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Probiotics as sustainable alternatives to antibiotic growth promoters: Mechanisms, applications, and future perspectives in livestock production

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Abstract

This narrative review systematically summarizes the current knowledge about probiotics as scientifically defined alternatives to conventional antibiotic growth promoters (AGPs) in modern livestock farming. Globally, there is an increase in demand for AGP-free livestock due to growing threat of antimicrobial resistance. Although ample literature is available on efficacy of probiotics as AGP alternatives, there is a paucity of comprehensive data on strain-specific modes of action, effective dose regimens, and standardized application protocols across wide variety of livestock species. Probiotics exert complex antimicrobial mechanisms, including direct pathogen antagonism, gut microbiome modulation, intestinal barrier enhancement, immune system stimulation, and metabolic optimisation. In ruminants, poultry, pig, and rabbits, the application of probiotics has repeatedly shown improved growth performance, increased resistance to disease, better breeding performance and so on. Most of the literature available shows positive results on rumen fermentation, caecal microbiota composition, nutrient digestibility, and immunological responses. Nonetheless, knowledge gaps remain in the areas of interaction with the environment, production standardisation, and long-term effects of sustainability. Sustainability elements include less development of antimicrobial resistance, reduced environmental impact due to more efficient resource use, and improved economic viability. However, variations in strain-specific probiotic efficacy, environment-dependent outcomes of probiotic applications, and lack of production standardisation remain the challenges. Emerging innovations, such as postbiotics, precision livestock farming integration using AI, Internet of Things, and multi-strain synbiotic formulations offer promising solutions. This review summarises opportunities and challenges associated with probiotic use in livestock production systems and underscores their potential to revolutionise sustainable animal agriculture, as well as deal with serious public health concern of antimicrobial resistance.

1. Introduction

Global livestock production systems are facing unique paradigm change due to rising concerns of antimicrobial resistance (AMR) and the consequent implications on animal and public health (Bava et al. 2024). Antibiotic growth promoters (AGP's) have been widely used in animal production to improve productivity and for infection control (Rodrigues et al. 2021). Nevertheless, its usage led to unplanned consequences due to increase in the growth of multidrug resistant pathogens that threatens the health of animals and humans (Ronquillo and Hernandez 2017). This disturbing trend has resulted in regulatory authority all over the world implementing strict restrictions on the use of antibiotics in animal production (Idowu et al. 2021; Beber et al. 2025). The European Union was the first to prohibit AGPs in animal feed in 2006 and some other countries and regions followed thereafter (Leistikow et al. 2022). As a result of such regulatory restrictions and growing consumer demand for antibiotic-free animal products, there has been accelerated search for effective alternatives to AGPs to sustain livestock productivity while addressing the AMR challenge (Almansour et al. 2023). Among the number of options investigated, probiotics has gained importance as a scientifically proven and commercially feasible dietary application providing a multifaceted strategy in managing the health and performance of animals (Chakravarty 2025).

When introduced in adequate amounts, these live microorganisms contribute to the health of host by a wide range of interactions, not only resistance to diseases. Over the last decade, research on probiotic use in livestock production expanded significantly, with numerous studies validating their effectiveness in multiple animal species such as poultry, ruminants, and swine (Fig. 1). Sachdeva et al. (2025) stated that probiotics positively influence the production parameters and immune function through gut-associated immune modulation, with more pronounced response in herbivore species than omnivores. This species-dependent diversity of responses emphasizes the need of specific design of probiotic formulations accounting for the physiology and microbial ecology of individual livestock species (Grumet et al. 2020). The mechanisms behind the action of probiotics are a complex network of several inter-related pathways all contributing to the enhancement of animal health and productivity (Kouhounde et al. 2022). The direct antagonistic action against pathogenic microorganisms is one of major mechanisms that is realized through competitive exclusion, production of antibacterial compounds, and changing environments by reducing pH level (Mazanko et al. 2022). Also, probiotics can influence the composition of the gut microbiome by favouring the growth of beneficial microbial populations while suppressing the harmful species through ecological niche competition (Idowu et al. 2025; Montazeri-Najafabady 2025). Such probiotic mechanisms are complemented by reinforcement of intestinal barrier

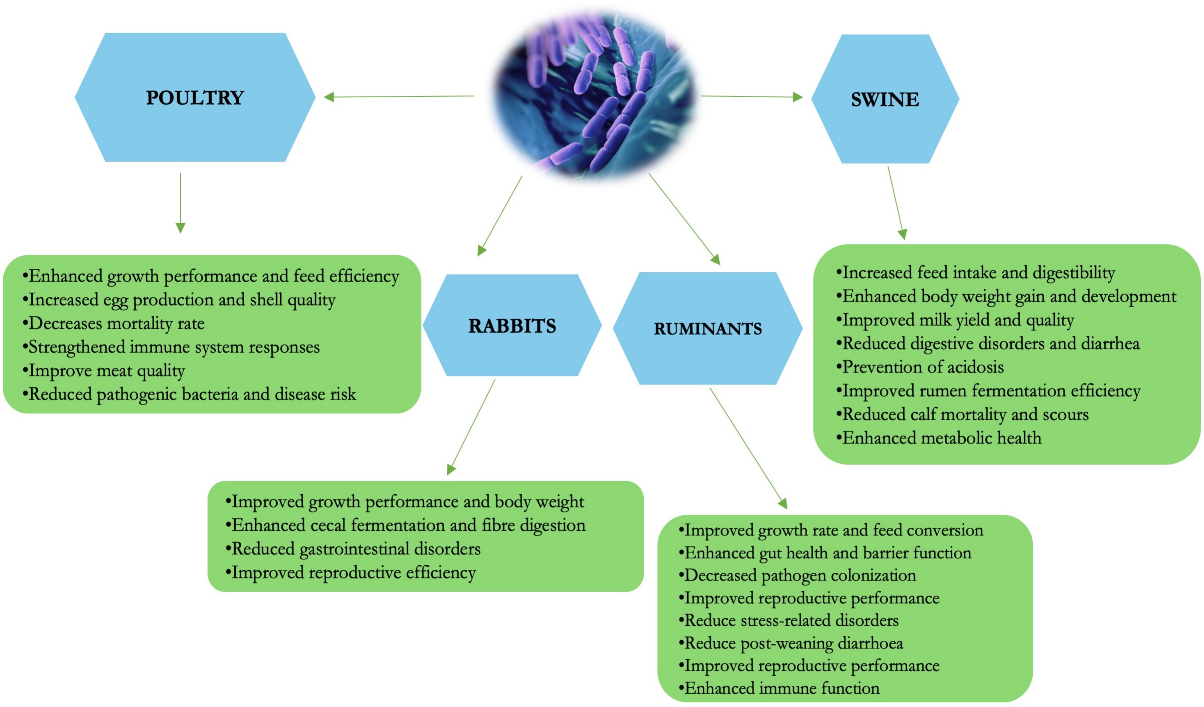

Fig. 1. *Efficacy of probiotics across diverse animal species*

function, immune regulation, and improved metabolic functions to form an integral biological foundation for the optimal performance of the animals (Park and Seo 2023; Zhao et al. 2024a; Idowu et al. 2025; Montazeri-Najafabady 2025).

The clinical and commercial success of probiotic interventions has been documented across multiple livestock species, with commonly used genera including *Lactobacillus*, *Bifidobacterium*, *Bacillus*, and *Enterococcus* (Williams et al. 2023). Currently, modern research is furthering the elucidation of strain-specific mechanisms, optimal dosage schedules, and synergy amongst various probiotic species, paving the way for next-generation formulations that would optimize therapeutic potentials with minimal side effects and better regulatory compliance. Even though the evidence supporting the efficacy of probiotics is heavily documented in the literature, limited information about the strain-specific mechanisms, dosing regimens, and application protocols across different production systems. Hence, this narrative review aims to: (i) summarize the mechanisms of probiotic action; (ii) explain species-specific responses and applications; (iii) identify sustainability and regulatory implications; and (iv) outline current knowledge gaps and future research priorities needed to advance the use of probiotics in sustainable livestock systems.

2. Materials and Methods

The objective of this review was to carry out a narrative synthesis of published data on probiotics as a sustainable alternative to AGPs in the livestock species, and critically assess the information.

2.1 Literature Search Strategy

Literature search was carried out from January 2015 to July 2025 using databases PubMed, Scopus, and Web of Science. The keywords used in combination with Boolean operators (AND, OR) included "probiotics", "dietary fed microbials", "live microorganism", "beneficial bacteria" "antibiotic growth promoters", "mechanisms of action", "sustainability", "gut microbiota" and "animal performance" and "livestock production". Bibliographies from relevant articles and reviews were also manually searched for identification of additional potential sources.

2.2 Inclusion and Exclusion Criteria

Articles were considered relevant if they: (i) reported experimental or observational results of probiotic application in ruminants, poultry, pigs, and rabbits; (ii) offered interpretations on mechanistic pathways, production performance responses, immunoregulatory outcome, microbial modulation and/or sustainability related implications; (iii) were published within the time frame from 2015 to 2025 in peer-reviewed journals; and iv) addressed feeding trials including well-controlled experiments taking into account multi-site experimental studies as well as molecular investigations. Non-English reporting studies, unpublished theses or abstracts without full texts available and studies not in the context of livestock production were ruled out

2.3 Data Extraction and Thematic Categorization

Key findings from included studies were summarised into three thematic areas:

- Mechanistic perspective: It involved competitive exclusion, antimicrobial metabolite synthesis, immune modulation, and gut microbiota regulation.
- Applications at production scale with implications on growth and reproductive performance, feed efficiency, and disease resistance.
- The sustainability aspect and environmental impact

Given the narrative nature of this review, meta-analytic procedures were not used; instead, evidences were critically screened to recognize broad patterns, emphasize emerging research directions, and identify voids deserving future study.

3. Probiotic and Diseases Resistance

3.1 Systemic Immune Function

The ban of AGP in livestock production has demanded the development of alternative strategies to maintain or improve animal health and productivity (Ronquillo and Hernandez 2017; Anee et al. 2021), with probiotics emerging as one of the most promising candidates for improving systemic immune function primarily through gut microbiome modulation (Fig. 2). This paradigm shift describes a fundamental change in animal feeding approaches, where livestock producers and consumers are demanding sustainable alternatives with livestock performance benefits along with improved food safety and reduced antibiotic resistance (Leistikow et al. 2022; Ibeagha-Awemu et al. 2025). In response to these expectations, probiotics regulate the immune response of livestock by establishing and maintaining beneficial bacteria within gastrointestinal tract (GIT), which induces a cascade of immune responses beyond the intestine. The gut-associated lymphoid tissue (GALT) is a major site for such immune responses, where probiotic bacteria interact with immune cells, such as dendritic cells (DC) and Macrophages, bringing about the systemic immune regulation (Mazanko et al. 2022). Several studies reported that probiotic feeding modifies the production of short-chain fatty acids (SCFAs) in pig cecum (Park and Seo 2023; Zhang et al. 2022), which in turn affect the blood immune markers and overall animal health. Probiotic strains such as *Lactobacillus* spp. have been observed to protect the epithelium by maintaining the porcine intestinal epithelial cell functions and enhance the resistance of the host against gastrointestinal pathogen and growth via competitive exclusion and antimicrobial metabolite production (Kober et al. 2022). In addition to classical immunity, certain probiotics exert psychobiotics effects by modulating the neuroimmune signalling and stress resilience. Such kind of effects, mediated via microbiota–immune–brain axis, influence productivity, behaviour, stress tolerance, and anxiety responses (Shini and Bryden 2021; Idowu et al. 2025). Also, lactic acid bacteria shift the faecal microbiota composition towards the beneficial bacterial families, such as *Clostridiaceae*, *Lachnospiraceae*, and *Bifidobacteriaceae*, associated with improved immunity and growth (Chiu et al. 2021; Mansilla et al. 2022). Dietary supplementation of probiotics promotes IgG production, enhance natural killer (NK) cell activity, elevate cytokines (IL-10 and IFN-γ), and suppress pro-inflammatory cytokines (TNF-α) (Rodríguez et al. 2023). These changes ultimately improve disease resistance, vaccine response, and stress tolerance (Markowiak and Ślizewska 2018;

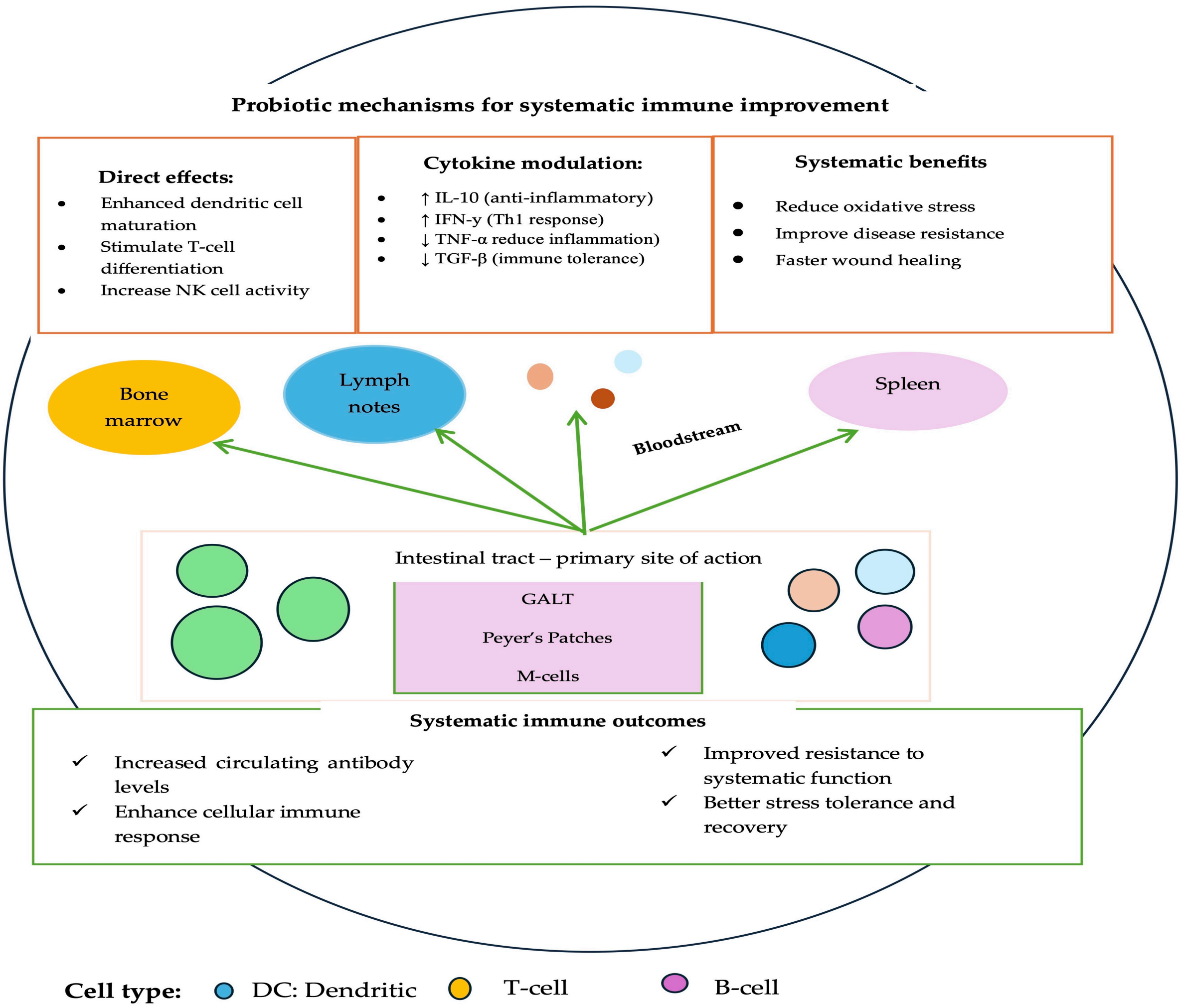

Fig. 2. *Probiotic mechanisms for systematic immune improvement*
GALT: Gut-Associated Lymphoid Tissue, DC: Dendritic Cells, Mac: Macrophages, NK: Natural Killer cells, IL: Interleukin, IFN-γ: Interferon-gamma, TNF-α: Tumor Necrosis Factor-alpha, TGF-β: Transforming Growth Factor-beta, IgG: Immunoglobulin G, WBC: White Blood Cells, Ab: Antibodies, Cyt: Cytokines

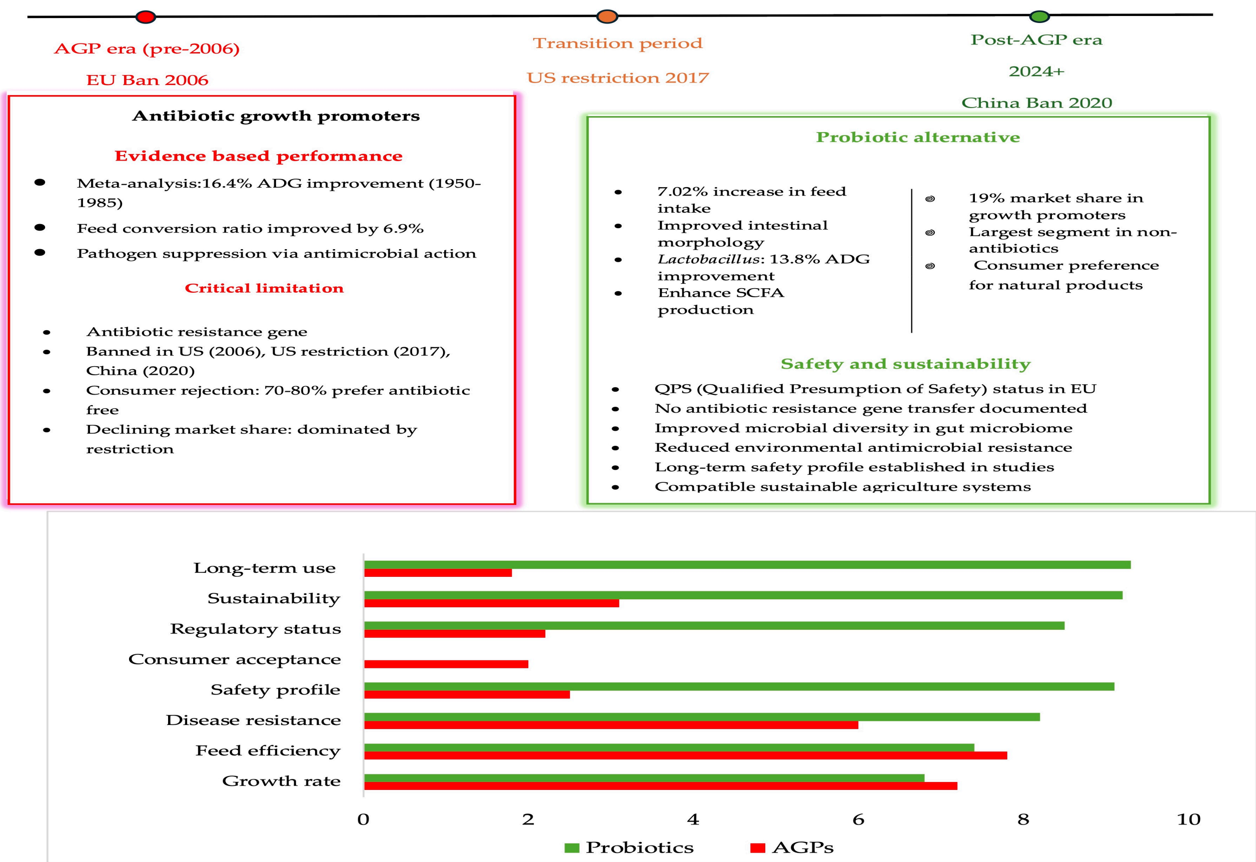

Fig. 3. *Evidence-based comparative performance: AGPs vs Probiotics (2017-2025) (Zhu et al. 2022; Liu et al. 2022; He et al. 2024; Sachdeva et al. 2025) Scores (0–10) shows normalized performance ratings across growth rate, feed efficiency, disease resistance, safety profile, consumer acceptance, regulatory status, sustainability, and long-term use. Higher scores indicate more favourable outcomes. Probiotics show superior performance in safety, sustainability, and long-term applicability, whereas AGPs perform slightly better in immediate growth and feed efficiency*

Liu et al. 2022). Fig. 2 summarizes the mechanisms driving systemic immune enhancement through probiotic intervention.

3.2 Probiotic as Alternatives to AGPs

Global prohibition of AGPs in livestock production demanded comprehensive evidence-based evaluations of probiotics as one of the scientifically validated dietary manipulation for maintaining animal health and productivity whilst addressing the antimicrobial resistance concerns (Beber et al. 2024). Meta-analytical assessments encompassing 71 empirical studies revealed that non-antibiotic feed additives, primarily probiotics, significantly enhanced production and immune function across livestock species. The study further observed that herbivores demonstrate more pronounced responses than omnivores, establishing their efficacy as viable AGP replacements (Liu et al. 2025). Probiotic strains such as *Lactobacillus* and *Bifidobacterium* effectively inhibit pathogens such as *Clostridium perfringens* and *Salmonella* in livestock, offering pathogen control comparable to AGPs, through antimicrobial compound production and competitive nutrient exclusion mechanisms (Sachdeva et al. 2025). Moreover, direct comparative studies revealed that *Lactobacillus plantarum* supplementation increased SCFA production compared to AGP's, which provided no such metabolic benefit, thus describe the superior nutritional enhancement mechanisms of probiotics (Peng et al. 2016; Wang et al. 2021; Herich et al. 2025). Improvement in body weight (2.94%) and feed conversion efficiency (2.91%), equalling or surpassing antibiotic effects, was observed in probiotic supplemented chicken (Baksi et al. 2019). Thus, probiotics provide long-term sustainability by promoting health, immunity, growth, nutrient digestibility, and microbial balance through competitive exclusion without contributing to AMR development unlike antibiotics (Terpou et al. 2019). Even though AGPs have been used for many years due to their low price, it is becoming increasingly difficult for poultry producers to continue using them due to stricter regulations aimed at discouraging their use based on AMR (Leistikow et al. 2022). Accordingly, probiotics have become an environmentally friendly replacement to continue maintaining animal productivity and address the issue of AMR, while meeting public concerns for safe animal products without harmful residues. Fig. 3 shows the evolution of AGP over the years.

3.3 Mechanisms of Probiotics Action in Livestock

Probiotics offer a multifaceted strategy for sustainable livestock production through interconnected mechanisms (Fig. 4). Direct antagonism forms the first defence, with strains such as *Lactobacillus plantarum* and *Bacillus subtilis* competing for nutrients and adhesion sites while producing antimicrobial compounds such as bacteriocins, organic acids, and hydrogen peroxide, which suppresses pathogens (Mazanko et al. 2022; Sachdeva et al. 2025). The second mechanism involves gut microbiome modulation, whereby probiotics restructure microbial communities to favour commensals and suppress pathogens including *Clostridium perfringens* and *Salmonella* via competitive exclusion (Liu et al. 2025). Also, probiotics enhance the gut barrier integrity by upregulating tight junction proteins and increasing mucin

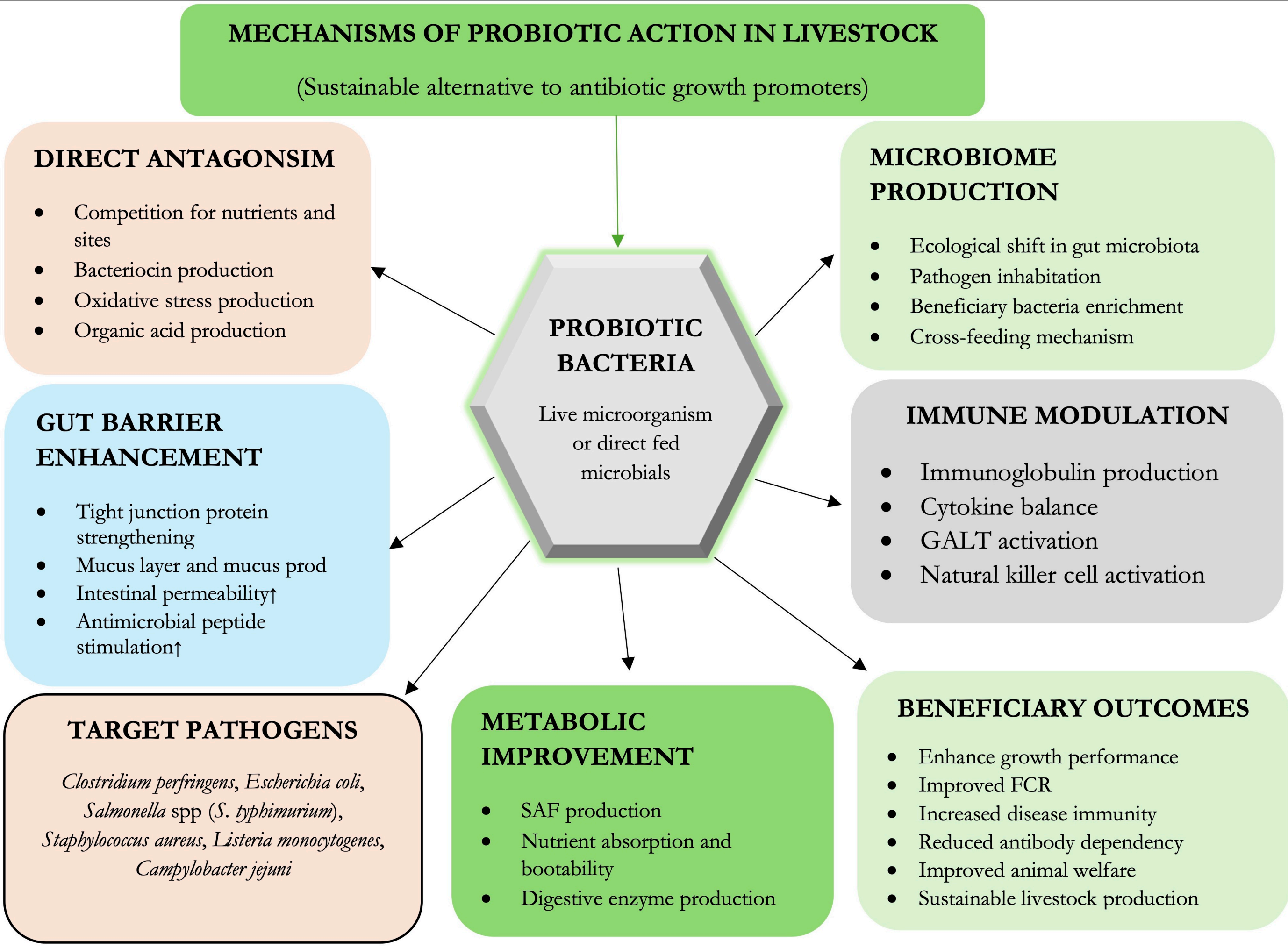

Fig. 4. *Mechanisms of action of probiotics in livestock*

secretion, which limit pathogen translocation (Park and Seo 2023; Wang et al. 2024). Furthermore, probiotics bring about immune modulation through elevated immunoglobulin (IgA, IgG, IgM) production, balanced cytokine regulation, and activation of GALT (Markowiak and Ślizewska 2018). Another mechanism is the metabolic enhancement which includes greater SCFA production, improved nutrient uptake, and increased digestive enzyme activity, resulting in improved growth performance, disease resistance, and reduced antibiotic reliance (Lee et al. 2025). Understanding this mechanistic processes is critical for optimizing probiotics as sustainable alternatives to AGPs in modern animal agriculture.

4. Species-Specific Applications of Probiotics

4.1 Probiotics Applications in Ruminant production

Dietary supplementation of probiotics has significantly improved rumen fermentation, gut morphology, immune modulation, and overall performance (Table 1). In early-lactation Holstein cows, supplementation with *Enterococcus faecium* at 1.1 × 10^9 CFU/g, improved dry matter, neutral detergent fibre, organic matter, crude protein, and non-structured carbohydrate digestibility. Further, the study observed an increased milk yield, feed efficiency, and serum glucose with reduced serum cholesterol and lowered *in vitro* methane production compared with a commercial strain (Azzaz et al. 2022). In Jintang black goats, dietary supplementation of *Bacillus amyloliquefaciens* and *Bacillus pumilus* significantly improved the histomorphology of GIT, increasing the length and width of ruminal papillae by 30-74% and 17-66%, respectively, and increasing the height of intestinal villi by 14–93% (Zhang et al. 2020). In addition to improving digestion, probiotics support immune function and overall health. For instance, supplementation of *Bacillus amyloliquefaciens* increased the oxidative burst capacity by 30.41%, paralleled by a rise in serum phosphorus (+13.84%), total protein (+17.0%), and in blood urea nitrogen (+56.3%) in Alpine goat kids (Lee et al. 2025), implying an improved metabolic status. In growth-retarded Hu lambs, co-fermented *Lactobacillus acidophilus* and *Bacillus subtilis* boosted antioxidant defences by increasing superoxide dismutase and glutathione peroxidase activities while reducing malondialdehyde levels, an indicator of oxidative stress (Mao et al. 2023). These physiological improvements were further reflected in robust immunomodulatory effects, such as elevated immunoglobulin G (IgG) levels and reduced pro-inflammatory cytokines such as interleukin-6 (IL-6), tumour necrosis factor-alpha (TNF-α), and interferon-gamma (IFN-γ) (Iddir et al. 2020). Probiotics particularly offer protective effects under stress conditions such as subacute ruminal acidosis. Combination of *Enterococcus faecium* and *Saccharomyces cerevisiae* helped maintain a more stable median ruminal pH and prevented milk yield decline in challenged cows (Tun et al. 2020). In sheep, *Bacillus subtilis* supplementation not only enhanced serum total protein, globulin, and

Table 1. Bacterial probiotic interventions: effects on ruminant performance, digestibility, immunity, and rumen fermentation

Ruminant type/ Breed	Age/ Stage	Probiotic strain	Study design	Key findings	References
Holstein dairy cows	Mid-lactation	*Saccharomyces cerevisiae* fermentation product (SCFP)	80 cows; 4 SCFP levels (0–180 g/d) for 10 wks	↑ Milk yield; ↑ N-conversion; ↑ total VFAs, acetate, propionate, butyrate; ↑ rumen fungi & cellulolytic bacteria; ↓ *S. bovis*, ↓ lactate-utilizing bacteria; ↑ urinary purine derivatives	(Zhu et al. 2017)
Jintang black goats	80-day-old weanlings	*B. amyloliquefaciens* fsznc-06; *B. pumilus* fsznc-09	30 goats; 3 treatments	↑ Ruminal papillae length (+30–74%) & width (+17–66%); ↑ villus height (+14–93%); ↑ microbial diversity; ↑ beneficial bacteria; ↓ pathogens; ↑ metabolic pathways	(Zhang et al. 2020)
Alpine goat kids	14-day-old weanlings	*B. amyloliquefaciens* CU33 Fermented Product (CU33FP)	40 kids; 4 inclusion levels	↑ BW gain (+26.0% pre-weaning at 0.1% level; +30.7% overall); ↑ FCR (−18.8%); ↓ fecal score (−18.5% pre-weaning, −20.9% post); ↓ coliforms (−35%); ↑ Bacillus-like spp. (+52.6%); ↑ serum P (+13.8%), ↑ protein (+17%), ↑ BUN (+56%); ↑ oxidative burst (+30%)	(Lee et al. 2025)
Lactating dairy cows (rumen-cannulated)	Mature, lactating stage	*Saccharomyces cerevisiae* fermentation products (SCFP): Original XPC and NutriTek (Diamond V)	In vitro (high pH >6.3 vs. depressed pH 5.8–6.0) and in vivo cross-over trial (8 cows; 14 g/d SCFP; SARA challenge)	XPC & NutriTek ↑ *Ruminococcus flavefaciens* and ↑ *Fibrobacter succinogenes* at both pH levels; NutriTek had stronger effect. ↑ *Prevotella brevis*, ↑ *R. flavefaciens*, ↑ ciliate protozoa, ↑ *Bifidobacterium* spp. SARA challenge ↓ microbial richness, ↓ diversity, ↓ Bacteroidetes, ↓ protozoa, and ↓ microbiota functionality	(Tun et al. 2020)
Sheep & lambs	Adults (40 kg) & lambs (9.4 kg)	*B. subtilis*, *B. licheniformis* (Enzimsporin™)	Sheep: 3 groups; Lambs: 2 groups for 30 d	Lambs: ↑ weight gain (+18.8%); ↑ serum protein (+5.3%), globulin (+10.8%), urea (+6.2%); ↑ Lactobacillus & Bifidobacterium; ↓ *E. coli*, Enterococcus. Sheep: ↑ protein, albumin, globulin; ↓ cholesterol, bilirubin; ↑ immune status	(Devyatkin et al. 2021)
Duhan hybrid lambs	3 months (24 kg)	*Bacillus subtilis* C-3102	10 lambs; 80-day trial	↑ ADG (+18.8%); ↑ rumen diversity; ↑ Bacteroidetes; ↓ Firmicutes; ↑ acetate, isobutyrate, butyrate; ↑ microbial protein synthesis	(Gao et al. 2022)
Hu lambs (growth-retarded)	50 days	Co-fermented *L. acidophilus* + *B. subtilis*	24 lambs; 60-day trial	↑ ADG & DMI; ↑ SOD & GSH-Px; ↓ MDA; ↑ IgG & GH; ↓ IL-6, TNF-α, IFN-γ; ↑ total VFA & acetate; ↑ beneficial genera (*Ruminococcus*, *Succiniclasticum*, *Acidaminococcus*)	(Mao et al. 2023)
Holstein dairy cows	Early lactation	*E. faecium* EGY_NRC1 (1.1×10^9 CFU/g) vs commercial strain	In vitro + 60-day in vivo	↑ DM & NDF digestibility; ↑ milk yield; ↑ feed efficiency; ↑ OM, XP & NSC digestibility; ↑ serum glucose; ↓ serum cholesterol; in vitro ↓ methane	(Azzaz et al. 2022)
Holstein dairy cows	Lactating (genomic analysis)	*E. faecium* (isolated strain, GWAS)	GWAS across 1,313 cows	Identified 95 SNPs for milk traits & SCC; pleiotropic SNPs on PLEC, PLEKHA5, TONSL, PTGER4, LCORL; key genes (GPAT3, CEBPB, AGO2, SLC37A1, FNDC3B) linked to fat metabolism & mammary development	(Wang et al. 2022)

Exp. – Experiment, d – Days, kg/d – Kilograms per day, ↑ – Increase, ↓ – Decrease, ± – Plus or minus standard deviation or error), ADG – Average Daily Gain, DMI – Dry Matter Intake, DM – Dry Matter, NDF – Neutral Detergent Fiber, XP – Crude Protein (CP), NH_3-N – Ammonia Nitrogen, VFA – Volatile Fatty Acids, SARA – Subacute Ruminal Acidosis, LBP – Lipopolysaccharide Binding Protein, SAA – Serum Amyloid A (acute-phase protein), SOD – Superoxide Dismutase (antioxidant enzyme), GSH-Px – Glutathione Peroxidase (antioxidant enzyme), MDA – Malondialdehyde (lipid peroxidation biomarker), GH – Growth Hormone, IgG – Immunoglobulin G, IL-6 – Interleukin-6 (pro-inflammatory cytokine), TNF-α – Tumor Necrosis Factor-alpha (pro-inflammatory cytokine), IFN-γ – Interferon-gamma (pro-inflammatory cytokine), B. – Bacillus, L. – Lactobacillus, E. – Enterococcus, S. – Saccharomyces, P. – Propionibacterium, CFU – Colony-Forming Unit (viable bacterial count), DFM – Direct-Fed Microbials (live microbial feed additives), GWAS – Genome-Wide Association Study, SNPs – Single Nucleotide Polymorphisms, PLEC – Plectin (gene), PLEKHA5 – Pleckstrin Homology Domain Containing A5 (gene), TONSL – Tonsoku-Like DNA Repair Protein (gene), PTGER4 – Prostaglandin E Receptor 4 (gene), LCORL – Ligand-Dependent Nuclear Receptor Corepressor-Like (gene), GPAT3 – Glycerol-3-Phosphate Acyltransferase 3 (gene), CEBPB – CCAAT/Enhancer Binding Protein Beta (gene), AGO2 – Argonaute 2 (gene, involved in RNA silencing), SLC37A1 – Solute Carrier Family 37 Member A1 (gene), FNDC3B – Fibronectin Type III Domain Containing 3B

urea concentrations but also favourably modulated gut microbiota composition (Devyatkin et al. 2021). At the same time, higher concentrations of urea indicate higher protein turnover, which suggests either better microbial fermentation or possible inefficiencies in nitrogen utilisation depending on dietary protein levels. The beneficial modulation of gut microbiota composition triggers the potential probiotic functions to stabilise rumen ecology, inhibit pathogenic species, and improve fermentative efficiency. In another study, *Clostridium butyricum* enhanced average daily feed intake and weight gain in pre-weaned calves (Wang et al. 2025). A consistent trend is observed among the studies reviewed that probiotics enhance the performance of ruminants *via* coordinated activities on rumen fermentation, gut integrity, metabolic status, immune regulation, lower indigestibility values, improved papillae and villi structure, and higher microbial fibrolytic activity (Azzaz et al. 2022). This explains that probiotics enhance both fermentative efficiency and nutrient absorption which contribute to improved milk yield, growth rate, and FCR across species. Concomitant metabolic changes (increased glucose and enhanced protein metabolism, increased antioxidant status) along with decrease in proinflammatory cytokines point to the ability of probiotic to cope with subclinical stress and enhancement of overall system resilience (El-Sayed et al. 2024). Nevertheless, the response is strain, dose, and stage of physiology dependent, there is need for more long-term studies to understand herd level effects. Although there is still limited knowledge on detailed mechanisms, the current evidence clearly indicates that directed use of probiotics can be used to improve productivity and health including rumen stability in ruminant production systems while promoting the transition toward more sustainable farming.

4.2 Probiotics Application in Poultry Production

The use of Probiotic in the poultry industry has become a viable means of enhancement of growth performance, feed utilization, gut health, and overall bird welfare and has been proposed as a major dietary strategy in the sustainable animal production as alternative to AGP's (Ayana and Kamutambuko 2024; Idowu et al. 2025). The use of multi-strain and multi-species probiotics have shown synergistic effects in the intestinal tract, resulting in enhanced performance of birds (Kwoji et al. 2021; Kim et al. 2024). *Pediococcus acidilactici* supplementation in Ross 308 broiler chicken yielded dose-dependent improvements in body weight and feed efficiency, increased villus height and crypt depth with improved peripheral blood mononuclear cell optimal performance at 1 × 10^9 CFU g^{-1} feed inclusion levels (Hosseinzadeh et al. 2025). Similarly, dietary supplementation combining *Pediococcus acidilactici* (0.1 g/kg), mannan-oligosaccharides (2 g/kg), and butyric acid (0.5 g/kg) improved broiler performance under *Salmonella Typhimurium* challenge by restoring feed intake and body weight gain to control levels along with decreased feed conversion ratio, cecal *Salmonella* counts at 14 and 21-days post-challenge, and heterophil: lymphocyte ratio (Jazi et al. 2018). Furthermore, dietary supplementation of *P. acidilactici* at 1 × 10^8 CFU/g improve villus height and Villus Height (VH): Crypt Depth (CD) ratio in the jejunum by 10–15% and restored amylase and protease activities (Dong et al. 2025). Similar findings were recorded by Jazi et al. (2018) when compared to pre-challenge levels. This indicates the ability of probiotics in enhancing intestinal structure and enzymatic function. In another study, fermented protein sources from *Bacillus amyloliquefaciens* CU33 improved growth by 11% over conventional sources in broiler chickens (Lee et al. 2022). Probiotics exert beneficial effects through complex mechanisms involving gut health and caecal fermentation modifications. Specifically, the cecum serves as a critical site for complex carbohydrate fermentation and short-chain fatty acid production (Zhang et al. 2022) and probiotic supplementation optimizes cecal microbial composition by reducing pathogens and enriching beneficial taxa (Hoepers et al. 2024). The *Limosilactobacillus fermentum* (10^8 CFU/mL) and *Saccharomyces cerevisiae* (10^7 CFU/mL) (1:1) supplementation improved body weight gain, enhanced feed conversion efficiency, increased WBC and hemoglobin levels, elevated HDL concentrations, and showed no tissue necrosis or adverse effects (Hati et al. 2023). Additionally, administration of synbiotics once a day to Mandarah one-day-old male chicks (0.25-0.50 mL per bird, single-dose through drinking water) improved gut health by increasing lactic acid bacteria populations with reduced *Escherichia coli* count, enhanced duodenal and ileal villus height and villus: crypt ratios without affecting intestinal length or pH (Youssef et al. 2024). The common strains including *Bacillus subtilis*, *Lactobacillus acidophilus*, and *Bifidobacterium bifidum* promote anti-inflammatory cytokines like IL-10 and TGF-β, while suppressing proinflammatory mediators (Krysiak et al. 2021). Moreover, *Bacillus licheniformis* and *B. subtilis* reduced Salmonella colonization in the ileum and cecum (Fathima et al. 2022), while *Bacillus coagulans* increased beneficial *Lactobacillus* and *Bifidobacterium* populations. These changes in gut microbial structure are associated with upregulation of Toll-like receptors TLR2 and TLR4, reinforcing mucosal immunity and long-term gut health (Soren et al. 2024). Table 2 shows that dietary supplementation of probiotics in poultry studies, generally improved growth performance, gut health, and immune responses. Nevertheless, the degree of effectiveness depends on strain, dose, and challenge model. Supplementation with *lactobacilli, bacilli, pediococci*, and yeast generally improved body weight gain, feed/gain ratio, villus height, and microbial equilibrium with decreased counts of pathogens and the induction of diarrhoea (Krysiak et al. 2021). Some studies also reported additive effects of probiotics when combined with vaccines, postbiotic, and prebiotic in chicken (Reuben et al. 2021; Shehata et al. 2022). Therefore, the available evidence shows that specific probiotic strains can potentially uphold gut health, immunity, and performance in broilers thus representing potential substitutes to antimicrobials feed additives.

4.3 Probiotic Application in Swine Production

In hybrid and crossbred pigs, multi-species formulations such as *Bacillus subtilis*, *Saccharomyces cerevisiae*, and lactic acid bacteria, increase beneficial microbial populations such as LAB and yeast, while reducing pathogenic bacteria like *E. coli* and *Clostridium spp.* (Rybarczyk et al. 2021; Kim et al. 2024). Improved gut microbial balance is associated with improved growth performance, such as enhanced ADG, FCR, and carcass traits, particularly in growing and weaned piglets (Dowarah et al. 2017; Kwak et al. 2021). The efficacy of probiotics appears to be strain-specific and age-dependent. Species-specific strains, such as *Pediococcus acidilactici* FT28, consistently outperformed generalist strains in early-weaned piglets by improving feed conversion, nutrient digestibility, and antioxidant status (Dowarah et al. 2018). Multi-strain combinations demonstrated synergistic effects on gut morphology, evidenced by increased villus height, enhanced villus: crypt ratios, and modulation of intestinal tight junction proteins (Kwak et al. 2021; Castillo Zuniga et al. 2024). These morphological adaptations suggest improved absorptive capacity and intestinal barrier integrity, which are crucial for nutrient uptake and pathogen defence. Some interventions improved antioxidative status, blood biochemistry parameters, and tight-junction integrity of intestinal (Gonzalez et al. 2024; Tang et al. 2024). Moreover, decreased pro-inflammatory cytokine expression was frequently reported across studies (Dowarah et al. 2018; Kwak et al. 2021). Reduction in diarrhoea incidence and modulation of faecal pH further explain the role of probiotics in maintaining gut homeostasis (Dowarah et al. 2018). Early-life administration, particularly during the nursery phase, appeared most effective, with transient reductions in enteric pathogens providing a critical window for gut colonization and immune system maturation (Rybarczyk et al. 2021). However, some strains, such as EM Bokashi, while increasing lean carcass proportion, slightly compromised certain meat quality traits (pH, drip loss) (Rybarczyk et al. 2016), suggesting that strain selection should consider both performance and product quality. Furthermore, probiotics administered to sows during gestation and lactation positively influenced reproductive outcomes, piglet birth and weaning weights, and overall litter performance, demonstrating trans-generational benefits (Mazur-Kuśnirek et al. 2023). Lastly, in a large-scale commercial study with 83,838 Landrace–Yorkshire × Duroc pigs fed from 78 d of age to slaughter (112 kg), feeding of *Bacillus licheniformis* and *B. subtilis* reduced mortality by 1.36%, improved ADG and feed efficiency, and shifted the gut microbiota toward greater beneficial bacteria and fewer pathogens while carcass quality was unaffected (Rybarczyk et al. 2021). In summary, these results validates that Bacillus-based probiotics can improve herd-level growth performance and health stability in intensive pork systems, although no effect on carcass characteristics were observed. Table 3 summarises the impact of dietary application of probiotics on growth performance, gut structure and microbial balance, immune function, and nutrient utilization in pigs at different stages of growth and production. *Bacillus, Lactobacillus, Pediococcus*, and multi-strain combinations, often increased weight gain, feed intake, feed efficiency, and villus height while they decreased diarrhea

Table 2. Bacterial probiotic interventions: effects on poultry performance, digestibility, immunity, and gut morphology

Poultry species	Age/ Stage	Probiotic strain	Study design	Key findings	References
Japanese quail	36–42 wk (laying)	*Bacillus subtilis* (0.1%)	CRD, 3 treatments × 5 replicates × 11 quails	↑ Egg production & weight, ↑ LDL & antibodies, ↓ cholesterol, ↑ villus height & villus/crypt ratio, ↓ cecal coliforms, *E. coli*, *Salmonella*	(Manafi et al. 2016)
Japanese quail	0–36 d	*Pediococcus acidilactici*	2 genetic lines, probiotic vs. control, behavioural & memory tests	↓ tonic immobility; both lines: ↑ memory performance	(Parois et al. 2017)
Japanese quail	14 wks	*L. casei* + *L. rhamnosus* (0.005%)	CRD, 4 treatments × 6 replicates × 10 quails	↑ Feed intake, water intake, feed efficiency, egg production; ↔ Egg mass	(Lokapirnasari et al. 2017)
Meat-type quail	0–28 d	Protexin (0.1 g/kg)	CRD, 5 groups, feed restriction ± probiotic	↑ Feed conversion, ↓ mortality; ↔ Carcass traits	(Soomro et al. 2019)
Ross 308 broilers	Starter to finisher	*Pediococcus acidilactici* (PA) ± mannan-oligosaccharides (MOS) ± butyric acid (BA)	420 male broilers assigned to six treatments: negative control, positive control (*S. Typhimurium* challenge), and additive diets (PA, MOS, BA, or PA + MOS + BA)	*S. Typhimurium* challenge ↓ feed intake, ↓ body weight gain, ↓ VH and VH:CD ratio, ↑ FCR. Additive combination (PA + MOS + BA) restored growth performance (↑ BWG, ↓ FCR), ↓ cecal *Salmonella* counts, ↓ heterophil:lymphocyte ratio, ↑ VH and VH:CD ratio, ↑ amylase and protease activity	(Jazi et al. 2018)
Ross 308 broilers	Starter to finisher	*Pediococcus acidilactici (PediGuard)*	300 chicks divided into four groups: control, probiotic-only, vaccine-only, and probiotic + vaccine	↑ PBMC viability; ↑ IL-1, IL-6, TNF-α, and IL-10 expression; ↑ antibody titres (synergistic with vaccination); ↑ body weight gain, ↑ specific growth rate, ↑ feed efficiency; ↑ villus height and crypt depth	(Hosseinzadeh et al. 2025)
Cobb 500 broilers	0–35 d	*B. coagulans*, *Lactobacillus* spp (1g/kg dried culture)	2 seasonal cycles, 5 treatments × 17 birds	FOS & MOS: ↑ Immune response, ↓ *E. coli* & *Salmonella*; ↔ Growth, feed efficiency	(Al-Khalaifa et al. 2019)
Arbor Acres broilers	0–21/35 d	*Bacillus amyloliquefaciens* CU33-fermented FFSMP	2 trials, CRD	↑ Weight gain & feed efficiency, ↑ villus height & villus/crypt ratio, ↓ blood urea N, ↑ creatinine	(Lee et al. 2022)
Cobb broilers	1–42 d	*Limosilactobacillus fermentum* (KGL4; 10^8 CFU/mL) and *Saccharomyces cerevisiae* (WBS2A; 10^7 CFU/mL)	96 broilers assigned to 4 groups: control (with immunomodulator + commercial probiotic), KGL4, WBS2A, or KGL4+WBS2A	KGL4-treated birds showed ↑ BWG (2583 g), ↑ FCR, ↑ WBC, ↑ hemoglobin, ↑ HDL; improvements in haematology; ↓ tissue necrosis; no major changes in cecal microbiota composition	(Hati et al. 2023)
Broiler	1–28 days (starter to grower)	*Lactobacillus fermentum* (1 × 10^9 CFU/kg) and *Bacillus coagulans* (1 × 10^{10} CFU/kg)	336 chicks assigned to 4 groups (control, *C. perfringens*-challenged, + *L. fermentum*, + *B. coagulans*); samples collected on days 21 and 28	*C. perfringens* challenge ↑ IL-1β, TGF-β4, and disrupted gut microbiota; probiotic supplementation ↓ inflammatory cytokine expression, ↑ IL-17, IFN-γ, and T-cell ($CD3^+$) counts; ↑ ileal and cecal microbial richness and ACE index; restored *Lactobacillus* abundance and reduced pathogenic genera (*Romboutsia*, *Ruminococcus_torques*); overall ↓ intestinal inflammation and dysbiosis	(Guo et al. 2021)
Arbor Acres broilers	33–42 d (finishing)	*E. faecium* 5×10^6 CFU/g (encapsulated & uncoated)	CRD, 4 treatments × 12 birds	↑ Intestinal colonization, ↑ SCFAs, ↑ villus height (encapsulated), ↑ feed efficiency	(Zhang et al. 2024)
Ven Cobb 400 broiler	1-42 days	*Bacillus subtilis* (200 g/MT) and *Saccharomyces cerevisiae* fermentation product (1.25 kg/MT)	324 chicks, 3 dietary groups (control, B. subtilis, SCFP) with 12 replicates per die	SCFP ↑ ADG and ADFI (days 1–14), ↑ overall FCR; ↓ cholesterol and corticosterone; ↓ *E. coli*, ESBL-producing Enterobacteriaceae, *Salmonella*; ↑ villus height and VH:CD ratio; ↑ antibody titers (NDV, IBDV); no change in carcass traits or blood biochemistry	(Soren et al. 2024)

CRD – Completely Randomized Design, RCT – Randomized Controlled Trial, d – Days, wk – Weeks, ↑ – Increase, ↓ – Decrease, ↔ – No significant change (no difference), LDL – Low-Density Lipoprotein (bad cholesterol), ADG – Average Daily Gain, N – Nitrogen (in context of blood urea N), CD3+, CD4+, CD8+ T-cells – Subsets of immune T-lymphocytes (CD3: general T cells; CD4: helper T cells; CD8: cytotoxic T cells), TLR2, TLR4 – Toll-Like Receptor 2 and 4 (innate immune receptors), SCFAs – Short-Chain Fatty Acids, MOS – Mannan-Oligosaccharides (prebiotic), FOS – Fructo-Oligosaccharides (prebiotic), CFU – Colony-Forming Unit (measure of viable bacteria)L. – Lactobacillus (abbreviation for genus), B. – Bacillus, E. – Enterococcus / Escherichia (depending on context: E. faecium vs E. coli), C. – Clostridium, S. – Saccharomyces, LTI – Long Tonic Immobility (genetic line of quail used for fear response), STI – Short Tonic Immobility (genetic line of quail), FFSMP – Fishmeal-Fermented Soybean Meal Product (used as fermented protein source in diet), LAB – Lactic Acid Bacteria. PBMC: peripheral blood mononuclear cell

occurrence rate as well as fecal pathogens count and inflammatory parameters. This depicts its ability to improve pig health and serve as barrier to disease entry. Collectively, existing findings support that properly selected probiotics or postbiotics may be successfully employed to improve swine production, gut function, and microbial balance as a reliable alternative to antibiotics used as growth promoters.

4.4 Probiotics Application in Rabbits

The dietary supplementation of probiotics in rabbit production improves growth, health, and immunity under various environmental and dietary conditions (Table 4). Multi-strain formulations combining

Table 3. Bacterial probiotic interventions: effects on swine performance, digestibility, immunity, and gut morphology

Breed	Age/ Stage	Probiotic strain	Study design	Key findings	References
Hybrid pigs (P-76 × Naïma)	Growing-finishing (28–164 d)	EM®Bokashi: *S. cerevisiae*, *L. casei*, *L. plantarum*	Controlled trial, 120 pigs, 2 groups	↑ Lean %, ↑ beneficial microbiota (LAB/yeast); ↑ Na, Mg, Se; ↓ Meat pH, ↑ drip loss, ↑ shear force	(Rybarczyk et al. 2021)
Crossbred piglets (Local × Landrace)	Early weaned (28 d)	*Pediococcus acidilactici* FT28	RCT, 36 piglets, 3 groups	↑ FCR, ↑ crude protein & fibre digestibility, ↑ RBC, WBC, glucose, total protein, albumin, globulin, ↑ Antioxidants (GSH, SOD, catalase)	(Dowarah et al. 2018)
Crossbred pigs (Local × Landrace)	28–180 d	*P. acidilactici* FT28 vs *L. acidophilus*	180-d trial, 36 piglets, 3 groups	↑ ADG, ↑ ADMI, ↑ G: F, ↓ Diarrhea, ↓ Fecal pH, ↑ Lactic acid, ↑ Villus height/crypt depth, ↑ Beneficial microbiota, ↓ Pathogens	(Dowarah et al. 2017)
Landrace × Yorkshire	Grower-finisher (28.7–89 kg, 61–151 d)	Multi-strain: *B. subtilis* H4 + *S. boulardii* + LAB complex	75-d trial, 80 pigs, 4 treatments	↑ ADG & FCR (growers, BSL), ↑ Nutrient digestibility, ↑ Fecal LAB, ↓ *E. coli*; ↔ Finisher performance	(Giang et al. 2011)
(Landrace × Yorkshire) × Duroc	Growing finishing (112 d)	Multi-species probiotic: *L. plantarum*, *L. fermentum*, *L. salivarius*, *Leuconostoc*, *B. subtilis*, *B. licheniformis*	42-d trial, 80 pigs, 2 groups	↑ Body weight, ↑ Feed efficiency, ↑ Jejunal ZO-1, ↓ IL-12, ↓ Serum/hepatic TG, ↓ Lipogenic genes, ↑ β-oxidation gene, ↑ Beneficial microbiota	(Kwak et al. 2021)
DanBred sows	Gestation & lactation	*B. subtilis* DSM25841 + *B. amyloliquefaciens* DSM25840	2-cycle study, 96 sows	↑ Sow feed intake, ↓ Body weight & backfat loss, ↑ BCS; ↑ Piglet birth & weaning weight, ↑ Litter weight gain, ↓ *E. coli*, ↓ Feed cost per piglet	(Mazur-Kuśnirek et al. 2023)
Large White pigs	Weaned piglets & pregnant sows	*E. coli* ED1a & Nissle 1917	4 trials	Modest ↓ ESCR *E. coli* (<1 log), good colonization; ↔ Long-term ESCR prevention	(Mourand et al. 2017)
Landrace-Yorkshire × Duroc	78 d–slaughter (~112 kg)	*B. licheniformis* + *B. subtilis* (BioPlus YC)	Large-scale, 83,838 pigs	↓ Mortality (↓1.36%), ↑ ADG, ↑ Feed efficiency, ↑ Beneficial bacteria, ↓ Pathogens; ↔ Carcass quality	(Rybarczyk et al. 2021)
Crossbreed Commercial Pigs	18–21 d weaned	*Lactobacillus plantarum* and *Pediococcus acidilactici* including microencapsulated *L. plantarum* (spray-dried)	240 neonatal pigs; oral administration of probiotics or antibiotics; compared fresh vs. spray-dried formulations under commercial feeding conditions	↑ Average daily gain and feed conversion ratio during nursery and grower phases; ↑ fecal *Lactobacillus*, ↓ *Enterobacteriaceae* counts; ↑ villus height and villus:crypt ratio (especially in jejunum); microencapsulation preserved probiotic viability and efficacy.	(Pupa et al. 2021)
Weaned crossbred pigs	Nursery (weaning–47 d)	Lactobacillus-based probiotic (LPr), Bifidobacterium-based postbiotic (BPo)	Complete block, 1040 pigs, 4 treatments	↑ ADFI (D0–10), ↑ Villus height: crypt depth, ↑ Lactobacillaceae abundance; ↔ Overall growth	(Castillo Zuniga et al. 2024)
Duroc × Landrace × Yorkshire	Weaned piglets	*Bacillus amyloliquefaciens TL106*	Randomized controlled design; TL106 isolated from Tibetan pigs and tested for probiotic potential	↑ Growth performance and nutrient digestibility (crude fiber, crude ash); ↓ diarrhea incidence; ↑ abundance of beneficial gut bacteria (*Lachnospiraceae*, *Peptococcaceae.rc4_4*, *Erysipelotrichaceae.L7A_E11*, *Mollicutes.RF39*); stabilized gut microbiota under weaning stress	(Du et al. 2022)

RCT – Randomized Controlled Trial, RCB – Randomized Complete Block design, d – Days, wk – Week, ↑ – Increase, ↓ – Decrease, ↔ – No significant change (no difference), ADG – Average Daily Gain, ADFI – Average Daily Feed Intake, ADMI – Average Daily Metabolizable Energy Intake (sometimes noted as Average Daily Metabolizable Intake, here context = feed intake), FCR – Feed Conversion Ratio, G:F – Gain-to-Feed ratio (efficiency measure), LAB – Lactic Acid Bacteria, ZO-1 – Zonula Occludens-1 (tight junction protein), IL-12 – Interleukin-12 (pro-inflammatory cytokine), TG – Triglycerides, BCS – Body Condition Score, RBC – Red Blood Cells, WBC – White Blood Cells, GSH – Reduced Glutathione (antioxidant), SOD – Superoxide Dismutase (antioxidant enzyme), ESCR – Extended-Spectrum Cephalosporin-Resistant (E. coli), B. – Bacillus, L. – Lactobacillus, P. – Pediococcus, S. – Saccharomyces, E. – Enterococcus / Escherichia (depends on context: E. faecium, E. coli), BioPlus YC – Commercial probiotic product containing B. subtilis and B. licheniformis, EM® Bokashi – Effective Microorganisms Bokashi (fermented feed additive containing yeast and lactic acid bacteria), ***log*** *– Logarithmic unit (base 10, for microbial counts)*

Lactobacillus, *Bacillus*, and *Saccharomyces cerevisiae* were more effective than single strains in enhancing body weight, increasing average daily gain, and feed conversion ratio (Mohamed et al. 2023; Phuoc and Jamikorn 2016; Tufarelli et al. 2025). These benefits were linked to better nutrient digestibility, improved intestinal villus structure, and efficient cecal fermentation. Probiotics also protect rabbits from dietary mycotoxins and metabolic disorders. Blends of *L. acidophilus*, *L. plantarum*, and *Bifidobacterium bifidum* reduced aflatoxin toxicity while supporting growth (Mohamed et al. 2023). Similarly, *Aspergillus awamori* countered ochratoxin A toxicity by improving intestinal morphology, digestibility, and antioxidant responses (El-deep et al. 2020). *L. acidophilus* supplementation was shown to normalize liver enzymes and lipid metabolism in high-cholesterol diets (Aziz et al. 2023). Probiotics consistently enhanced immunity, increased RBC counts, lymphocyte proliferation, and phagocytic activity (Fathi et al. 2017). *Lactobacillus casei* strengthened gut immunity in suckling rabbits by increasing vermiform appendix length and TLR-9 expression (Shen et al. 2019). Probiotics improve reproduction and production efficiency economically. A commercial probiotic ZOOVIT increased fertility and litter size while reducing pre-weaning mortality (Dimova et al. 2017). Similarly, *S. cerevisiae* supplementation lowered the cost per kilogram of weight gain (Ezema and Eze 2015) and *A. awamori* supplementation at 0.15% in doe diets enhanced milk quality and kit weaning weights (Refaie et al. 2022). Probiotics also mitigated heat stress-related performance losses (Ayyat et al. 2018). Overall, probiotic supplementation enhances growth, immunity, reproduction, and economic returns in rabbit production systems. Table 4 demonstrates that probiotic supplementation consistently improved rabbit performance in controlled trials, mostly increasing growth rate, gut morphometry, immune response, and microbial balance or decreasing pathogen load as well as improving nutrient digestibility or stress tolerance. Use of multi-strain Bacillus products provided the most consistent benefits between studies. Altogether probiotics enhanced body-weight gain, feed conversion ratio, digestibility and villus height and villus: crypt ratio presenting better profile of gut structure and health.

5. Sustainability Aspects and Environmental Impact of Probiotics

Combining probiotics with livestock feeding systems is a feasible and scientific approach of enhancing agricultural sustainability, characterized by low environmental emissions, high production efficiency, and enhanced animal well-being. Probiotics serving as a substitute to AGPs decrease challenges posed by AMR without affecting animal productivity. Hence, the usage of probiotics is in line with the priorities for global antimicrobial stewardship (Leistikow et al. 2022; Mârza et al. 2025). Probiotic supplementation has been shown to lower enteric methane by 10-20% depending on strain and dose, largely through shifts in rumen fermentation pathways that enhance fibre degradation and redirect hydrogen away from methanogenesis. Nitrogen use efficiency also improves, reducing urinary and faecal nitrogen excretion by 8-15%, which in turn decreases ammonia and nitrous oxide emissions from manure. Evidence from controlled trials confirms that optimizing nutrient use can reduce ammonia volatilisation by 24-65% (Abd El-Hack et al. 2018). Recent life cycle assessment modelling further reinforces these findings in dairy cows, where the supplementation of *Saccharomyces cerevisiae* Actisaf reduced the carbon footprint of milk production by 5% during the supplementation period and by 2.9% when extrapolated annually, alongside reductions in land use (2.05%), water use (2.47%), resource use (1.67%), acidification (2.28%), and eutrophication impacts (2.14-2.28%) (Salah et al. 2024). These reductions were attained with minimal cost, as production of the probiotic contributed only 0.005-0.016% to total farm carbon emissions (Salah et al. 2024). Also, optimized feed conversion entails lower feed, land, and water use per unit of output and contributes to improved health, reduced morbidity, as well as less need for veterinary care over time (Almeida et al. 2022). Long-term profitability is also augmented by enhanced weight gain, reproduction efficiency, and adherence to more stringent antibiotic-free production requirements (Ezema and Eze 2015; Dimova et al. 2017). Taken together these findings assert that probiotic supplementation can serve as a powerful multi-purpose sustainability dietary measure to reduce environmental emissions, increase efficiency, and meet regulatory and market trends while ensuring no detrimental impact on animal performance.

6. Challenges and Limitations

Despite substantial progress, several interrelated challenges still exist as hinderances in the widespread application of probiotics in livestock production systems. Primarily, variability in strain-related efficacy generates a high level of complexity, since different species of probiotic have a range of effects on different species of livestock, which would require a tailored formulation approach for optimal outcomes (Dowarah et al. 2018). Environmental conditions such as temperature, moisture, and management practices play a critical role on the probiotic survival and colonisation success. Additionally, the regulatory standards are inconsistent across jurisdictions which hinders implementation of standardised protocols by the producers.

Table 4. Bacterial probiotic interventions: effects on rabbit performance, digestibility, immunity, and gut morphology

Breed	Age/ Stage	Probiotic strain	Study design	Key findings	References
White New Zealand	Does + weaned rabbits	“ZOOVIT” probiotic	50 does + 36 weaned rabbits, 2 groups	Increased fertility (85.7%), lower mortality, higher daily growth rate, increased live weight	(Dimova et al. 2017)
ITELV2006	Male & female, 60-day trial	*Lactobacillus rhamnosus* GG, *Bifidobacterium animalis* BB-12, *Saccharomyces boulardii*	60 days, with 30-day withdrawal	Improved body weight, blood parameters, sex-specific effects observed	(Kadja et al. 2021)
New Zealand White	Summer & winter	*Bactocell®, Bio-Mos®*, yeast	100 rabbits, 10 groups, factorial 2×5	Improved FI, blood parameters, reduced heat stress impact in summer	(Ayyat et al. 2018)
New Zealand White	25 days trial	Enterococcus spp., *E. coli* strains	Compared to antimicrobial-fed group	Probiotic group had fewer multidrug-resistant strains, restored microbiota	(Cunha et al. 2017)
New Zealand White	AFB1-exposed rabbits	*AVI-5-BAC* + ajowan	40 rabbits, 5 diet groups	Improved BW, digestibility, serum protein; detoxified AFB1 effects	(Mohamed et al. 2023)
Cross-bred rabbits	45–120 days	*Sporothermin* + girasole beet pulp	30 rabbits, 3 groups	Increased muscle mass (up to 20.15%), better meat composition and growth	(Kurchaeva et al. 2019)
New Zealand White	Weaned (28 days)	*B. subtilis*, *L. acidophilus*	64 rabbits, 4 diet groups	LA & BL groups showed improved BWG, digestibility, gut flora; BS alone less effective	(Phuoc and Jamikorn 2016)
Jabali, V-line, crossbreds	8 weeks	*B. subtilis* (200 & 400 g/t)	80 rabbits, 4 × 3 factorial	Higher carcass % with 400 g/t; improved blood & immunity; no significant BWG	(Fathi et al. 2017)
Italian White	6–12 weeks	Multi-strain Slab51 (MS-Prob)	86 rabbits, 2 groups	Higher BW, better feed efficiency, antioxidant status, reduced harmful bacteria	(Tufarelli et al. 2025)
APRI maternal line	5–13 weeks	*Aspergillus awamori*	48 rabbits, 4 groups	Counteracted OTA toxicity; improved immunity, gut morphology, antioxidants	(El-deep et al. 2020)
Crossbred	13 weeks	*Saccharomyces cerevisiae*	20 rabbits, 4 groups (0.08–0.16 g/kg)	Group B (0.12 g/kg) had highest weight gain, lowest cost/kg BWG	(Ezema and Eze 2015)

NZB / NZW – New Zealand White, BW – Body Weight, BWG – Body Weight Gain, ADG – Average Daily Gain, FI – Feed Intake, VH:CD – Villus Height to Crypt Depth Ratio, d – Days, wk. – Weeks, g/t – Grams per Ton (of feed), ADFI – Average Daily Feed Intake, MDR – Multi-Drug Resistant, AFB1 – Aflatoxin B1, RCB – Randomized Complete Block, LA – Lactobacillus acidophilus, BL – Bacillus licheniformis, BS – Bacillus subtilis, OTA – Ochratoxin A, D0–10 – Days 0 to 10 (time period of measurement)

Standardisation in production remains a challenge with respect of the number of viable cells, authenticity of the strains, and the shelf-life stability. However, the technological developments in encapsulation and delivery systems gradually conquered these restrictions to a considerable extent (Rashidinejad et al. 2022). Subsequently, cost considerations and limited farmer education represent additional barriers, though increasing regulatory support and consumer demand for antibiotic-free products continue driving adoption rates higher.

7. Future perspectives

The inherent limitations of probiotic applications in livestock feeding are being overcome with the emergence of next generation approaches, which transform the probiotic applications beyond the conventional live microbial supplementation. For instance, postbiotic formulations, in which metabolic products of probiotic fermentation, provide benefits without the viability and stability issues of live organisms (Zhao et al. 2024b). The implementation of precision livestock farming technologies such as the Internet of Things (IoT) (Kaur and Virk 2024) and real-time sensors (Halachmi et al. 2019) enables continuous monitoring of animal health parameters. This further reduces environmental variability and optimize the timing and dosage of probiotic application (Fenster et al. 2019). Additionally, the development of multi-strain synbiotic formulations containing probiotics with prebiotics show improved efficacy by synergistic effects. Advances in genetic engineering enable the design of probiotics with specific functions, such as increased resistance to pathogens and increased nutrient utilization (Parvin and Sadras 2024). Lastly, precise probiotic strategies according to the animal microbiome profile should be adopted and probiotic selection and optimisation using artificial intelligence and machine learning applications can enable more advanced and effective livestock health management systems (D'Urso et al. 2024; Asar et al. 2025).

8. Conclusions

Based on the scientific evidences, probiotics represent a viable alternative to AGPs with a significant impact on ruminants, poultry, swine, and rabbit. They enhance immunity, facilitate nutrient utilisation, growth performance, disease resistance, reproductive efficiency, and maintains gut integrity, thereby contributing to the solution of antimicrobial resistance. While there are concerns about strain-specificity and standardisation, recent developments such as postbiotics and precision farming technologies offer new hope for progress. This shift towards biologically inspired interventions supports sustainable livestock production by promoting animal welfare, food safety, economic viability, and environmental stewardship. Therefore, probiotics are considered key components to future-proof agricultural production practices.

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