Immunomodulation Potential of Probiotics

Subjects: Agriculture, Dairy & Animal Science | Immunology

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The use of probiotics in livestock has been suggested to significantly improve their health, immunity, growth performance, nutritional digestibility, and intestinal microbial balance. Furthermore, it was reported that the use of probiotics in animals was helpful in equilibrating their beneficial microbial population and microbial turnover via stimulating the host immune response through specific secretions and competitive exclusion of potentially pathogenic bacteria in the digestive tract.

livestock | healthy growth strategy | probiotics | immunoregulatory effects of probiotics

1. Introduction

Antimicrobial resistance represents a global health problem that contributes to tens of thousands of deaths per year. Furthermore, the global demand for meat and dairy consumption is increasing at a rapid and unprecedented rate [1]. To fulfill this demand, many countries are shifting to intensive livestock production systems that use antimicrobial (AM) drugs to keep animals healthy and increase their development and productivity [2][3]. For example, Van Boeckel et al. (2015) found that between 2010 and 2030, the global consumption of AM agent for livestock industry increased by 67%, while on the other hand, the increase in AM agent consumption in the BRICS countries (Brazil, Russia, India, China, South Africa) will be 67%. Furthermore, Denmark was the foremost nation to report authorized antimicrobial agent manufacturing/sales data in 1996, under the name of Danish Integrated Additive Manufacturing Resistance Monitoring and Research Program (DANMAP). In 2011, the European Medicines Agency Surveillance of Veterinary Consumption group (ESVAC) published the first report on veterinary AM sales in eight countries (Czech Republic, Denmark, Finland, France, Netherlands, Norway, Sweden, UK) since 2005. The latest 2017 report provides an overview of AM sales across all EU countries. Furthermore, North American countries and Canada began collecting sales data for AM resistance monitoring in 2008 for the Canadian Comprehensive Program (CIPARS), which reports AM resistance and AM use. In Asia, Japan was the first country to launch the Japan Veterinary AM Monitoring System (JVARM) to report AM agent use [4]. In addition, current global trends in the use of AM agents in livestock animal feeds were represented in **Figure 1**. Therefore, the establishment of AM-free feeding system by using probiotics has been required for secure and healthy livestock production. The most commonly used probiotics in livestock are the strains of lactic acid bacteria (LAB) and *Bifidobacterium* [5]. In addition, gastrointestinal tract (GI) infections in livestock are considered a major global problem, with a negative economic impact on livestock farmers [6]. In this regard, the likelihood of using feed supplements to attain a healthier animal, welfare, and yield by manipulating the gut microbiota has received considerable attention over the past 30 years. Antibiotics have been applied widely to prevent and treat GI infection in livestock; however, the random uses of antibiotics in livestock are responsible for the development of antibiotic resistance, which has a long-lasting effect on the human body, as well as the destruction of gut microflora [7][8][9]. Probiotics might be used as a potential alternative therapy to treat gastrointestinal tract disorders and to enhance the endogenous immune function of the host (**Figure 1**).

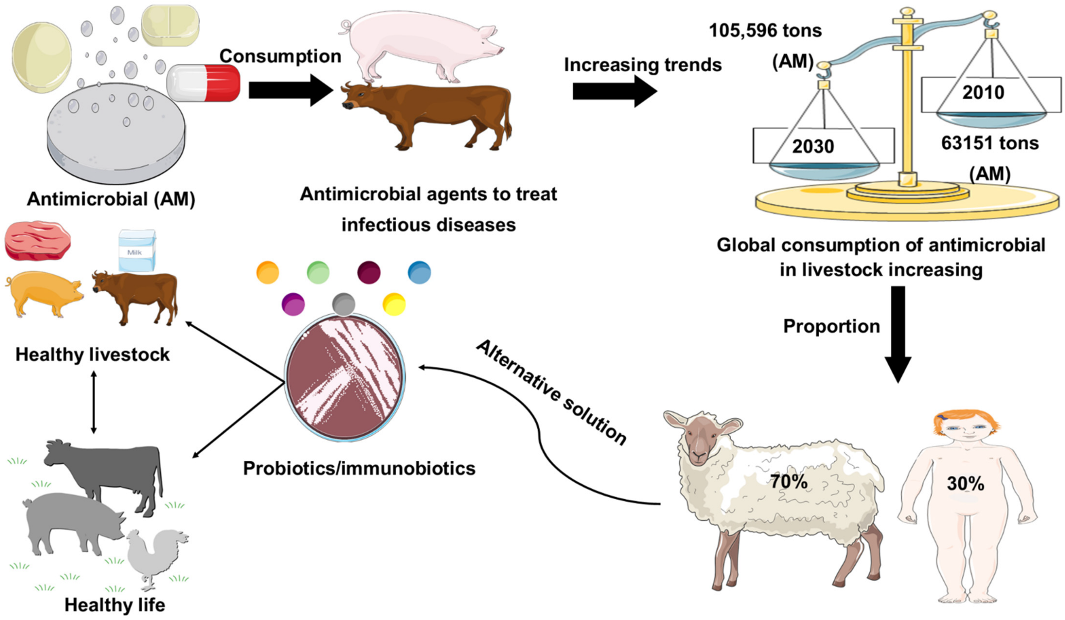

Figure 1. Role of probiotics in livestock healthy growth strategy. Global consumption of AM (AM) in livestock production was estimated in 2010 and is projected to rise by 67%, by 2030. Global increase (67%) in AM consumption is due to the growing number of animals raised for meat and milk production. Probiotics used as a safer alternative to conventional antibiotic drug therapy.

Numerous probiotics might be used to improve the performance of ruminant and pig animals. Numerous studies have demonstrated that probiotics can exert an AM effect against pathogens and improve animal health, as well as productivity [10][11]. Earlier, the team established a porcine intestinal epithelial (PIE) cell line and demonstrated that PIE cells are a useful in vitro tool for the selection of immunomodulatory LAB (immunobiotic LAB). Furthermore, the team has demonstrated that the in vitro and in vivo immunobiotic LAB is a good alternative to improve resistance against GI pathogens in the porcine host. Additionally, the laboratory has shown that the probiotic *Lactobacillus* with immunoregulatory functions can beneficially modulate the immune response in the gut through controlling the functions of PIE cells [10][11][12][13][14][15]. This contrasts with previous studies that recommend the modulation of gut microbiota and piglet immunity via appropriate probiotic strains, which will lead to better growth performance. Therefore, it is necessary to establish a non-toxic feeding system and a food safety system to ensure the safe and healthy production of animal husbandry. A study suggested that the probiotic-supplemented diet significantly improved the health status, growth performance, and intestinal morphology in pigs [16]. Similarly, it was suggested that the multi-species probiotic diet has excellent potential to endorse the growth performance and healthy status of pigs via modulation of gut microbiota [17].

2. Application of Probiotics in Livestock Production

In recent decades, some studies were conducted to illustrate the new scope in the field of probiotics and to discover the potential probiotic microbes. According to Sun et al., (2021) multi-species probiotics consisting of *L. acidophilus, L. casei*, *B. thermophilum*, and *E. faecium* were successfully used to reduce the diarrhea caused by enterotoxigenic *E. coli* (ETEC) F18^{+} in newly weaned pig [18]. In addition, multi-species probiotics were helpful in enhancing growth performance through a reduction in intestinal inflammation, oxidative stress, and morphological damages. Sobrino et al. (2021) attempted to study AM substitutes in pig production. They used *Ligilactobacillus salivarius* strain retrieved from sow's milk and fed it to pregnant sows and piglets. The results suggested that there was a notable reduction in the presence of antibiotic-resistant *Lactobacillus*, which became apparent in the treatment group [19]. In recent studies, it was suggested that *Prevotella* exerted positive consequences in pig production by enhancing growth performance and immune response [20][21][22][23]. The *Lactobacillus, Escherichia, Shigella*, and *Bacteroides* dominate the small intestine microbiota, while on the other hand, the *Prevotella* dominates the large intestinal microbiota during the newborn stage. Furthermore, the *Prevotella* dominates the pig's small and large intestines after weaning [24]. Additionally, it was reported that the non-diarrheic piglets were found to have a considerably higher abundance of intestinal *Prevotella* than diarrheic piglets. *Prevotellacea* UCG-003 was the key bacterium in the non-diarrheic microbiota of piglets, according to co-correlation network analysis [23]. Ngo et al. (2021) used a new probiotic (*B. amyloliquefaciens* H57) in high concentrate feed pellets that reduces volatile fatty acid production and prevents flavor in pellet feed. That facilitates higher feed intake in ruminant animals [25]. In recent studies on anaerobic fungi, it was demonstrated that it contributes essentially to ruminal fiber utilization by degrading plant cell walls in two ways, i.e., enzymatically and mechanically [26][27]. Remarkably, ongoing exploration showed the affinity of fungal CAZymes for stubborn fiber, which might clarify the specific use of anaerobic fungi when lower quality forages were fed to ruminants. Therefore, this can also be used as a potential probiotic in ruminant nutrition [28]. Studies on the utilization of *B. subtilis* as a spore-shaping probiotic bacterium in livestock nutrition have shown no unsafe impacts and have exhibited the viability of its utilization as a probiotic, mostly because of its demonstrated AM, mitigating cell reinforcement and exhibiting enzymatic, and immunomodulatory action [29]. A study by Cai et al. (2021) enumerated that *S. cerevisiae* and *C. butyricum* and their blend enhanced rumen conditions by expanding the pH and diminishing oxidation and upgraded rumen maturation capacities by expanding absorbability of supplements and further developing VFA production; from that point on, further enhancements in production growth of heat-stressed goats were observed [30]. The *Debaryomyces hansenii* is also gaining attraction as a new potential probiotic for both terrestrial and aquatic animals. The oral delivery of *D. Hansenii* has been linked to probiotic features, such as immunostimulatory effects, gut microbiota regulation, increased cell proliferation, differentiation, and improved digestive function. Its bioactive molecules have been identified and linked to its immunomodulatory effect, including cell wall components and polyamines [31]. Therefore, there are many potential probiotic microbes that are still to be discovered, which might play an evolutionary role in livestock production.

3. Modes of Action of Livestock Probiotics

There are numerous proposed modes of action of livestock probiotics [32][33][34][35][36][37][38][39][40]. However, the major mechanisms of action proposed for probiotics are considered in the following segments (summarized in **Figure 2**).

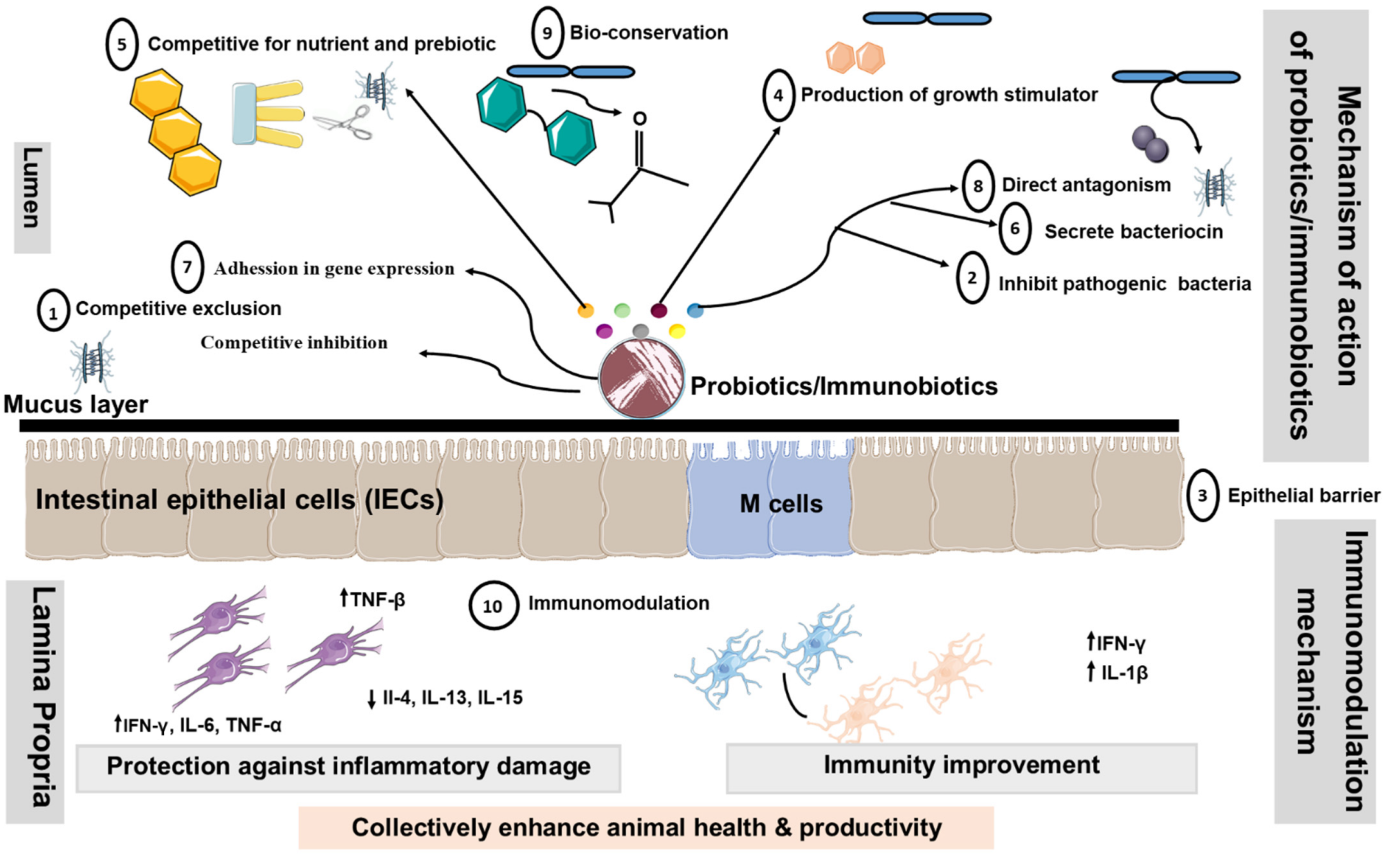

Figure 2. Proposed modes of action of livestock probiotics. Schematic diagram illustrating potential mechanisms, whereby oral administration of probiotics might promote beneficial effects by changing the composition of intestinal microbiota, altering intestinal barrier function, bile salts, and production of Th1 cytokines. Additionally, probiotics containing LAB may down-regulate the expression of pro-inflammatory cytokines and chemokines. Decrease in the translocation of bacteria may occur as a result of the ability of probiotics to tighten the mucosal barrier. Probiotics disallow colonization by pathogenic bacteria through competition for nutrients, immune system up-regulation, and production of antitoxins. These mechanisms include ① Competitive exclusion for binding sites, ② Adhesion to the GIT, ③ Enhancement of the epithelial barrier, ④ Increase in digestion and absorption of nutrients, ⑤ Competing with pathogenic bacteria for nutrients in the gut, ⑥ Production of AM substances, ⑦ Alteration in gene expression in pathogenic microorganisms, ⑧ Bacterial antagonism, ⑨ Bioconversion and ⑩ Immunomodulation. Abbreviations: ↑, increased; ↓, decreased; Th1, Type 1 T helper; Th2, Type 2 T helper; IEC, intestinal epithelial cells; DC: dendritic cell.

① *Modification of the microbial population of the GIT*: Probiotics might boost the population of beneficial microbes, such as *Lactobacillus and Bifidobacterium*, which subsequently restrict the growth of harmful bacteria by creating inhibitory chemicals and by competing for binding sites [41][42]. ② *Adhesion to the GIT wall to prevent colonization by pathogenic microorganisms*: The majority of enteric pathogens might colonize the intestinal epithelium and cause disease as a result [43]. As a result, *Lactobacillus* can adhere to the gut epithelium and compete with pathogens for adhesion receptors, such as glycoconjugates [44]. The *Lactobacillus* and *Bifidobacterium* have hydrophobic surface layer proteins that assist the bacteria non-specifically by adhering to the animal cell surface [45]. ③ *Enhancement of the Epithelial Barrier:* The experimental studies in model animal have shown that probiotics *P. acidilactici* improve intestinal barrier function by reducing the permeability of the intestinal epithelium translocation of enterotoxigenic *E. coli* to mesenteric lymph nodes in post-weaning piglets as compared to the control group after ETEC challenge

④ *Increase in digestion and absorption of nutrients*: In this case, the spore-forming bacteria enhance the production of extracellular enzymes, which facilitate nutrient digestion [48][49]. ⑤ *Competing with pathogenic bacteria for nutrients in the gut*: Probiotic bacteria might compete with pathogenic bacteria for nutrients and absorption sites by rapidly utilizing energy sources, potentially shortening the log phase of bacterial development [42]. ⑥ *Production of antimicrobial substances*: Several probiotic bacteria, particularly those that produce lactic and acetic acids, have the ability to suppress harmful microorganisms [50][51]. ⑦ *Alteration in gene expression in pathogenic microorganisms*: Probiotics might influence pathogenic bacteria's quorum sensing, hence altering their pathogenicity. Fermentation products from *L. acidophilus* La-5 significantly suppressed the extracellular production of a chemical signal (autoinducer-2) by human enterohaemorrhagic *E. coli* serotype O157:H7, leading to inhibition of the virulent gene (LEE—locus of enterocyte effacement) expression in vitro [52]. ⑧ *Bacterial antagonism:* Probiotic microorganisms, once established in the gut, may produce organic acids, hydrogen peroxide, lactoferrin, and bacteriocin, which may exhibit either bactericidal or bacteriostatic properties [53].⑨ *Bactericidal activity/Bioconversion*: *Lactobacillus* convert lactose to lactic acid, lowering the pH to a point where pathogenic bacteria cannot survive. Furthermore, living yeasts compete with lactic acid-producing bacteria to digest sugars obtained from starch breakdown, thereby stabilizing rumen pH and minimizing the danger of acidosis [54][55][56]. ⑩ *Immunomodulation*: It is shown that probiotic LAB with immunoregulatory functions can beneficially modulate the immune response in the gut by modulating the functions of PIE cells [12][57][58]. In addition, probiotic LAB have proven to be capable of acting as immune modulators by enhancing macrophage activity [57], increasing local antibody levels, inducing the production of anti-inflammation cytokines (interleukin (IL)- 10, interferon (IFN)-γ, β, IL-1β, TGF-β), reducing IL-4, IL-6, IL-8, MCP-1, and activating killer cells [11][59][57].

Immunomodulation properties appear to be strain dependent, which means that dissimilar probiotics might have parallel mechanisms of action, whereas a single strain may have multiple mechanisms of action. Quite a lot of probiotic strains, for example, have comparable impact on the microbial community of gastrointestinal tract, although the mechanisms of action of certain probiotics are mostly unknown. The exact mode of action of probiotics is not well understood in the majority of studies on their impact on performance. Therefore, the mechanisms must be explored on a case-by-case basis because closely interrelated probiotics appear to have diverse ways of action. Probiotic effects are a result of the interaction between the host and the probiotic microorganism. As a result, more research into the host–microbe interaction could shed light on the probiotic mode of action. Rapid improvements in molecular techniques and genome sequencing for microbial ecology research will substantially aid our understanding of probiotic mechanisms of action.

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