Lactobacilli* can modify the response of antibody to antigens in chickens and also plays a part in stimulating the transforming growth factor β production [2]. Gastrointestinal diseases such as salmonellosis, necrotic enteritis, and coccidiosis were prevented by the addition of probiotics [81–84]. Improved nutrient digestion and enhanced cecal microbial population were observed in broilers by adding probiotics [85]. Enhanced immunity was recorded by several studies by the probiotic administration in poultry [86–89]. In the studies of Zhang et al. [90], improved chicken growth rate, immune system, and antioxidant quantity were observed by the addition of *Lactobacillus casei*, and *Bifidobacterium* in the food.

4. Challenges to the Application of Probiotics in Animal Feeding

The commonly used probiotic microbials in animal feedstuff are usually considered harmless. The greatest risks associated with probiotic microbes used in animal feed are, increased chance of spreading antibiotic resistance by the existence of communicable antibiotic resistance genes in some probiotic bacteria, and contagions from probiotic microorganisms along with the occurrence of entero and emetic toxins. Most probiotic research publications usually deal with their efficiency rather than safety. The maximum information available regarding the wellbeing of probiotics is only based on *Lactobacillus* and *Bifidobacterium* [91]. Hence further research is needed in relation to the safety and use of probiotics.

Even though probiotics used in animal feed are relatively safe in protecting animals, humans, and the environment, precautions should be taken with unsafe or harmful microorganisms. Hypothetically, menaces connected with the use of probiotics in animal feed are as follows [92].

- i. GIT infection of the animal that nourished the probiotic;
- ii. GIT infection of the consumers who had animal products that are produced by probiotic fed animals;
- iii. From probiotics, antibiotic resistance transmission to other pathogenic microbes;
- iv. Infections in the animal and animal food handlers;
- v. Sensitization/irritation of skin or eye in the administrators of probiotics;
- vi. Production of toxins by probiotics causing harmful metabolic or toxic effects in the host;
- vii. Susceptible hosts hyper-stimulation of the immune system.

Before considering microorganisms as probiotics, animal feed containing probiotics should be evaluated against the above-mentioned risks. The identification of microorganisms up to strain level is needed to evaluate the specificity of particular bacteria and to understand its beneficial properties.

While evaluating the safety of microorganisms to use as probiotics in animal feed, a few concerns have to be addressed, as presented in Figure 2. Primarily used probiotic microorganisms such as *Lactobacillus* and *Bifidobacterium* are usually safest because these microorganisms have been used extensively and traditionally in several fermented foods for a long time [93]. These microbes are generally present to a large extent in the GIT of humans and animals, and infections related to these micro-organisms are very rare. Moreover, the

US Food and Drug Administration states that *L. acidophilus* and *L. bulgaricus* are “Generally Regarded as Safe” (GRS). A few *Bacillus* species, including *B. subtilis*, *B. megaterium*, *B. licheniformis*, and *B. coagulans*, were recognized to be safe according to European Food Safety Authority (EFSA) due to an absence of toxins. Even though *Enterococcus* bacteria have numerous beneficial effects, they have related to few infections in humans such as community and hospital-acquired infections. Therefore, there is a need for stringent safety evaluations before the use of the *Enterococcus* bacteria as probiotics [94].

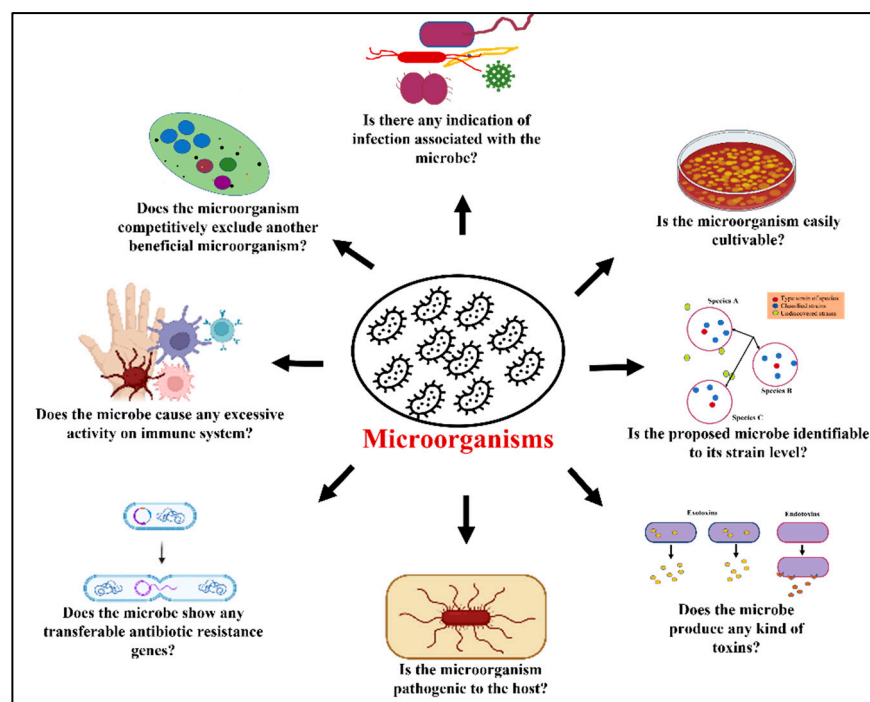


Figure 2. Criteria for evaluation of potential probiotics in animal feeding.

5. Application of Prebiotics to Complement the Effects of Probiotics in Poultry

Prebiotics are described as non-digestible food ingredients that beneficially impact the host by selectively stimulating the growth and/or activity of one or a limited number of beneficial bacteria or probiotics in the colon [95]. These nondigestible polysaccharides are not hydrolyzed by the endogenous digestive enzymes in the small intestine of monogastric animals such as poultry and thus find their way intact into the large intestine and caeca where they are hydrolyzed by bacteria in the large intestine and caeca. Large beneficial bacterial populations or probiotics which exist within the GIT of poultry use these indigestible carbohydrates (oligo or polysaccharides) and in turn confer their beneficial effects to poultry.

The dominant prebiotics in the industry were reported by Jiang et al. and Huang et al. [96,97]. These include mannanoligosaccharides (MOS), fructooligosaccharides (FOS), galactooligosaccharides and glucooligosaccharides (GOS), xylooligosaccharides (XOS), oligofructose, mannose, stachyose, and even peptides, proteins, and lipids, especially the short chain fatty acids (SCFAs). These oligosaccharides are naturally occurring constituents of plants and vegetables and they include bananas, onions, chicory root, Jerusalem artichoke, and bamboo shoots. The prebiotics therefore provide energy and are a source of carbon to gut microorganisms [98] and therefore modulate metabolism of gut flora or probiotics. The trend of the profile of the gut flora primarily dictated by the diet is the primary influence on the microbial profile and their associated functions [99].

According to Hajati and Rizaie [100], of prebiotics, lactose qualifies as a prebiotic because it consists of glucose and galactose which have prebiotic effects in chickens. Chickens do not possess the lactase enzyme, whereby the lactose enters the lower segments of

the GIT, the large intestine and caecum where it is used during microbial fermentation. While these prebiotics benefit gut microorganisms directly by providing their nutrients, they also benefit the host by providing useful nutrients such as the short chain fatty acids (SCFAs) from the microbial fermentative breakdown of the prebiotics such as MOS, FOS and GOS, and by modulating the development and functionality of the digestive in terms of mucosal morphology and mucin dynamics. They also benefit the host by enhancing the immune system through production of antimicrobial peptides and pro-inflammatory cytokines [101].

When a product contains both probiotics and prebiotics causing a synergism that favors or enhances the functionality of the probiotic compound, the term *synbiotic* is used to describe the combination of the two products [56]. The effect of synbiotics on poultry, both broilers and layers, has been researched extensively [101,102]. Prebiotics have also received some attention as candidates for free-range broiler feeds [101–103]. Sequencing of the gut microbiome to ascertain the impact of prebiotics on bird performance revealed the presence of lactic acid bacteria and bifidobacteri, a which were associated with improvement in bird performance [102]. Several performance traits of free-range broilers were also evaluated and positive response to prebiotics feeding was reported [102,103].

6. Future Directions

Research has provided evidence that probiotics are a significant source of health-promoting antimicrobials, which also serve as a source of nutrients in animal production. Probiotics can act as substitutions to growth-stimulating antibiotics, therefore enhancing the immunological capacity of the animals. Even with the current knowledge of the effects of probiotics on organisms, there is ongoing research to explain further some of the modes of action of probiotics. In future, to counter a specific aspect of growth or animal performance, the mechanism of action of probiotics is very important. Further research on specific gene expression pathways or metabolic pathways related to the effect of probiotics can reveal numerous applications of probiotics and their specific diagnostic and therapeutic uses. Targeting specific applications of probiotics can also provide answers for several disease related concerns in both humans and animals.

New and advanced molecular techniques, such as transcriptome and metabolomics, provide in-depth information on mechanisms of action of probiotics, which illuminates the beneficial effects of these probiotics and how they improve bird performance. Further research on specific gene expression pathways, including those revealed through metabolomics assays associated with the effect of probiotics, also reveal numerous applications of probiotics and their specific diagnostic and therapeutic uses. Targeting specific applications of probiotics can also help to evaluate answers for several disease related concerns in both humans and animals.

While probiotics have been hailed as beneficial in enhancing animal performance, including health, there are limitations associated with feeding probiotics to animals. Several probiotic organisms, such as enterococci, may harbor transmissible drug resistant genes and others, such as *Bacillus cereus*, produce enterotoxins which may be harmful to the host [104,105]. Other key challenges to use of probiotics are a lack of understanding of the possible interactions of the probiotics with host cells, and their respective safe doses. There is therefore a need to enhance studies to establish the use of probiotics more effectively and in the right doses depending on conditions of the host organisms or target recipients.

7. Conclusions

Current trends in poultry production call for a more robust approach to producing meat and eggs efficiently and yet delivering acceptable poultry products, meat and eggs to the consumer. This can only be assured by both a reduction in production cost through feeding and ensuring that poultry flocks are healthy. It is fair to imply that an efficiently managed and healthy flocks can cut down the cost of production ensuring affordability and safe poultry and poultry products for the consumer. There is enough evidence, as docu-

mented in this review, pointing to the use of probiotics and prebiotics in poultry production. Studies demonstrated the potential for probiotics to modify and maintain homeostasis of the gastrointestinal flora of birds increasing the level of beneficial microorganisms such as *Lactobacillus Acidophilus*, *Bifidobacterium*, *L. plantarum*, and *B. subtilis* while reducing the pathogenic microorganisms such as salmonella and campylobacter.

The excessive use of therapeutic levels of antibiotics in the poultry industry has been associated with antimicrobial resistance. In the search for alternatives to antibiotics, probiotics have been evaluated quite extensively, and they seem to have antimicrobial potential in addition to providing additional nutrients from their metabolites. Much effort should therefore be directed toward establishing diets for poultry which support the microbiomes of interest, because it is well established that diet has a significant influence on the profiles of the gut flora. Using current next generation sequencing technology, transcriptomics and metabolomics, further evaluation of microorganisms with the potential and properties of probiotics should be pursued.

This review has also spelled out the potential for prebiotics such as MOS, FOS, GOS, XOS, and SCFAs in poultry production. Their benefit to the bird is two-fold, they are a source of energy to probiotics and their byproducts of bacterial fermentation can be absorbed as nutrients usable by the host. Therefore, the combination of probiotics and prebiotics has potential in the poultry industry especially in enhancing efficiency of feed use and health through competitive exclusion and exhibition of antimicrobial properties.

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