The Potential Use of Probiotics to Improve Animal Health, Efficiency, and Meat Quality: A Review

Sarmad G. Al-Shawi 1, David S. Dang 2, Asraa Y. Yousif 3, Zena K. Al-Younis 1, Teif A. Najm 2 and Sulaiman K. Matarneh 2,*

1 Food Science Department, Agriculture College, Basrah University, Basrah 00964, Iraq; sarmad.mohammed@uobasrah.edu.iq (S.G.A.-S.); zena.issa@uobasrah.edu.iq (Z.K.A.-Y.)
2 Department of Nutrition, Dietetics and Food Sciences, Utah State University, Logan, UT 84322, USA; ds.dang24@gmail.com (D.S.D.); taefnajeem@yahoo.com (T.A.N.)
3 Animal Production Department, Agriculture College, Basrah University, Basrah 00964, Iraq; asraa.yousif@uobasrah.edu.iq
* Correspondence: sulaiman.matarneh@usu.edu; Tel.: +1-435-797-2114

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Abstract: 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.

Keywords: 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 [1]. 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 [2]. 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 [3]. However, the use of antibiotics and growth promotants has brought about concerns over the development of antibiotic resistant microbes [4], increase in foodborne allergies [5], and the negative impacts it has on the environment such as agricultural runoff [6]. Furthermore, although still debated, there is a rising concern among increasingly wary consumers on the effects of antibiotics and growth promotants on human health [7]. 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” [8]. 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 [33,36,37], 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 [38,47]. 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-α (TNF-α), interleukin-4 (IL-4), and interferon-γ (IFN-γ) [53]. In this regard, the intake of probiotics has been shown to improve disease resistance and reduce metabolic stress and mortality [13,54]. 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 × 10^5 and 1.4 × 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-α, IFN-γ, 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 [60–64], 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.

4. Application of Probiotics in Animal Growth and Production

There are several factors that can dictate the growth of an animal such as genetics, age, diet, and sex [71]. Among those factors, providing an adequate nutritional plane is essential for the growth and development of an animal [72]. Not only does an animal require optimal amount of feed, but also improving the digestibility of feedstuff is crucial for maximizing growth. Meng et al. [73] reported an increase in growth performance of growing and finishing pigs that were supplemented with the probiotics *Bacillus subtilis* and *Clostridium butyricum*. The increase in growth performance was attributed to better nutrient digestibility in the probiotic supplemented pigs compared to their control counterparts. Similarly, greater nutrient absorption was observed in pigs that were supplemented with a strain of *Bacillus* culture [74]. In this case, after four to five months of supplementation, pigs that received the probiotic showed a 10% increase in protein utilization compared to pigs that did not receive any supplementation. Improved weight gain and performance was also reported in broiler chicks [75–78] and calves [79,80] when supplemented with probiotics. Abdel-Azeem [45] indicated that turkeys supplemented with *Bacillus amyloquefaciens* experienced increased feeding frequency and duration. Although the exact mechanism is still unclear, more recent research on the relationship between the gut microbiome and the brain (the gut–brain axis) have indicated the possibility of neurological changes occurring in which the microbiota could affect the feeding behavior of farm animals [25].

One of the biggest challenges to overcome in advocating the use of probiotics is their effectiveness and reliability in comparison to antibiotic growth promoters and implants. Ran et al. [81] demonstrated that steers that were given implants alone or in combination with antibiotics had approximately 10% more growth than those given a supplement containing fermented *Lactobacillus* products. Although several studies have reported positive effects on growth and performance from the use of probiotics, the efficacy of probiotics must be taken with great consideration. This is because the efficacy of probiotics is influenced by many factors such as differences in microbial composition (e.g., single or multi-strains) and viability, method of administration and dosage, environmental stress factors, as well as animal’s age and health status. For example, Zhang et al. [82] reported no improvement in average daily gain, dry matter intake, and nutritional digestibility in Holstein calves supplemented with *Lactobacillus plantarum* GF103 and *Bacillus subtilis*. The authors suggested that the efficacy of the probiotics was hindered as all calves used in the study were healthy, a notion that has been supported by other studies [83,84]. Table 1 lists the results of recent studies on the effect of probiotics on growth and performance of farm animals. The table also provides information on strain, dosage, and duration of probiotic treatment which is essential for improving the effectiveness of probiotics.
Table 1. Effects of probiotics on growth and performance of farm animals.

| Host          | Host Age                      | Probiotic Strain                                      | Administration/Dosage | Duration | Outcome                      | Ref. |
|---------------|-------------------------------|-------------------------------------------------------|------------------------|----------|------------------------------|------|
| Broiler Chicks (1 day old) | Mixed: B. subtilis (CPB 011, CPB 029, HP 1.6, and D014) B. velezensis (CPB 020 and CPB 035) | 1 × 10^9 CFU/g feed | 35 days | ↑ LW and FCR | [85] |
| Broiler Chicks (1 day old) | Mixed: L. bulgaricus L. plantarum S. faecium B. Bifidum S. cerevisiae | 100 g/ton starter 50 g/ton finisher | 42 days | No improvement | [86] |
| Broiler Chicks (1 day old) | Mixed: L. acidophilus L. casei E. faecium B. thermophilus | 10^9 CFU/g of feed | 42 days | ↑ FI and LW | [87] |
| Layer Hens (15 months old) | B. licheniformis | 10^7 CFU/g of feed | 12 days | ↑ Egg production | [88] |
| Bovine Calves (8 days old) | Single: L. plantarum GF103 | Oral 1.7 × 10^10 CFU/day | 83 days | ↑ FCR and CP digestibility | [82] |
| Bovine Calves (10 days old) | Mixed: L. casei DSPV 318T L. salivarius DSPV 315T P. acidilactici DSPV 006T | Oral 10^6 CFU/kg LW/day | 35 days | ↑ LW | [89] |
| Bovine Dairy cows * | Mixed: L. casei Zhang and L. plantarum P-8 | 6.5 × 10^10 CFU/day | 30 days | ↑ Milk production | [90] |
| Porcine Piglets (36 days old) | Single: L. plantarum GF103 Single: B. subtilis B27 Mixed: L. plantarum GF103 B. subtilis | 8.6 × 10^8 CFU/kg feed | 35 days | All treatments ↑ FCR | [91] |
| Porcine Piglets (1 month old) | Mixed: L. reuteri ZJ625 L. reuteri VBA4 L. salivarius ZJ61 S. salivarius NBRC 13956 | Oral (10 mL)/week 6.8 × 10^10 CFU/mL 5.5 × 10^10 CFU/mL 5.5 × 10^10 CFU/mL 2.9 × 10^10 CFU/mL | 30 days | ↑ GP | [92] |
| Porcine Piglets (35 days old) | Mixed: L. amylovorus E. faecium | Oral (3 mL/day 10^9 CFU/mL) | 35 days | ↑ FI and FE | [93] |

FCR = feed conversion ratio; CP = crude protein; FI = feed intake; FE = feed efficiency; LW = live weight; GP = growth performance; (↑) increase in respective outcome; (*) study did not include age of the animal.

The early postnatal period is thought to be a “critical window” for modifying the gut microbiota as it is the period in which the microbiome is more responsive to internal and external stimuli [40]. This is apparent in germ-free animals bred and kept in a sterile environment in which their immune system is underdeveloped due to lack of exposure to microbes [94]. Jørgensen et al. [42] indicated that the highest sensitivity to probiotic supplementation was seen during the weaning and growing stages of pigs, while it was less effective during the finishing stages. The same study also revealed that probiotic supplementation was most effective when pigs were offered a lower energy diet, which reaffirms the idea that the nutritional status of an animal must be considered when evaluating the efficacy of probiotic supplementation.

The addition of dietary probiotics has also been shown to improve the yield and quality of milk and eggs [52]. The addition of *Bacillus subtilis* and *Bacillus licheniformis* significantly improved the fat and protein content of sow’s milk during feeding [95]. Similarly, supplementing dairy cows with...
Aspergillus oryzae culture increased the percentage of protein and dry fat-free solids in their milk [96]. Not only does the quality of milk seem to improve when supplemented with probiotics, but several authors have also reported greater milk yield in lactating cows, sows, ewes, and does [95,97–99]. Xu et al. [90] observed an increase in milk yield by 37% in dairy cows supplemented with Lactobacillus casei Zhang and Lactobacillus plantarum P-8. Alhussien and Dang [100] attributed the increased milk yield in probiotics-supplemented dairy animals to a greater absorption of microbial-derived amino acids and reduced mammary gland inflammation and mastitis. In laying hens, adding the probiotics Bacillus licheniformis and Bacillus subtilis in the diet increased egg production by 3% and lowered cholesterol levels in egg yolk by 35% [101]. Furthermore, the inclusion of $10^7$ CFU/g of probiotic Bacillus licheniformis in laying hen’s diet alleviated the adverse effects of heat stress as more egg production, higher feed intake, and better immune response was observed [88].

5. Probiotics Effect on Meat Quality

Meat quality is an overarching term that describes traits influencing consumer purchasing decision and eating experience. Such traits include meat color, texture, and water holding capacity (WHC) [102]. These characteristics are influenced throughout the stages of life of an animal, and during harvesting, fabrication, and preparation of the final meat product. In addition to improving animal’s growth and production capabilities, probiotics showed a positive effect on quality aspects of both fresh and processed meat products [103,104]. Such effects include improvement in product quality and safety, extending shelf-life [105], imparting unique sensory qualities [106], and providing health benefits [105].

Meat color is considered the most important quality attribute in dictating consumers’ purchasing decision. This is because consumers use color as an indicator of the overall freshness and wholesomeness of meat products [107]. The variation in meat color is influenced by both pre- and postmortem handling of the animal and carcass and is highly associated with the rate and extent of pH decline postmortem [108]. Normally, pH gradually declines from an initial value of 7.2 to an ultimate pH near 5.6. Abnormal pH decline can lead to defects such as dark, firm, and dry (DFD) meat in which the ultimate pH remains relatively high (pH > 6.0). In contrast, a rapid decline in pH while carcass temperature is still high leads to the development of pale, soft, and exudative (PSE) meat defect. In this case, the meat exudes water along with water soluble proteins such as myoglobin, thereby producing a product that has a pale unsavory appearance [109]. In recent years, researchers have explored the potential use of probiotics to improve meat color stability and pH. Zheng et al. [110] reported higher pectoralis major muscle pH at 45 min postmortem of broilers that were supplemented with Enterococcus faecium. The increase in pH was associated with redder pectoralis meat. Similarly, Meng et al. [73] reported darker and redder meat of pigs supplemented with probiotics. The relationship between probiotic supplementation and postmortem pH decline remains unclear. However, the resulting pH seems to be influenced by the type of microorganism used and method of administration [111]. Ivanovic et al. [112] showed an increase in breast ultimate pH of broilers fed with Bacillus cereus IP 5832, whereas a diet containing Streptococcus faecium cernelle 68 was associated with a lower ultimate pH compared to the control diet. Furthermore, Pelicano et al. [113] indicated differences in ultimate pH when probiotics were supplemented in the diet compared to drinking water. In totality, by preventing a rapid decline in pH early postmortem, the use of probiotics could help solving this issue that has been plaguing the pork and poultry industries.

In general, the ability of meat to retain water increases with increasing the ultimate pH of meat. Within the normal range of ultimate pH (5.4–5.8), an increase in pH is often associated with increase in the meat’s WHC, which results in a final product that is considered more tender, juicier, and firmer [114]. Evidently, the use of probiotics has been shown to improve WHC and tenderness in meat [115,116]. Along with the improvement in tenderness, it was reported by Cramer et al. [117] that the use of probiotic supplementation has the potential to mitigate detrimental defects in meat with animals that have undergone oxidative stress. This notion is supported by studies that showed an increase in antioxidant capacity [118] and reduction in lipid oxidation [119] and reactive oxygen species [120] in
meats from animals that were supplemented with probiotics. With increasing evidence indicating beneficial effects of probiotics in improving meat quality, researchers have begun to explore other ways to implement these microbes in meat products.

Beyond the benefits on using probiotic cultures in fresh meat products, researchers have recently given attention to using fermented meat products as a carrier of probiotics for human consumption [121]. By using fermented meat as a carrier for these beneficial cultures, there is a prospect of improving human health and inhibiting potential growth of pathogenic and spoilage microorganisms in the food product [122]. Unlike fresh meat products that are usually cooked prior to consumption, processing of fermented meat products requires little to no heat treatment, making them suitable carriers for live cultures [121]. However, the appropriate strain of microbes must be selected in which it could survive the low pH and water activity and high salt conditions of fermented meat products. The most important probiotic bacteria in fermented meat products are Lactobacilli because of their ability to acidify meat, which contributes to its preservation. Many studies have shown the possibility of using probiotic Lactobacillus strains in fermented meat products such as Lactobacillus plantarum, Lactobacillus curvatus, and Lactobacillus sakei [121]. Erkkilä [123] showed that the use of Lactobacillus rhamnosus GG, Lactobacillus rhamnosus E-97800, and Lactobacillus rhamnosus LC-705 probiotic cultures inhibits E. coli 0157:H7 growth in dry sausage, while at the same time produces high quality sausage that had no adverse effect on technological and sensory properties. In a similar case, Lactobacillus rhamnosus GG showed an inhibitory effect on the growth of Enterobacteriaceae during the fermentation process when applied at $10^5$ and $10^7$ CFU/g [124].

In fresh and fermented meat products, lipid oxidation is a major concern as it negatively impacts the sensory quality of the product, and subsequently, its acceptability by the consumer [125]. Free fatty acids are the main precursors for lipid oxidation in fresh and fermented meats [126,127]. The potential to use probiotics as protective agents against lipid oxidation comes from their ability to produce bacteriocins that inhibit lipolytic microbes from forming free fatty acids [128,129]. Özer et al. [130] demonstrated that the use of Lactobacillus plantarum at $10^5$ CFU/kg in fermented sucuk showed significantly lower levels of thiobarbituric acid reactive substances (TBARS), a marker of lipid peroxidation, compared to control samples. A similar conclusion was made by Trabelsi et al. [103] in which lower TBARS were measured in minced meat that was inoculated with Lactobacillus plantarum. Similarly, the addition of Lactobacillus acidophilus and Bifidobacterium lactis in fermented sausage reduced the occurrence of lipid oxidation and contributed to positive organoleptic qualities [131–133].

6. Conclusions

Based on this review which summarizes many previous studies, it can be concluded that there are many positive benefits of using probiotics in animal feeding and fresh and processed meat products. Using probiotics seems to improve gut microbiota composition, immune response, nutrient digestibility and absorption, animal growth, and meat quality. Furthermore, many studies showed that probiotics improve the quality of fermented meat products and their sensory properties. Although there were some cases where the use of probiotics in animal feeds and meat products had no clear effect, this sheds some light on the complexity on the use of probiotics. Thus, further studies characterizing specific strains, identifying the optimal dosage, and understanding the network of interactions between probiotics and the gut microbiota could help in formulating more effective probiotic mixtures to be used in animal feed and meat products.

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