

# The Potential Use of Probiotics

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To address the rapidly growing use of probiotics in animal agriculture, this review discusses the effect of probiotics on animal growth and development, immune response, and productivity. Several benefits have been associated with the use of probiotics in farm animals, such as improved growth and feed efficiency, reduced mortality, and enhanced product quality. While the mechanisms through which probiotics induce their beneficial effects are not well understood, their role in modifying the gastrointestinal microbiota is believed to be the main mechanism. The use of probiotics in fresh and fermented meat products has been also shown to reduce pathogenic and spoilage microorganisms and improve sensory characteristics. Although many benefits have been associated with the use of probiotics, their effectiveness in improving animal performance and product quality is highly variable. Factors that dictate such variability are dependent on the probiotic strain being utilized and its stability during storage and administration/inoculation, frequency and dosage, nutritional and health status as well as age of the host animal. Therefore, future research should focus on finding more effective probiotic strains for the desired use and identifying the optimum dose, administration time, delivery method, and mechanism of action for each strain/host.

probiotic

gut microbiota

immune response

growth

efficiency

meat quality

## 1. Introduction

The increase in demand for animal products due to the growing world population has been an ongoing challenge for the animal production sector worldwide <sup>[1]</sup>. Tremendous progress has been achieved over the past five decades in this regard, mainly due to improvements in genetic selection, health status, nutrition, and the use of antibiotics and growth promotants <sup>[2]</sup>. Indeed, the use of the latter two practices in commercial animal production has improved the health status and feed efficiency of farm animals, which has led to approximately 18% increase in the overall growth performance <sup>[3]</sup>. However, the use of antibiotics and growth promotants has brought about concerns over the development of antibiotic resistant microbes <sup>[4]</sup>, increase in foodborne allergies <sup>[5]</sup>, and the negative impacts it has on the environment such as agricultural runoff <sup>[6]</sup>. Furthermore, although still debated, there is a rising concern among increasingly wary consumers on the effects of antibiotics and growth promotants on human health <sup>[7]</sup>. To that end, researchers have been investigating alternative ways to improve the quantity, quality, and homogeneity of farm animals and their products. One such alternative is the supplementation of probiotics, as single or mixed strains, to the diet of farm animals.

The term “probiotic” was coined by Metchnikoff in 1908 and derived from the two Greek words “pro” and “bios”, which means “for life” <sup>[8]</sup>. Probiotics are defined as living microbial supplements that advantageously influence the

host through improving its intestinal microbial composition [9]. A more modern definition was adopted by FAO/WHO in 2002 [10], which states “mono or mixed strains of living microorganisms which confer a desirable health benefits on the host when used adequately”. To regard a microorganism as probiotic, it should be nonpathogenic, able to give a viable cell count, has a positive effect on the health of the host, and enhance the functions of the intestinal tract. The most commonly used probiotics are *Lactobacillus acidophilus*, *Lactobacillus lactis*, *Lactobacillus plantarum*, *Lactobacillus bulgaricus*, *Lactobacillus casei*, *Lactobacillus helveticus*, *Lactobacillus salivarius*, *Bifido bacterium* spp., *Enterococcus faecium*, *Enterococcus faecalis*, *Streptococcus thermophilus*, *Escherichia coli* bacteria, and other probiotic fungi such as *Saccharomyces cerevisiae* and *Saccharomyces boulardii* [11][12]. Decades of research have indicated that the use of probiotics in farm animals is beneficial as it improves feed efficiency, weight gain, and immune response [13][14]. However, the overall effectiveness of probiotics is dictated by factors such as optimal selection of microbial strains, the use of a suitable dose, and the species and age of the host [15][16]. Thus, careful consideration must be taken prior to any implementation of probiotics in the diet of farm animals. The aim of this review is to discuss the administration of probiotics in animal feed, either as supplements or additives, and their effect on animal health, growth and productivity, and product quality. We will also briefly review the use of probiotics in fresh and fermented meat products.

## 2. The Gut Microbiota

Within the gastrointestinal tract (GIT) of animals, there exists a microbial population that is widely diverse [17]. Microbial density and diversity vary throughout the GIT, with maximal populations in areas where the pH range is close to neutral [17][18]. Such areas include the pre-gastric rumen of ruminants and post-gastric cecum of ruminants, horses, pigs, and fowls. Depending whether the animal is a ruminant or monogastric, the GIT can sustain up to several thousand unique microbial species including bacteria, archaea, fungi, and protozoa [19]. Because bacterial species are the most commonly used microorganisms as probiotics [20], we intend to focus our review to bacteria.

Most gut bacteria belong to two main phyla, Firmicutes and Bacteroidetes, but species from the phyla Actinobacteria, Proteobacteria, and Verrucomicrobia are also present [21][22]. For instance, Firmicutes and Bacteroidetes species account for approximately 99% (42% and 57%, respectively) of the total microbiota in bovine rumen [23], and 96% (49% and 47%, respectively) in ovine rumen [24]. However, Firmicutes are predominant in the hindgut of pigs and cecum of chickens, with only a small percentage (< 2%) of Bacteroidetes [25]. The commensal (indigenous) gut microbiota plays important roles in the animal's overall health, growth and development, and productivity through promotion of immune system development and response and facilitating nutrient extraction from the diet [26]. The latter is obvious in ruminant animals as the gut microbiome provides approximately 70% of their daily energy requirements [27]. A large proportion of that percentage comes from microbial fermentation of carbohydrates, which generates volatile fatty acids that are absorbed and used as an energy source. Moreover, the microbial populations themselves can be utilized as a source of protein (microbial protein) as they leave the rumen and are digested in the small intestine [28].

The gut microbiota is known to interact with the host immune system [29]. However, communication between the two “systems” is indirect and relies on intestinal epithelial cells residing in the lumen and immunomodulatory cells

in the lamina propria [29][30]. The microbiota and immune cells are separated by an intestinal epithelium that has two essential functions. The first is to physically segregate any foreign substances or microbes from the host immune cells. The second is to deliver signals to immune cells in response to metabolites produced by the gut microbiome, which, in turn, invokes an immune response [31]. Thus, the relationship between the gut microbiota and the host's health is complex as it involves "cross-talk" between the residing commensal microbiota, epithelium, and innate immune system.

Sustaining an abundant and diverse microbiota is beneficial to the animal's welfare [32]. An "imbalanced" microbiota (dysbiosis) in which the population of pathogenic bacteria is higher than beneficial commensal bacteria, leads to impairment of gut health, and ultimately, overall health, behavior (feeding, social, and stress response), and growth of an animal [25]. Several factors are known to influence the enrichment and diversification of the microbiota, including diet, environment, and host genetics [33]. The relationship amongst these three factors is complex, as one factor could be more influential than other, depending on the circumstances. For instance, the GIT of a newborn animal is "sterile" right after birth, but is rapidly colonized by microbial communities from the environment and the mother [33][34]. In an example where a change in diet modulates gut microbiota, Hildebrandt et al. [35] observed an increase in the phyla Firmicutes and Proteobacteria and a decrease in Bacteroidetes in mice that were fed a high fat diet. Although studies have shown that genetics also contributes to modulating the microbiota [36][37], its contribution is likely confounded with environmental and dietary factors [33]. In addition to the aforementioned, the use of probiotic supplementation is known to diversify and modulate the gut microbiome [38]. It has been shown that probiotics can enrich and restore beneficial commensal microbes during a period of dysbiosis [39]. However, one of the challenges in the use of probiotics is determining when the appropriate modification should be implemented during the lifecycle of an animal. Some have argued that the intervention to modify the microbiome should occur when the animal is young, because changes in the microbiome during adulthood are rather subtle [40][41]. Thus, the effectiveness of probiotic on the gut microbiota is closely related to the time of implementation, such as during the weaning, growing, or finishing stages of growth [42].

### 3. Proposed Mechanism of Action for Probiotics

The use of probiotics for animals has been increasing since the mid-1970s [43]. Probiotics have been used as therapeutic supplements in farm animals in order to decrease morbidity and mortality [44], improve feeding behavior [45], and increase production (meat, milk, and eggs) yield [44][46]. Furthermore, due to their ability to inhibit a wide variety of pathogenic microorganisms, derived from the environment and diet, the use of probiotics has expanded into the food industry as well [47]. There are at least two proposed mechanisms by which probiotics can combat unwanted microorganisms: the production of inhibitory compounds and/or direct cell-to-cell interactions [48]. Probiotics produce antimicrobial compounds, such as organic acids, hydrogen peroxide, bacteriocins, and biosurfactants, all of which can inhibit the growth of pathogenic microorganisms [49]. The most commonly produced compounds by probiotic bacteria are lactic and acetic acids that reduce the pH, thereby making it less favorable for pathogen growth. Additionally, probiotics enhance resistance to intestinal pathogens via competitive colonization of intestinal adhesion sites and nutrients [50][51].

Probiotics, like other organic nutrients in the intestine, are partly digested and broken down, thus, only a small population is viable. Yet, probiotics have shown to be effective against microorganisms that negatively impact the host's health. Systemic stimulation of the immune system is an important role for probiotics against the pathogenic invading microorganisms [52]. Probiotics are suggested to participate in a complex stimulatory mechanism of the innate immune system through increasing expression of toll-like receptors (TLRs), which results in the release of cytokines such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-4 (IL-4), and interferon- $\gamma$  (IFN- $\gamma$ ) [53]. In this regard, the intake of probiotics has been shown to improve disease resistance and reduce metabolic stress and mortality [54]. A plain diet supplemented with a mixture of probiotics containing *Lactobacillus casei*, *Lactobacillus acidophilus*, *Bifidobacterium thermophiles*, and *Enterococcus faecium* increased the concentration of immunoglobulins (Ig) M and G in turkeys, which enhanced their resistance against diseases as well as growth performance [55]. Moreover, an increase in intestinal IgA of sows and piglets supplemented with *Bacillus cereus* for 56 days at  $2.6 \times 10^5$  and  $1.4 \times 10^6$  cfu/g of feed, respectively, was also reported [56]. Secretion of mucosal IgA prevents microorganisms and toxins binding to epithelial cells, a mechanism known as immune exclusion [57]. In a different study, Yi et al. [58] showed that the dietary administration of *Bacillus velezensis* JW in fish (*Carassius auratus*) increased the activity of several enzymes involved in immune response such as acid phosphatase, alkaline phosphatase, and glutathione peroxidase in serum, as well as expression of regulatory cytokine genes including TNF- $\alpha$ , IFN- $\gamma$ , and IL-1, 4, and 10 in head kidney. In addition, the same study showed that when challenged with a pathogenic bacterium, *Bacillus velezensis* JW-supplemented fish had improved survival rate.

One of the most widely used probiotics are *Lactobacillus* cultures, which have been shown to control gastrointestinal pathogenic microbial populations [59]. A variety of *Lactobacillus* strains are effective in decreasing *Escherichia coli*, *Salmonella*, and coliform counts in poultry [60][61][62][63][64], and *Clostridium* sp. in piglets [65]. In beef cattle, feeding  $10^9$  CFU of *Lactobacillus acidophilus* NP51 per day to steers for 126 days was shown to reduce *E. coli* O157:H7 shedding by 37% [66]. Further, the use of *Lactobacillus rhamnosus* has been shown effective against a virulent strain of *Aeromonas salmonicida* in aquaculture [67]. The reduction of pathogenic microbes in the gut by *Lactobacillus* is usually attributed to its ability to exclude other microorganisms by competing for adhesion sites and nutrients [68]. For a more in-depth review on the mechanisms involved in competing for adhesion sites, please refer to relevant reviews by Lebeer et al. [69] and Vélez et al. [70]. In totality, the use of probiotics seems to improve the health and immune system function of farm animals.

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