Chapter

Advances on Probiotics Utilization in Poultry Health and Nutrition

Janvi Hemant Kadam, Rasika Sagar Pawar, Mohd Fadhil Md. Din and Vasudeo Zambare

Abstract

Poultry is one of the most rapidly expanding food production sectors, especially in developing countries. The poultry birds are safe and in good health due to the antibiotic supplemented feeds. However, the increasing awareness regarding antibiotic resistance has led to a dire need for the development of antibiotic-free poultry. Therefore, in addition to the daunting challenge of sufficing the need for poultry food of the increasing population, the industry should also ensure that the production is based on sustainable practices. In poultry farming there are several alternatives to the antibiotics, and one of them is probiotics. Probiotics are beneficial and safe microorganisms for preservation of the host's health and well-being. There are convincing experimental shreds of evidence that discuss the impact of probiotics on the positive modulation of poultry's immunity, growth performance, feed utilization and general health condition. Therefore, this review shed light on the concept, impact and mode of action of probiotics in sustainable poultry production. By discussing the results obtained from the recent studies about the ability of probiotics to maintain the poultry animal's performance level, this chapter condensed the valuable information and open several avenues for further investigation and development of the probiotic applications in the poultry industry with a special focus on its effect in improving poultry's' health and nutritional value.

Keywords: poultry, probiotics, growth promoters, mode of action, immunity

1. Introduction

Poultry is crucial in supplying the burgeoning urban population's growing need for protein rich foods and is one of the young and quickly expanding subsets of animal husbandry [1]. The significance of raising poultry in enhancing the socio-economic conditions of regions that lack sufficient resources cannot be exaggerated. This is due to its features like swift bird generation, concentrated expansion, remarkable efficiency, reduced labor expenses, and minimal resource needs [2]. However, with increasing commercialization, poultry production continues to shift from subsidence agricultural practices to intensive food production, which has consequently increased the occurrence of diseases [3].

Antibiotics (when administered at sub-therapeutic levels) have been shown to be a successful method for illness control, growth enhancement, and feed conversion efficiency during the last several decades [4]. However, unrestricted use of antimicrobial drugs has resulted in their accumulation in terrestrial habitat, which has led to extensively documented adverse outcomes, including the rise of antibiotic-resistant bacterial strains, the accumulation of antibiotic remnants in the meat, and changes to the beneficial microbiota of the poultry [5]. There is always a risk associated with transmittance of resistant bacterial genes from poultry animals to humans via non-pathogenic bacteria to human pathogens. Therefore, the use of antibiotics in poultry has become risky [6].

Antibiotic resistance and concerns about food safety associated with overusing antibiotics prompted the European Union in 2006 to prohibit their use in animal feed [7, 8]. As a result, there has been a rise in the search for and use of alternatives to antibiotics, in order to safeguard poultry and human health.

The use of probiotics, often known as beneficial bacteria, suppress diseases in a number of ways, and is increasingly viewed as a substitute for antibiotics [9]. However, the significance of probiotics employed in poultry is not confined to the gastrointestinal system; they also play a noteworthy part in the enhancement of the organism's overall health. This study therefore compiles and assesses the current state of knowledge on probiotics for poultry's health and nutrition.

2. Probiotics

Probiotics are "live organisms and its substances that contribute to gut's microbial equilibrium," as Parker put it in 1974. Definition of probiotics as "a live microbial feed additive that beneficially impacts the host animal by enhancing its gut microbial balance" was established in 1989. More recently, "live microorganisms that when administered in suitable proportions impart a health benefit on the host" has been used to characterize probiotics [10].

Probiotics that are regularly utilized include strains of *Bacillus subtilis, Lactobacillus, Saccharomyces cerevisiae*, *Bifidobacterium, Aspergillus*, and *Streptococcus* all of which are capable of growth promotion and antimicrobial activity against pathogenic bacteria like *Escherichia coli, Salmonella typhimurium, Clostridium perfringens*, *Staphylococcus aureus*, etc. [11]. These strains can be isolated from fermented products or animal body such as breast milk, gut, fecal matter. *Lactobacillus plantarum* and *Leuconostoc mesenteroides* probiotics from some non-conventional sources of vegetables and fruits are also reported [12].

In the past, the probiotic market was predominantly led by the Asia Pacific region, with Europe following suit. The worldwide probiotic market's estimated worth is approximately USD 57.8 billion in 2022, and projections indicate a compound annual growth rate (CAGR) of 7.5% [13]. Apart from being consumed by humans, probiotics are also experiencing a growing application in animals, particularly in the context of poultry farming. The rising demand for poultry probiotic components in poultry diet can be attributed to the growing interest of consumers in eating more protein-rich meals like eggs. Probiotics employed in poultry farming may consist of a solitary strain or a blend of two or more strains, serving the purpose of disease prevention, health enhancement, and the augmentation of poultry growth and efficiency. It is widely recognized that utilizing a combination of multiple strains of probiotics can yield synergistic advantages [14]. Probiotics exist in different forms such as granules, powder, liquid paste and gel out of which dry forms are better for gastric environment and longer shelf life [11]. Several review articles have reported that probiotic use in poultry has provided various benefits such as improvement in growth performance, along with immune enhancement, and sustainability of gut microbes.

3. Mode of action

There is no one method via which probiotics exert their benefits; nevertheless, they are essential to the health of the host immune system and the interaction involving the gut microbiota and the immune system of the host. Probiotic microorganisms used in poultry production should fulfill several requirements to be considered effective, including being indigenous to the host, adhering to the intestinal epithelium, surviving gastric juices and mucous, engaging in competition for colonization within the gastrointestinal tract alongside other microorganisms, and exhibiting high viability under storage conditions [15].

Probiotics have been shown to improve digestion, increase the production of digestive enzymes, and even detoxify substances in the diet, resulting in improved growth and performance in poultry. Next possible action is: by prevention of pathogens via competition for adhesion sites; producing organic acids which can alter gut pH (predominantly probiotics containing *Lactobacillus* strains), which can promote absorption of minerals and protein, volatile fatty acids and antibacterial substances like bacteriocins, hydrogen peroxide, defensins, etc.; and physiological effect by regenerating intestinal mucosa, and immunological effects by modulating antigen presenting cells, regulatory T and B cells, and regulation of pro-inflammatory cytokines [16].

Furthermore, probiotics might alter the development and composition of the microbiota in the gut by restraining the impact of harmful bacteria, as well as bolster the body's natural defense mechanism through the synthesis of inhibitory substances. The adhesion of specific probiotics to the gut mucin, a glycoprotein, suggested the competitive suppression or colonization resistance of infectious pathogens adhering

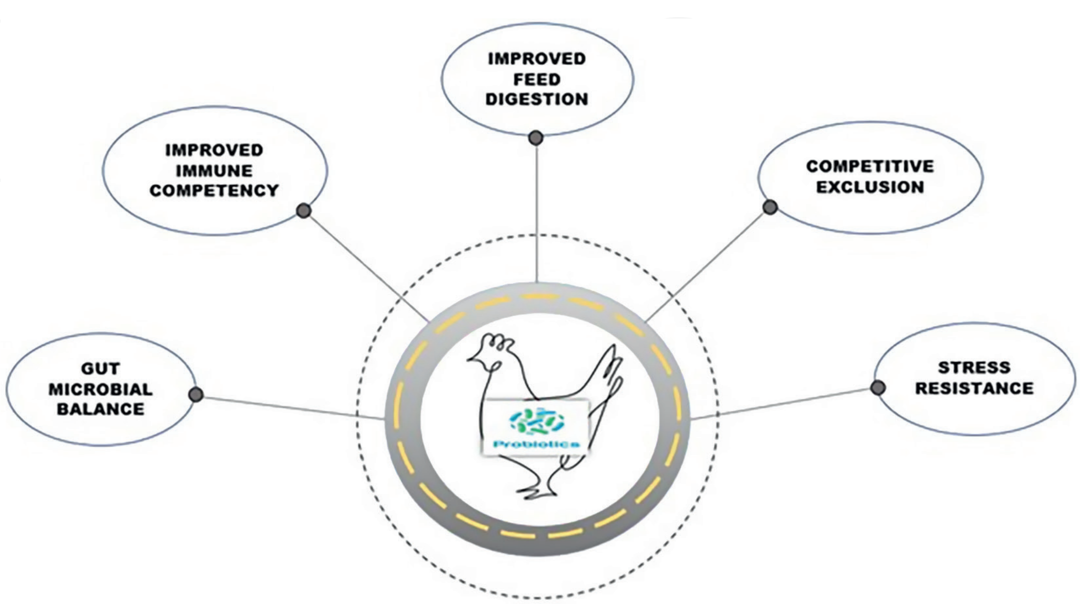

Figure 1.
Mode of action and impact of probiotics in poultry.

to the shared route/site [17]. Thus, it out competes the proliferation of opportunistic pathogenic microbes. Lastly, increasing the resistance of poultry animals against stress caused by temperature as a potent environmental factor.

The aforementioned mechanism of action provided foundational data for understanding the positive benefits on the poultry animals (**Figure 1**). However, transcriptome and proteomic analyses are needed in future studies of probiotic and host interaction to better understand probiotic activity.

4. Effect on growth performance

It has been theorized that probiotics can help poultry birds continue their typical development and functioning by serving as a source of vitamins, nutrients, and digestive enzymes that have a beneficial impact on feed utilization, nutrient absorption, and growth rates. Various commercial probiotic supplements have been tested for their growth-promoting effects in poultry. Studies have demonstrated that probiotics such as *Bacillus subtilis* and Lactobacillus species have the potential to improve feed conversion ratios (FCR) and increase body weight gain in broilers. These effects are attributed to the mechanisms mentioned earlier, which collectively lead to optimized nutrient utilization and enhanced growth rates. Zymospore® (Vetanco Brazil, *B. subtilis*), a commercially available direct-fed microbe, increased the bacterial variety of the broiler's gut microflora evidenced by heightened levels of lactic acid bacteria and clostridiales, thereby promoting feed digestion and growth, even under experimentally challenging conditions [18]. Broiler chickens given commercial probiotics (Lacto sacc, Alltech, Inc.) showed a considerable feed conversion ratio, as determined by Georgieva et al. [19].

In addition to single-strain probiotic supplements, herbal probiotic additives have also been explored for their growth-enhancing properties. These additives often combine beneficial microorganisms with herbal extracts, creating a synergistic effect on growth performance. For example, a study reported significant effect on weight gain in broilers provided with diet supplemented with Promix® (*B. subtilis*, *Bifidobacterium bifidum*, *Bifidobacterium longum*, *Lactobacillus acidophilus*, *S. cerevisiae*) and herbs (*Curcuma xanthorriza*, *Curcuma aeruginosa*, *Zingiber offocinale*, *Curcuma domestica*, *Kaempferia galanga*), a commercial herbal probiotic feed additive [20]. When challenged with Salmonella pullorum, a recent study observed significant effect ($P \leq 0.05$) on feed intake, mortality, and gut microflora in dwarf male chicks fed with basal diet supplemented with probiotic fermented herbal blend [21].

Nutrient adequacy and nutrient deficiency conditions controlling the probiotic growth. In cases of nutrient deficiency, probiotics assist in enhancing nutrient absorption and utilization, effectively compensating for reduced nutrient availability in the diet. This suggests that probiotics could play a crucial role in improving growth rates even in challenging dietary situations. A study observed, significant improvement in Feed conversion ratio (FCR), Feed intake (FI) and on weight gain (WG) in broiler hens fed with triticalate-based diet enriched with probiotics and enzymes [22]. The ileal digestibility coefficient of proteins, starch, and gross energy all improved significantly ($P \leq 0.05$) when the probiotic (*Bacillus subtilis*) was added to the broiler diets of both nutrient-adequate and nutrient-deficient birds [23]. Similarly, significant ($P \leq 0.01$) increased in body weight and decreased FCR was observed in chicks fed with meal supplemented with *Bacillus subtilis* probiotic, the results were in comparison with conventional feed additive—bacitracin methylene disalicylate (BMD). However, when challenged with *E. coli*, both *B. subtilis* and BMD did not compensate for the growth deterioration in chicks [24]. A study showed that the overall performance of white Pekin ducks was enhanced when a probiotic combination of *L. acidophilus* and *Lactobacillus casei* was added to a diet low in protein [25]. Bidura et al. reported that enrichment of basal diet with probiotics, *Saccharomysces sp.* Increased growth performances along with decreased levels in serum and meat cholesterol in male duckling [26]. It has been found out that dietary supplementation with probiotics, *Pediococcus acidilactici* and *B. subtilis* in water, improves the intestinal health and resistance of chickens against coccidiosis-causing *Eimeria species* [27].

In contrast, as in Ref. [28], it was discovered that supplementing with probiotics had no discernible effect on chicken carcass, growth performance or meat quality. Recently, endeavors have been directed towards the identification and isolation of lactic acid bacteria possessing probiotic capabilities, to engage in interactions, whether direct or indirect, with aflatoxin-producing fungi, or to detoxify aflatoxins themselves. A study reported beneficial effect of probiotic, *Lactobacillus plantarum* 299v in subsiding the toxic effect of poultry fed contaminated by aflatoxins [29]. According to research, supplementing the diets of dual-purpose hens with the probiotics *Lactobacillus paracasei sparacasei* and *Lactobacillus rhamnosus* significantly ($P < 0.05$) increased both body weight gain and FCR [30]. Khabirov et al. postulated that supplementing broiler feed with Normosil, i.e., live cultures of *Lactobacillus* and *Enterococcus* strains improved overall growth, nutrient digestibility and hematological characteristics [31]. Another study reported that *Bacillus licheniformis* as probiotic in basal diet showed better improvement in broiler chickens' body weight gain and production efficiency factor in comparison with *Bacillus subtilis* [32].

The effects of Probiotics on poultry's growth performance are summarized in **Table 1**.

Probiotics	Effect	References
Effect on growth performance		
Zymospore® Direct feed microbials, *Bacillus subtilis*	Improvement in feed intake, feed conversion ratio and body weight	[18]
Bacillus based triticale diet	Increased feed intake, feed conversion ratio and weight gain along with cellular immunity	[22]
B. subtilis	Improvement in intestinal digestibility coefficients for starch, crude protein, and overall gross energy	[23]
B. subtilis	Positive impact on growth performance, improved gut health	[24]
L. plantarum and *L. paracasei* fermented herbal blend	Avoid death, improved growth performance, enhanced immunity and controlled intestinal flora	[21]
L. plantarum	Protective effect against hepatotoxicity and oxidative stress caused by aflatoxin along with improvement in growth performance	[29]
L. paracaseis sparacasei, *L. rhamnosus*	Improvement in the growth performances	[30]
Lactobacillus, Enterococcus strains	Improved overall growth, nutrient digestibility and hematological characteristics	[31]
B. licheniformis	Improvement in body weight gain and overall production	[32]
L acidophilus, L. casei	Compensated for protein deficient diet	[25]

Probiotics	Effect	References
Saccharomysces sp	Increased growth performances along with decreased levels in serum and meat cholesterol	[26]
P. acidilactici, *B. subtilis*	Improvement in intestinal health and resistance against *Eimeria sp*.	[27]
Promix®	Excellent effect on weight gain	[20]
B. subtilis PB6 (CLOSTAT)®	Improvement in skeletal health and meat quality	[33]
Effect during stress conditions		
S. cerevisiae	Reduction in erythrocyte osmotic fragility, lipid peroxidation and higher expressionof superoxide dismutase activity	[34]
B. subtilis	Reduce breast muscle oxidative degeneration and improved meat quality	[35]
B. subtilis	Immunity suppression both in thermoneutral and heat-stimulated situations, improved food conversion ratio	[36]
Lactobacillus strains	Prevalence of *Lactobacillus sp*. on heat stressing	[37]
S. cerevisiae, L. acidophilus selenium enriched	Inhibited hepatic oxidation	[38]
L. acidophilus, S. cerevisiae selenium enriched	Improved body weight gain and bone health	[39]
L. casei, *L. acidophilus*, and *Bifidobacterium lactis*	Gene regulation for immunity, and metabolism of glucose and lipid	[40]
Lactobacillus acidophilus, Lactobacillus plantarum, and *Enterococcus faecalis* (RUYIRUYI) ®	Improved intestinal integrity, ameliorated inflammatory response	[41]
Effect on immune modulation		
Lactobacillus sp.	Effective against reducing pathogenic enterobacterial colonization, downregulation of pro inflammatory cytokines	[42]
L. acidophilus, S. cerevisiae selenium enriched	Upregulation of IFN-gamma mRNA genes thus protecting against *E. tenella* infection	[43]
L. fermentum, *L. plantarum*, *S. cerevisiae, Enterococcus faecium, Pediococcus acidilactici*	Upregulation of mRNA anti-inflammatory genes thus protecting against *P. multocida*	[44]
L. fermentum, L. acidophilus	Downregulation of pro inflammatory cytokines	[45]
L. acidophilus, *L. casei*	Improvement in overall immunity	[25]
L. plantarum	Improvement in both cell mediated and humoral immune response	[46]
Probiotic with diclazuril	Increase in the levels of interleukin and immunoglobulin	[47]

Table 1.
Probiotics' effects on poultry.

5. Effect during stressful conditions

The term "stress" is used to refer to the body's reactions to extrinsic or environmental factors that threaten homeostasis, or the body's normal state of physiological equilibrium. Environmental stressor normally weakens the immune system of animals and increases their susceptibility to disease occurrence. Poultry birds respond to stress by modifying their behavior, biochemistry, and physiology in an effort to restore body's equilibrium. Based on the above, poultry animals normally suffer from one important stressful condition i.e., heat stress.

The deleterious effects of heat stress on the immunology, physiology, and microbiology of birds leads to negative impact on poultry industry. Genes like heat shock protein (HSP) are likely to have their expression altered in response to heat stress. Various studies have confirmed effective use of probiotics to supplementation in eliminating poultry heat stress. Ogbuagu et al. reported that combination of fisetin and probiotics, decreased erythrocyte osmotic fragility, lipid peroxidation and increased superoxide dismutase, thus reducing the effects of oxidative stress alterations in broiler chickens exposed to heat stress [34].

During chronic heat stress supplementing broiler with dietary probiotic of *B. subtilis* improved meat quality and alleviate oxidative deterioration of breast muscles [35]. A study similarly reported dietary *B. subtilis* (1×10^6 CFU/g feed) significantly improved broiler performance with respect to FCR and alleviation of immune response under both thermoneutral and heat stimulated conditions [36]. Terminal-RFLP analysis was used in another study to see how probiotic. The Cecal and jejunal microbiota of broiler chickens under heat stress is affected *Lactobacillus* strains. The study found out that there was no significant abundance in the microbial population but the supplementation did show higher prevalence of *Lactobacillus sp*. in heat stressed chickens in comparison with the controls [37].

Oxidative stress is a common consequence of heat stress in poultry. The combination of antioxidant compounds and probiotics offers a multifaceted approach to combating oxidative stress. One of the trace elements, selenium, is essential for the body's functioning. It shields red blood cells against the detrimental impacts of free radicals and constitutes a component of the robust antioxidant known as glutathione peroxidase. In summary, the normal development of enzymatic systems like superoxide dismutase and glutathione peroxidase is reliant on its presence. Khan et al. postulated that supplementing basal diet of broiler with selenium enriched probiotics (SP) (*S. cerevisiae* and *L. acidophilus*) enhanced antioxidant system to effectively inhibit hepatic oxidation during heat stress; probiotics alone were not as effective [38]. In another study SP, significantly improved body weight gain and bone health by up-regulating the expression of DIO2 and T3 in heat stressed broiler [39]. The effect of probiotic use in stressed poultry are summarized in **Table 1**.

Moreover, stress can disrupt the balance of the gut microbiota, affecting nutrient absorption and overall gut health. The role of probiotics in moderating this impact is explored in the following section.

6. Immuno-modulatory effect of probiotics

The gut of poultry serves as a critical interface where the immune system interacts with the external environment, including ingested feed and potential pathogens. This dynamic environment houses a diverse microbial community that plays a pivotal role in shaping the host's immune responses. Probiotics, as beneficial members of the gut microbiota, have gained attention for their immunomodulatory effects, influencing the intricate relationship between the gut and the immune system.

Certain probiotic strains have the ability to stimulate immune cells in the gut-associated lymphoid tissue (GALT), including dendritic cells, macrophages, and T cells. This activation enhances the recognition and response to pathogens while preventing excessive immune activation [48]. Moreover, Probiotics have been explored as potential adjuvants to enhance the efficacy of vaccines in poultry. By promoting a balanced immune response, probiotics can improve the recognition and memory formation of vaccine antigens. This leads to increased vaccine effectiveness and protection against pathogens. In a study conducted by Sarwar, it was observed that the vaccination against infectious bursal disease (IBDV) and Newcastle disease (ND) in combination with probiotic strains of *L. paracasei*, *L. casei*, *L acidophilus, Bifidobacterium*, *Streptococcus thermophiles* showed antibody titer improvement when compared control broiler groups administered with only with vaccine and probiotic alone [49].

Numerous studies have put forth the hypothesis that probiotics could potentially function as a viable substitute for antibiotics in the diets of poultry and are anticipated to boost animal immunity and health status. In a study, Penha Filho et al. presented that *Lactobacillus*-based probiotics proved to be effective against heavy infection of pathogenic enterobacterial infection of *Salmonella enteritidis* (SE) in chickens, by reducing SE's colonization in chicks. Study reported immunomodulatory effect of the probiotic such as, decrease in the levels of pro-inflammatory cytokines. It further showed stimulation of TLR2 expression in caecal tonsils which can further pay way to consider this probiotic as TLR2 based adjuvant with injectable vaccines [42]. On the other hand, a study observed upregulation of IFN-gamma mRNA genes in selenium enriched probiotic supplemented chickens when challenged with *E. Tenella*, thus providing protection against infection. Here the immunomodulatory response was linked with the increased antioxidant capacities [43]. Similarly, another research observed elevation of mRNA anti-inflammatory genes HIF1A (hypoxia inducible factor 1 alpha) and TSG-6 (tumor necrosis factor- (TNF) in the caecal mucosa of broilers fed with dietary probiotics when they were challenged with *P. multocida* [44]. *Lactobacillus fermentum* and *L. acidophilus* decrease the expression of pro-inflammatory cytokines in broilers affected with necrotic enteritis [45].

White pekin ducks' nonspecific immune responses were greatly boosted when their diets were supplemented with probiotics. Additionally, incorporation of probiotics to a low crude protein diet increased duck immunity to the same levels as those on a high crude protein diet [25]. Dietary addition of *L. plantarum* probiotic (1×10^8 CFU) showed significant ($p < 0.05$) improvement in both cell mediated and humoral immune response thus protecting the chickens against coccidiosis [46]. Additionally, rise in the levels of interleukin and immunoglobulin was observed by Memon et al. in broiler chicks thus explaining the synergistic activity of probiotic with synthetic drugs such as diclazuril, under induced Eimeria infection [47]. **Table 1** highlights the influence of probiotics on the immunity of poultry.

7. Metagenomics and metaproteomics in poultry

The gut microbiota of birds plays a pivotal role in various aspects of their health and performance. Advances in metagenomic and metaproteomic techniques have enabled scientists to delve deeper into the complex microbial communities residing within the avian gut. Metagenomics involves analyzing the genetic material present in a sample to identify and characterize the diversity of microorganisms. On the other hand, metaproteomic focuses on the proteins expressed by these microorganisms, providing insights into their functional roles [50].

In a study, the fecal microbiota of broiler growers with varying FCR was analyzed using shotgun sequencing. The analysis revealed distinct microbial compositions between low and high FCR birds, with differences in phylum-level abundances and gene proportions related to metabolism, stress response, and virulence. Notably, certain genes associated with improved feed efficiency were found to be overrepresented in low FCR birds, providing insights for enhancing poultry feed efficiency and formulation strategies [51]. Yet another study, investigated the influence of heat stress on the gut microbiota of caged laying hens using metagenomics sequencing. Firmicutes, Bacteroidetes, and Proteobacteria were dominant phyla. Heat stress reduced Firmicutes and increased Bacteroidetes, leading to altered metabolic pathways like cysteine and methionine metabolism. The findings provide insights into potential interventions for mitigating heat stress effects in poultry [52]. Moreover, a study explored how diet affects the protein content of chicken gastrointestinal tract (GIT) using label-free metaproteomics. The crop section showed Lactobacillaceae dominance, irrespective of diet, while Veillonellaceae increased with phosphorus supplementation. In the ceca, Bacteroidaceae proteins rose with phosphorus, and Eubacteriaceae decreased; protein patterns indicated thriving communities with supplementation, highlighting GIT dynamics [53].

Thus, metagenomic and metaproteomic analyses can assist in the selection of probiotic strains with specific functional attributes. By identifying strains that promote beneficial metabolic pathways or produce bioactive compounds, researchers can tailor probiotic formulations to address particular challenges, such as enhancing nutrient absorption or modulating immune system.

8. Limitations on the use of probiotics in poultry

Because the potential of probiotics supplements varies depending on the species and is not shared between different genera and species of microbes, the widespread adoption of probiotics as part of poultry diets has brought it with some unique difficulties. Furthermore, there is always the possibility that the probiotics will be inadequately handled after they have reached the market, rendering them ineffective. Loss of viability of probiotics may occur when they come into touch with disinfectants in water or other interacting compounds in feed during oral administration. The probiotics' lack of availability until they reach the site of action in the host body is another potential barrier [54].

9. Conclusion

The studies discussed above indicate that probiotics may play a significant role in sustainable poultry farming. In all likelihood, they will serve as the superior replacement for antibiotics in poultry industries. Significant work and studies have proved that probiotics help in maintaining health status in poultry animals as they improve gut conditions and enhance nutrient absorption, thus overall improving the growth performance. Probiotics provide protective effect against stress conditions by enhancing antioxidant potential of enzymes. Probiotics also modulate immune response and provide protecting against infections through various mechanism of action. In future, probiotics in poultry feed should continue to determine the optimal conditions and standard methodology under which their application will have the greatest possible beneficial impact on meat quality. Additionally, more clinical trials may be conducted to investigate other potential areas of benefit.

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Conflict of interest

The authors declare no conflict of interest.

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