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Review Article

# Roles of Probiotics in Farm Animals: A Review

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ABSTRACT

There are many reports of the positive effects of probiotics on gastrointestinal tract (GIT) microorganisms and the immunological systems of their hosts. Probiotics have prophylactic and metaphylactic properties. The two main mechanisms of action of probiotics seem to be the release of compounds with beneficial effects and direct interaction with the cells of the host. The aim of this review was to evaluate the benefits of probiotic use in farm animals and to identify how they influence farm animal performance. The published data suggest that dietary supplementation of probiotics can improve the growth performance, nutrient digestibility, and immune response of farm animals, including cows, sheep, goats, pigs, aquacultures, and poultry. In ruminants, studies have shown that probiotics can significantly enhance the immune response, milk yield, food digestibility, and weight gain, particularly in ruminants exposed to stressful conditions. This is also the case in aquaculture as probiotics have been shown to enhance growth and reproduction traits, provide protection against pathogens, have positive effects on immunity, optimize digestion, and increase water quality. In horses, there is still controversy about the advantages of probiotic supplementation. In addition, some studies showed valuable effects of using probiotics on treatments of GIT diseases, and some studies showed adverse effects of supplementation of probiotics in horses. In poultry, balancing the intestinal microflora is not achievable but controlling the population of microflora is possible and studies showed that supplementation of probiotics could influence some aspects such as improving performance and health parameters. So, probiotics are used by almost all farmers who are trying to reduce antibiotic resistance. In conclusion, dietary supplementation of probiotics to farm animals has many notable influences on their performance, immune system, and diet digestion.

## 1. Introduction

Probiotics have attracted great attention due to their positive effects on gut microbiota and immunological systems in humans and livestock[1,2]. Probiotics are used as prophylaxis and for therapeutic purposes in both humans and farm animals[3,4], with probiotics being a safe and viable alternative to the use of antibiotics growth promotors which can result in antibiotic resistance in microbial population[5]. When used in farm animals instead of antibiotics dietary supplementation of probiotics can improve growth performance, nutrient digestibility, and immune response and reduce the incidence of diseases[1,5]. In addition, probiotics increase the oxygen concentration in the gastrointestinal tract (GIT) in a multiplicity of ways including through depleting oxygen-scavenging compounds, such as nitrates. Moreover, probiotics secrete hydrolytic enzymes that can hydrolyze bacterial toxins and inactivate toxin receptors, decreasing toxin-mediated infections in farm animals[6].

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Probiotics decrease pathogenic microorganisms in the GIT and establish a better microbial population in the GIT[7]. Probiotics can also activate an immunologic response and assemble a series of immunoregulatory molecules[8]. Some probiotics produce metabolites that can modulate metabolic pathways in the cell, such as bacteriocins, amines, and hydrogen peroxide. These metabolites can adjust cell apoptosis, proliferation, inflammation, and differentiation[9].

Microorganisms used as probiotics should be nonpathogenic, optimize host health, and improve GIT function. The microorganisms commonly used as probiotics in animal diets are principally bacteria, usually Gram-positive bacteria. *Lactobacillus* and *Enterococcus* are natural flora in the alimentary tract of most animals and are usually present in amounts of $10^7$-$10^8$ and $10^5$-$10^6$ CFU/g, respectively[10]. *Lactobacillus acidophilus (L. acidophilus), L. lactis, L. plantarum, L. bulgaricus, L. casei, L. helveticus, L. salivarius, Bifidobacterium* spp., *Enterococcus faecium* (*Ent. faecium*), *Ent. faecalis, Streptococcus thermophilus, Escherichia coli* are the most commonly used probiotic bacteria in farm animals and probiotic fungi such as *Saccharomyces cerevisiae* (*Sacc. cerevisiae*) and *Sacc. boulardii* are the most commonly used probiotic fungi [11-15]. Of note, no other substances should be supplemented during the administration of probiotics such as antibiotics, disinfectants, and materials that interrupt with the mechanism of action of probiotics. In addition, water used for dilution should not contain chlorine or disinfectants[10]. Factors, such as the optimal selection of microbial strains, host species, and age are critical in the selection of probiotics[15]. Given the positive effects of dietary supplementation of probiotics on farm animals' immune response and growth performance, the current study aimed to review the benefits of probiotics and their mechanism of action in farm animals.

## 2. Usage and benefits

The microbial balance of the GIT in farm animals is critical as it can affect feed efficiency, digestive welfare, and health in farm animals[10]. It is influenced by many factors including diet ingredients, farm management, feeding practices, and productivity limitations, for example, a change of diet from forage to a high-concentrate diet results in a change in the microbial population of the rumen and can cause ruminal acidosis. Weaning is a critical period for the gut microbiota as the change in the diet results in major changes in that microbiota, which if not properly managed increases the susceptibility of young animals to pathogen colonization[16].

Animal products are closely related to consumers' health. Animal intestinal pathogens, such as *Campylobacter, Salmonella, Listeria,* and *Yersinia*, contaminate foods and result in zoonosis disease, outbreaks of which threaten public health and bring about economic loss[17]. Thus, producers need to provide animal breeding methods to increase meat quality and safety, pay attention to animal welfare, and respect the natural environment.

According to some estimations, the world population is growing very fast and could reach 10 billion in 2050. Therefore, it could cause food security challenges worldwide[18]. Antibiotics have been used commonly to change the alimentary tract microorganism and increase productivity and animal growth[19]. Prolonged use of antibiotics results in the development of drug-resistant microbiota. Drug-resistant development in microbiota threatens consumers' health, causes a negative impact on the environment, and increases foodborne allergies[20-22]. To confront these challenges, probiotics could be beneficial. Therefore, the use of probiotics as dietary supplementation has received great concerted attention.

## 3. Mechanism of action

The metabolic activities, survivability of the probiotic throughout the gut, and the used strains are essential for the optimal efficacy of probiotics[23]. The combination of several mechanisms has indicated the positive effects of probiotics on animals' health. Two main mechanisms of action of probiotics include the release of compounds with inhibitory effects and direct interaction between cells[24-26]. Notably, organic acids, such as lactic or acetic acid, produced by bacterial probiotics can reduce the GIT pH, making it less favorable for pathogen colonization, particularly in monogastric animals[27]. Some probiotics release antimicrobial compounds, such as peptides (bacteriocins), hydrogen peroxide, and biosurfactants that prevent the pathogenic bacteria growth or release enzymes hydrolyzing bacterial toxins[28]. Probiotics have a higher affinity to adhesion sites and compete with GIT pathogens for nutrients[29,30]. In addition, studies have revealed that probiotics positively affect immune response[31]; for instance, administration of *L. acidophilus*, *L. salivarius*, and *L. plantarum* at the dosage of $10^7$-$10^8$ CFU/g can reduce the occurrence of diarrhea in calves[32].

Moreover, dietary probiotic supplementation increases disease resistance and decreases metabolic stress and mortality[27]. Some probiotics play an influential role in detoxifying and metabolizing inhibitory chemicals, such as nitrates and amines or oxygen scavenging[23], which is of utmost importance for GIT anaerobic ecosystems. The mechanism of probiotics action in human GIT is almost similar to that of animals and has health and nutrition benefits[10].

## 4. Probiotics in farm animal

### *4.1. Probiotics in ruminant*

Most bacteria in the ruminant intestinal tract belong to the phyla *Bacillota* (*Firmicutes*) and *Bacteroidota (Bacteroidetes)* with species in the phyla *Actinobacteria*, *Proteobacteria*, and Verrucomicrobia also being common. For example, the microbiological ecosystem of the rumen in the ruminant is complex, yet nearly 99% of bovine rumen bacteria and 96% of ovine rumen bacteria belong are *Firmicutes* or *Bacteroidetes*[15]. Nevertheless, the composition of the rumen microbiota is sensitive to change based on feed and animal status. So, the key times for which probiotics have been shown to be especially beneficial are at weaning, after calving, and after dietary changes from forage to high-concentrate diets for the gut microbiota. Probiotics, such as *Sacc. cerevisiae*, *lactobacilli*, *Aspergillus oryzae*, *Bacillus,* and *Enterococcus*, are commonly at such time points and positively affect the ruminant health[12,14,23,33-37]. Some studies have indicated that milk yield increases in cows fed a diet supplemented with probiotics. In addition, some probiotics can enrich milk secretion, such as *Bacillus subtilis*, *Sacc. cerevisiae*, and *Ent. faecalis*[38]. *Bifidobacterium bifidum* also prevents milk allergy reactions in dairy cows[39]. According to a previous study[40], probiotics containing *Ent. faecium* and *Sacc. cerevisiae* increases milk yield by 2.3 liters per dairy cattle daily. Another study showed that active yeast probiotics could enhance milk yield by nearly 1.2 g/kg of body weight in dairy cows, and increase dry matter intake in the ruminant by about 0.44 g/kg of body weight, but had no effects on milk protein percentage[41]. A study on goats revealed that the probiotic combination of *L. reuteri* DDL 19, *L. alimentarius* DDL 48, *Ent. faecium* DDE 39, and *Bifidobacterium bifidum* DDBA obtained from healthy goats could enhance the body weight of goats fed the combination for two months by 9%[42]. Some probiotics, such as *B. subtilis* and *B. amyloliquefaciens* can upgrade intestinal maturation by stimulating GH/IGF-1 hormone[43]. Some studies have indicated that probiotics can enhance the feed digestibility and immune system in ruminants[16-18]. A previous study revealed that probiotics (a mixture of *L. acidophilus* NP51 and *Propionibacterium freudenreichii* NP24) could increase the digestion of neutral detergent fiber, crude protein, and milk production by nearly 7.6% in ruminants[44]. In juvenile calves, mixed probiotics of *L. acidophilus*, *L. salivarius*, and *L. plantarum* at a dosage of $10^7$-$10^8$ CFU/g reduced diarrhea occurrence[45]. *Lactococcus lactis* produces an antimicrobial peptide named nisin, and infusion of nisin in the mammary gland in dairy cows can treat mastitis caused by *S. aureus*[46]. Teat spray containing *Lactobacillus* spp. has a positive effect on mammary gland condition and improves the functions of the teat sphincter[47]. Adding probiotics to young stressed calves' diet can improve immunity response and alleviate acidosis in cows with rumen acidosis[48]. In addition, probiotics release antimicrobial components that can reduce zoonotic pathogens and limit the risk of foodborne diseases in humans[10]. Probiotics can control ammonia production in the rumen [13]and mitigate methane excretion, which reduces ruminants' negative impact on the environment as methane produced by ruminants is responsible for 3-5% of global warming[17]. Probiotics containing *Rhodopseudomonas palustris* improve the growth performance of rumen microorganisms, increase microbial fermentation and optimize rumen microbial balance[49,50].

### *4.2. Probiotics in non-ruminant*

Bacterial strains that are commonly used in probiotics in monogastric animals are *Lactobacillus* spp., *Enterococcus* spp., *Pediococcus* spp., *Bacillus* spp., and yeasts, such as *Sacc. boulardii* that target cecum and colon [17].

The large intestine of horses is home to different microorganisms composed of bacteria, protozoa, and fungi that significantly impacting horse health and performance[51,52]. In equine medicine, some diseases such as acute colitis, equine grass sickness, and laminitis have all been linked to changes in the equine intestinal microbiota[52,53]. The most common phylum that exists in equine feces is *Firmicutes* which accounts for 46-70% of all identified bacteria following *Bacteroidetes*, *Proteobacteria*, *Verrucomicrobia*, *Actinobacteria*, and *Spirochaetes* each accounting for 0-15% [52,54,55]. Compared to horses with diarrhea, healthy horses have higher levels of *Actinobacteria* and *Spirochetes* and higher levels of *Fusobacteria*[52,56]. The large colon is the site of several diseases in equines and probiotics are intended to target this site. Species *Lactobacillus, Bifidobacterium*, and *Enterococci* which are commonly used in probiotics are not the most abundant species in the horse's large intestine[52,55]. Probiotics can modulate the immune system by strengthening the intestinal barrier, promoting the survival and growth of epithelial cells, and inducing the production of IgA and β-defensin, which inhibits pathogen growth[57]. Horses receiving *Sacc. boulardii* had a shorter period of watery diarrhea, but this finding should be interpreted with caution since only seven animals were used per group, and there was no difference between treatment groups[58]. Furthermore, obtained results may be confounded by treatment protocols. In another study, *Sacc. boulardii* was administered as an adjunctive therapy to horses with antimicrobial-associated enterocolitis, and no differences were observed in the treatment groups[59]. Another study assessed *L. pentosus* in a randomized placebo-controlled clinical trial, and its use was associated with a higher incidence of diarrhea, fever, and anorexia[60]. Two other studies report similar findings that the use of probiotics increased the risk of diarrhea in neonatal foals[61,62]. In contrast, A study that used multistrain equine-derived probiotics reported an increase in weight gain and a decrease in the incidence of diarrhea in neonatal foals[63]. Two studies on horses tried to determine the effect of probiotics on Salmonella shedding, but the results were not promising[64,65].

Various hypotheses have been mentioned as the reason why probiotics do not work effectively in horses, compared to other animals, especially ruminants. Most probiotics supplements in horses fail to meet the FAO and WHO (2002)[56] definitions because of quality control issues and a lack of specific research currently existing. Several studies indicated that some probiotics in the market that are used as a supplementation in horses did not contain the amounts specified on the product label and any quantity of some microorganisms[62,66,67].

Another hypothesis related to the lack of effectiveness of probiotics in horses is that physiological decreases in gastric pH protect horses from potential pathogenic microorganisms, but this reduction of gastric pH can also negatively impact the viability of microorganisms in equine probiotics[68]. Another disadvantage of using probiotics in horses is that many products are based on human studies data that may not apply to horses. Furthermore, several studies have shown that antimicrobial-resistant genes could transfer by probiotics in horses[67], which needs further investigation. Thus, more studies are required to capitalize on probiotics' potential benefits in equines.

Supplementation of probiotics could reduce diarrhea prevalence in piglets and prevent the colonization of pathogenic organisms, such as *E. coli, Clostridium difficile, Clostridium perfringens, Salmonella,* and *Listeria*, parasites (*Isospora* and *Cryptosporidium*) or viruses (*Coronavirus* and *Rotavirus*)[69]. These pathogenic organisms can result in growth reduction and diarrhea in piglets[17]. Weaning is a critical moment in pig production when animals are exposed to the stressful condition such as nutrition changes from milk to a vegetable base die, and animal transfer to another farm cause stress and consequently disturb immunological function and harms pigs' GIT. Studies reported that probiotics positively influence the immune system in stress conditions in pigs notably during weaning[69,70]. Probiotic supplementation can increase dry matter intake and average live weight in pigs[71].

### *4.3. Probiotics in aquaculture*

FAO (2020) reported that 179 million tons of fish were produced worldwide in 2018, which cost about 400 billion USD. Antibiotics are commonly used in the aquaculture industry to meet the increasing demand for fish meat. However, antibiotics increase the drug-resistant bacteria transmitted by food to humans[72,73]. The probiotics supplementation in water-dwelling animals enhances growth and reproduction, provides protection against pathogens, exerts positive effects on immunity, optimizes digestion, increases water quality, and can be an alternative to antibiotics[74]. One of the probiotics commonly used in fish farms is *Bacillus subtilis*, which can prevent harmful pathogens, such as *Vibrio*, *Pseudomonas, Aeromonas, Clostridium, Streptococcus, Flavobacterium, Acinetobacter*, and white spot syndrome virus in fishes[75]. Some bacterial strains, such as *L. lactis*[76] and *L. plantarum* VSG-3[77] are commonly used in fish farms. Gram-negative facultatively anaerobic bacteria are dominant in the GIT of aquaculture.

According to some studies, microalgae, such as *Dunaliella tertiolecta, Dunaliella salina, Isochrysis galbana, Phaeodactylum tricornutum*, and *Tetraselmis suecica*, increase the survival rate of aquatic animals[78,79]. A few studies reported the positive effects of *Sacc. cerevisiae* (yeast) on aquatic animals[80,81]. Farmers supplement probiotics to water circulation or to the fish diet [82]. Some probiotics are immunostimulants in aquatic animals that could be a single or combination of bacterial strains[83,84]. Proper timing and dosage are essential for obtaining the expected outcomes in fish farms[18]. Probiotics could optimize the health conditions of aquatic animals. Some studies reported that probiotics containing *B. pumilus*[85] and *L. plantarum*[86] can improve the health conditions of Nile tilapia and decrease the fatality rate caused by Lake Virus infection in Nile tilapia[87]. Probiotics containing *Pseudomonas* I-2 can decrease diseases caused by *vibrio* microorganisms in fishes[88]. Probiotics with *Pediococcus acidilactici* can prevent vibriosis in white-leg shrimp[89]. Dietary supplementation of probiotics plus *Lactobacillus, Saccharomyces, Bacillus, Bifidobacterium*, and Photosynthetic bacteria can increase the growth rate and larval metamorphosis in white-leg shrimp larvae. These strains also decrease the number of *vibrio* pathogens and encumber incomplete molting during the development in White leg shrimp larvae[90]. Some studies showed that probiotics containing *Pediococcus pentosaceus* and *S. hemolyticus* could reduce the prevalence of white spot syndrome virus in white leg prawns[91]. The *Sacc. cerevisiae* can be substituted as live food in the cultivation of some fish species, such as clownfish, Catla, hybrid striped bass, Japanese flounder, and Nile tilapia [18,92-95]. Probiotics have a positive effect on immune responses in fish. For instance, probiotics, including *Bacillus* activates, the humoral and cell-mediated immunologic response in fish[96,97]. In addition, probiotics can increase digestive enzyme processes, growth performance, and feed intake in aquaculture. Probiotics produce enzymes that improve nutrient digestion in aquacultures, such as protease, carbohydrase, and lipase[98,99]. Some studies showed that probiotics could enhance water quality by nourishing water properties, preventing diseases, and optimizing fish habitats, also reducing metabolic wastes[100,102]. Moreover, probiotics can increase water quality by decreasing pathogen microbes, nitrogen, and phosphate contamination in the sediments[103-105].

### *4.4. Probiotics in poultry*

The global concern about antibiotic residue in poultry products is increasing, and it is clear that more attempts should be conducted to find the best antibiotic alternative in poultry diets. One of the best approaches to control poultry diseases, perform prophylactic treatment, and recover the birds after antibiotic treatment is definitely probiotic bacteria[106].

However, balancing the intestinal microflora is not achievable but controlling the population of microflora is possible to occur. The administration of the beneficial bacteria is possible via different methods in the poultry industry[107-109]. Currently, *in ovo* administration of probiotics is considered in the poultry production system. This method of administration aimed to stimulate the immune system, control intestinal microflora, and improve the characteristics of gut health in the first days of life. *In ovo* feeding and vaccination are studied along with the administration of probiotics in hatchery[109].

The hatched chickens are exposed to many different bacteria in the first days of growing. Oral administration of probiotics or even prebiotics can improve performance and health parameters[106-109]. The probiotic agents usually do not absorb from the intestines and have no side effects on internal organs. However, some of these agents, such as *Sacc. cerevisiae*, can reduce the adverse side effects of aflatoxins on the liver[109,110]. The most common agents of synthetic probiotics in poultry industries are *Lactobacillus* spp., *Bifidobacterium bifidum*, *B. sabtilis*, *S. salivarius* sub spp. *Thermophilus*, *Asperigillus oryzae*, and *Sacc. cerevisiae*[106,109-110]. The positive effects of some natural sources of probiotics and phytobiotics, such as Kefir, are also reported[111,112]. The most important challenge of probiotic administration in different growing ages is administration duration. There are many commercial recommendations for using industrial products in a limited time to improve poultry performance parameters. However, it seems necessary to design some structured protocol studies on supplementing probiotics in different ages and health conditions.

## 5. Risks of using probiotic

Safety evaluation of probiotic strains is essential because probiotics might lead to adverse effects, such as transferring virulence factors and antimicrobial resistance, hemolytic potential, and production of toxic biochemicals[113]. Moreover, the potential risk of transmission of antimicrobial-resistant genes in the probiotic strain to other bacteria of the normal flora of GIT is more critical. Rarely, infectious diseases such as endocarditis, bacteremia, pneumonia, meningitis, and septic arthritis caused by *Lactobacillus* and *Enterococcus* are reported in immuno-compromised patients[114]. Some authors discourage using Enterococcus as a probiotic because Enterococcus is more likely to develop vancomycin-resistant genes and can transfer to neighboring pathogens and enhance the virulence of those pathogens[6]. Discovering the origin of GIT microbiota is necessary for ascertaining the source of a microorganism in the GIT. In addition, the possibility of probiotic supplementation that could contaminate the human food chain must be considered.

## 6. Conclusion

In conclusion, farmers use additives to increase growth performance due to the increasing demand for meat production. Some additives, such as antibiotics, are growth stimulators but have adverse effects on human and animal health, such as increasing antimicrobial resistance. Alternatives like probiotics could be a safer substitution. Probiotics are used as prophylaxes and for therapeutic purposes in humans and farm animals. In addition, probiotics positively affect the immune system, particularly in stressful conditions, increase performance such as growth performance and milk yield, enhance nutrient digestibility, and prevent some diseases in farm animals. More studies are needed to evaluate the effects of probiotics on farm animal performance and the safety of these additives.

## Declarations

### *Competing interests*

There is no conflict of interest.

### *Authors' contribution*

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### *Ethical considerations*

Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by all the authors.


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