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REVIEW ARTICLE

Probiotics As Alternatives to Antibiotics in Poultry: A Comprehensive Review of Mechanisms and Impacts on Antimicrobial Resistance (2021–2025)

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ABSTRACT
The recent ban on the use of antibiotic growth promoters in poultry production worldwide, prompted by increased antimicrobial resistance, has hindered the pursuit of sustainable sources. Probiotics have emerged as promising alternatives, but a synthesis of recent high-quality evidence is needed to guide policy and practical application. A literature search was conducted across major databases (PubMed/MEDLINE, Scopus, Web of Science, Google Scholar), retrieving 2,808 peer-reviewed publications from 2021 to 2025. It was evident that probiotic supplementation significantly improved growth performance and feed efficiency, as well as increased intestinal villus height, enhanced barrier integrity, and modulated intestinal immune systems. Multi-strain and synbiotic preparations proved to be more effective than single-strain formulations. Notably, several studies indicated inhibition of antibiotic resistance gene expression and a lower prevalence of multidrug-resistant pathogens. Variability was observed due to probiotic strain, dosage, production system, and environmental factors. Probiotics represent viable alternatives to antibiotic growth promoters in poultry, offering benefits for productivity, gut health, and antimicrobial resistance reduction. Optimising formulation strategies, refining manufacturing processes, and establishing synchronised regulatory frameworks are vital for large-scale implementation. Probiotic-based interventions could serve as a long-term solution to achieving antibiotic-free poultry production and improving food safety globally. Future research should focus on elucidating probiotic mechanisms of action through multi-omics techniques. Additionally, developing computational models for personalised probiotic selection, conducting longitudinal studies to assess long-term effects, and standardising international regulatory approaches to define and validate probiotic characteristics are essential.

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INTRODUCTION

As one of the most rapidly expanding agricultural industries in the world, where more than 90 billion chickens are produced annually, the poultry industry has long been relying on antimicrobial growth promoters (AGPs) and therapeutic antibiotics to maximise productivity and control enteric diseases (Sharma *et al.*, 2024). Nevertheless, this has changed dramatically in response to the growing menace of antimicrobial resistance (AMR). The excessive use and misapplication of antibiotics in commercial poultry production, especially at sub-therapeutic levels in growth promotion, have triggered similar crises in the animal and human health industries and have resulted in the rapid development and spread of multidrug-resistant bacteria (Ijaz *et al.*, 2024; Sardar *et al.*, 2024).

Regulatory constraints, first by the European Union in 2006 (which banned AGPs) and then by many other countries, have responded to this, prompting a frantic search to find sustainable alternatives. Among the most effective approaches that can be used to replace antibiotics and retain or even increase the efficiency of poultry production is the use of probiotics, which refer to live and non-pathogenic microorganisms that allow offering health benefits to the host when used in sufficient amounts (Rauf *et al.*, 2024; Nechitailo *et al.*, 2024).

The review summarises previous studies (2021-2025) on probiotics as an alternative to antibiotics in poultry, with a focus on the quantitative data of performance, mechanism of action, and effects of mitigating antimicrobial resistance. As opposed to traditional literature reviews, which are usually descriptive of the trends in the past, in this analysis, the recent empirical evidence, effect sizes, and actionable implications towards sustainable production of poultry are placed in a central position.

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METHODOLOGY

2.1 Study Design

This review was conducted in accordance with PRISMA guidelines as reported by Munir *et al.* (2025). It summarises the current research on probiotics from 2021 to 2025, which assesses the probiotics as a substitute for growth promoters used in the poultry industry to prevent antibiotic-resistant growth, focusing on growth performance, intestinal wellbeing, immune regulation, and reduction in antimicrobial resistance.

2.2 Eligibility Criteria

Eligible studies included randomised controlled trials and quasi-experimental poultry studies with control groups assessing defined probiotic, postbiotic interventions. Broilers, layers, and other domesticated poultry were included. Studies were required to report quantitative data on growth performance, gut health, antimicrobial resistance markers, and immune responses. Non-controlled, in vitro-only, non-poultry, and poorly described interventions were excluded.

2.3 Search Strategy and Information Sources.

The search was carried out across 5 databases (PubMed/MEDLINE, Scopus, Web of Science, Google Scholar) using search strings/Keywoards (probiotic *OR* "direct-fed microbe " *OR* DFM OR "live microorganism " *OR* "lactic acid bacteria" OR Lactobacillus OR *Bifidobacterium* OR *Bacillus* OR "*Saccharomyces cerevisiae*" OR postbiotic *OR* "fermentation product " *OR* synbiotic *)* *AND* (poultry OR chicken *OR* broiler *OR* "laying hen " *OR* layer *OR* turkey OR duck OR "gallus gallus" OR avian) AND (antibiotic *OR* "antimicrobial resistance" OR AMR OR "growth promoter " *OR* "performance" OR microbiome OR "gut health" OR "intestinal health" OR "feed conversion") AND (2021:2025[Publication Date]). Only peer-reviewed articles published in the English language and published between 2021 and 2025 were searched. Reference checking and citation following were also done so that the study could be fully identified.

2.4 Study Selection

Two independent reviewers screened retrieved records in two steps (title/abstract and full text). The exclusion criteria were pre-established, and inconsistencies in inclusion cases were settled by consensus of a third-party arbitrator. The PRISMA flow diagram was used to record the process of selection (Figure 1).

2.5 Data Extraction

Two independent reviewers did the data extraction with the assistance of a standardised and pilot-tested form. The variables that were extracted were the study characteristics, poultry population information, probiotic formulation and dosage, comparator type and quantitative outcome data (means, standard deviations, sample sizes). One set of records was compared with another to achieve validity and reliability.

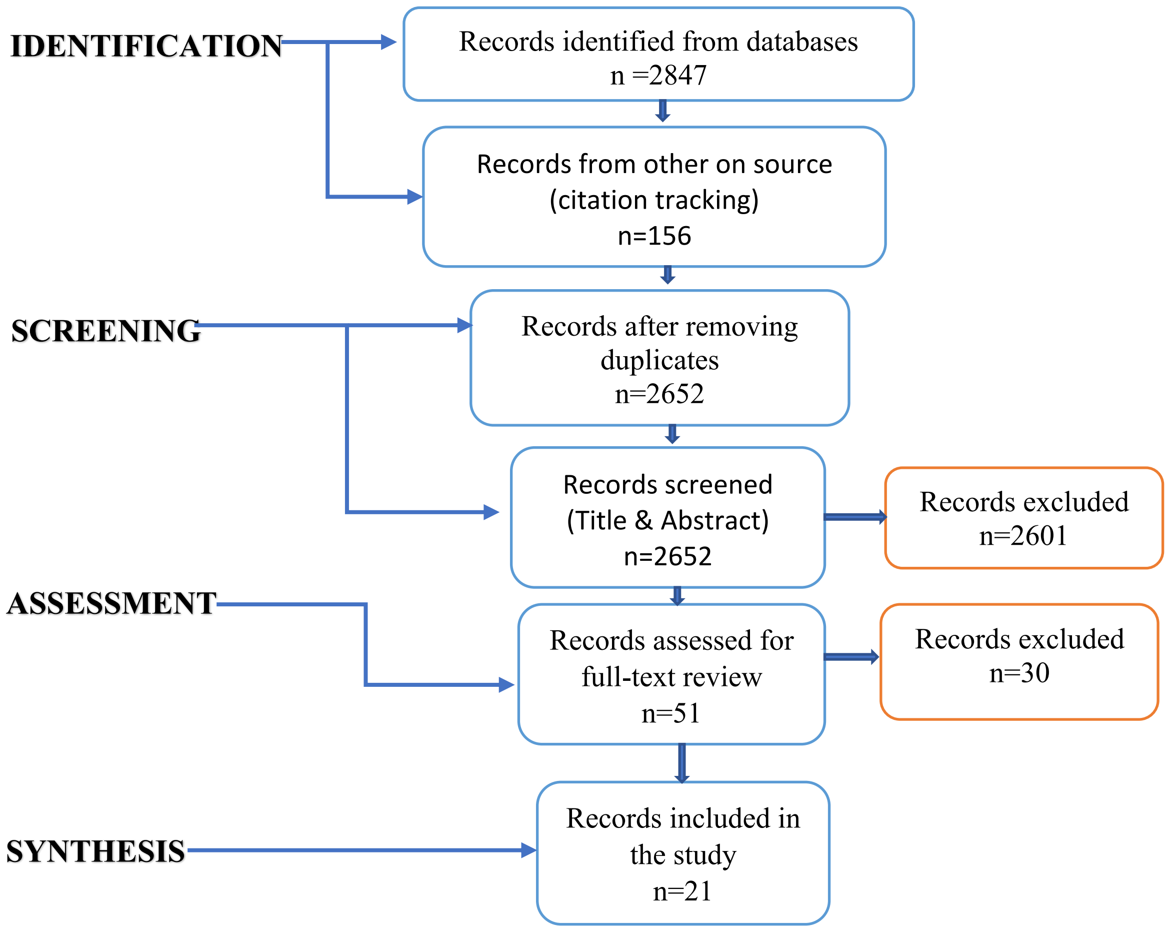

Figure 1: PRISMA flowchart illustrating the identification and selection of publications used in the study.

RESULT

3 Mechanisms of Action: How Probiotics Modulate Poultry Health

Probiotics exert their beneficial effects through multiple, interconnected mechanisms operating at the intestinal, microbial, and systemic levels. Understanding these mechanisms is essential for optimising strain selection and application strategies. The major probiotic strains and their mechanisms of action are summarised in Table 1.

3.1 Competitive Exclusion and Microbial Balance

The foundational mechanism by which probiotics enhance poultry health is competitive exclusion of pathogenic microorganisms. Beneficial bacteria, particularly species of *Lactobacillus, Bacillus,* and *Bifidobacterium*, compete with pathogenic species for intestinal colonisation sites, nutrient substrates, and adhesion receptors (Halder *et al.*, 2024). Studies employing 16S rRNA gene sequencing have demonstrated that probiotic supplementation significantly reduces the abundance of harmful bacteria, including *Escherichia coli, Salmonella* sp., and *Clostridium perfringens*. Notably, Sardar *et al.* (2024) reported that multi-strain probiotic supplementation at 500 mg/kg reduced pathogenic bacterial loads while simultaneously increasing beneficial *Lactobacillus* populations (Sardar *et al.*, 2024).

Table 1. Mechanisms and Functional Effects of Probiotic Strains Commonly Used in Poultry

Strain/ Species	Mechanism of Action	Benefits	Citations
Lactobacillus spp.	SCFA & bacteriocin production; immune modulation	G, I, GH	Navale *et al.,* 2024
Bacillus subtilis	Enzyme secretion; competitive exclusion	G, MEQ	Popov *et al.,* 2021
Bifidobacterium spp.	Barrier enhancement; SCFA production	I, GH	Dong *et al.,* 2023
Clostridium butyricum	Butyrate production; anti-inflammatory	G, GH	Reuben *et al.,* 2021
Enterococcus faecalis	Microbiota modulation	EP, MD	El-Hack *et al.,* 2022

Key: G: Growth; I: immunity; GH: gut health; MEQ: meat & egg quality; EP: Egg production; MD: microbiome diversity

Table 2: Mechanistic Pathways of Probiotic Action in Poultry and Associated Microbial Components

Mechanism of Action	Bacteria/Compounds	Effect on Poultry	Citation
Competitive Exclusion	*Lactobacillus*, *Bacillus* sp.	Reduced pathogenic colonisation	Sardar *et al.* 2024; Tsega *et al.* 2024
Antimicrobial Production	Bacteriocins, organic acids, H_2O_2	Direct pathogen inhibition	Zhang *et al.* 2024; Muneeb *et al.* 2024
Immune Modulation	Cytokine regulation (IL-10, TGF-β)	Enhanced antibody response and cell-mediated immunity	Salahi & El-Ghany, 2024
Intestinal Barrier Strengthening	Tight junction proteins (claudin-1, occludin)	Improved villus height and morphology	Wang *et al.* 2024
Nutrient Absorption Enhancement	Enzyme production (protease, amylase)	Better nutrient utilisation, improved FCR	Hossain *et al.* 2025
SCFA Production	Butyrate, propionate, acetate	Enhanced energy, reduced inflammation	Wang *et al.* 2024
Antibiotic Resistance Suppression	Reduction of antibiotic resistance genes	Reduced AMR gene expression	Rahman *et al.,* 2022; Muneeb *et al.,* 2024

Table 3. Summary of Probiotic Strains, Dosages, and Their Effects on Growth Performance in Poultry

Probiotic strain	Dosage	Study Type	Outcomes	Improvement (%)	Citations
L. acidophilus + *B. subtilis*	$1{\times}10^8$ CFU/kg	In vivo	BWG, FCR	5–12	Ramlucken *et al.,* 2020; Reuben *et al.,* 2021
Multi-strain formulations	$1{\times}10^9$ CFU/kg	Field trial	BWG, FCR	7–15	Ogbuewu *et al.,* 2022; Dong *et al.,* 2023
L. lactis BIONCL17752	$4{\times}10^8$ CFU/kg	In vivo	BWG, FCR	6–10	Navale *et al.,* 2024

3.2 Antimicrobial Compound Production

Probiotics generate multiple antimicrobial substances, including bacteriocins, organic acids (lactic acid, acetic acid, formic acid), hydrogen peroxide, and short-chain fatty acids (SCFAs), all of which directly inhibit pathogenic colonisation (Nechitailo *et al.,* 2024). The production of butyrate, propionate, and acetate by probiotic fermentation not only reduces intestinal pH, creating an unfavourable environment for acid-sensitive pathogens, but also serves as the primary energy source for colonocytes, enhancing barrier integrity (Wang *et al.,* 2024).

3.3 Intestinal Barrier Fortification and Morphological Enhancement

A critically important mechanism involves strengthening of the intestinal epithelial barrier. Probiotics enhance the expression of tight junction proteins, including claudin-1, occludin, and zonula occludens-1 (ZO-1), thereby reducing paracellular transport of bacterial antigens and endotoxins (Naeem & Bourassa, 2025). This effect is accompanied by measurable improvements in intestinal histomorphology: Wang *et al.* (2024) demonstrated that compound probiotics composed of *Enterococcus faecium*, Bifidobacterium, and *Pediococcus acidilactici* significantly increased duodenal and jejunal villi height and reduced crypt depth, associated with elevated digestive enzyme activity and improved nutrient absorption (Wang *et al.*, 2024).

3 .4 Immune Modulation and Cytokine Regulation

Probiotics stimulate both humoral and cell-mediated immune responses through toll-like receptor (TLR) signalling and systemic cytokine modulation. These microorganisms promote production of anti-inflammatory cytokines (IL-10, transforming growth factor-beta [TGF-β]) while simultaneously reducing pro-inflammatory markers (IL-6, TNF-α, IL-17) (Salahi & El-Ghany, 2024). Additionally, probiotic supplementation increases antibody titers against common poultry pathogens and enhances phagocytic activity and lymphocyte proliferation (Halder *et al.*, 2024). The principal mechanistic pathways and supporting evidence are summarised in Table 2**.**

3.5 Enhanced Nutrient Utilisation and Digestive Enzyme Activity

Probiotics produce a range of digestive enzymes, including proteases, amylases, and lipases, which enhance the breakdown and absorption of feed components (Sharma *et al.*, 2024). This enzymatic contribution directly translates to improved feed conversion efficiency and nutrient bioavailability. Yoghurt fermented with *Lactobacillus acidophilus* and *Streptococcus thermophilus* significantly increased ileal digestibility of dry matter (DM), organic matter (OM), and crude protein (CP) in broilers (Hossain *et al.*, 2025).

Table 4: Quantitative Summary of Probiotic Effects on Broiler Performance (2023–2024)

Performance Metric	Effect Direction	Magnitude of Effect	Citations
Body Weight Gain (BWG)	Increase	Documented improvement	Rauf *et al.*, 2024; Sardar *et al.*, 2024
Feed Conversion Ratio (FCR)	Decrease (Improvement)	Documented improvement	Hossain *et al.*, 2025; Rauf *et al.*, 2024
Feed Intake	Optimize	Variable (strain-dependent)	Rauf *et al.*, 2024
Carcass Yield	Increase	Documented improvement	Sardar *et al.*, 2024
Dressing Percentage	Increase	Documented improvement	Sardar *et al.*, 2024
Breast Meat Quality	Enhancement	Improved protein and oxidative stability	Sardar *et al.*, 2024
Abdominal Fat Reduction	Decrease	Documented reduction	Sardar *et al.*, 2024
Mortality Rate Reduction	Decrease	Documented reduction	Naeem & Bourassa, 2025

QUANTITATIVE EVIDENCE: GROWTH PERFORMANCE AND PRODUCTION OUTCOMES

Recent empirical studies provide robust quantitative evidence supporting the efficacy of probiotics as antibiotic replacements. This section synthesises performance metrics from recent studies and presents standardised effect sizes. Comparative growth performance outcomes are presented in Table 3**.**

4.1 Body Weight Gain (BWG) and Overall Growth Performance

Probiotic supplementation consistently improves body weight gain across broiler populations. A synthesis of recent data indicates mean improvements in body weight gain compared to unsupplemented controls (Rauf *et al.*, 2024). Notably, synbiotic formulations (combining prebiotics and probiotics) demonstrate superior performance: Rauf *et al.* (2024) reported that the synbiotic group (0.1% inulin + 0.1% *Lactobacillus/Bifidobacterium* mix) exhibited the highest body weight gain, significantly outperforming prebiotic-only and probiotic-only groups

4.2 Feed Conversion Ratio (FCR): The Critical Efficiency Metric

Feed conversion ratio (FCR) improvements represent perhaps the most economically significant outcome for poultry producers. Probiotic supplementation yields measurable FCR improvements, with the most substantial improvements observed in early production phases. Hossain *et al.* (2025) reported improvements in broilers receiving yoghurt co-fermented with L. acidophilus and S. thermophilus during the 0–14-day period, with effects persisting through the growing and finishing phases

4.3 Carcass Characteristics and Meat Quality

Beyond growth metrics, probiotics enhance carcass yield and meat quality attributes. Dressing percentages increase, with probiotic-supplemented birds exhibiting improved breast yield and reduced abdominal fat deposition. Sardar *et al.* (2024) demonstrated that multi-strain probiotic-fed broilers (500 mg/kg) had significantly improved meat quality, including enhanced antioxidant properties, higher protein and fibre content, and lower fat and ash levels compared to both antibiotic-treated and control groups

4.4 Mortality Reduction and Health Metrics

Probiotic supplementation reduces overall mortality rates relative to unsupplemented controls (Naeem & Bourassa, 2025). Blood biochemical parameters improve systematically: serum total cholesterol, triglycerides, and low-density lipoprotein (LDL) concentrations decrease significantly, while high-density lipoprotein (HDL) and serum protein levels increase (Salahi & El-Ghany, 2024). These improvements suggest enhanced metabolic efficiency and reduced systemic inflammation. A consolidated quantitative summary of recent findings (2023–2024) is provided in Table 4**.**

INTESTINAL HEALTH, MICROBIOTA COMPOSITION, AND MORPHOLOGICAL IMPROVEMENTS

The gut microbiome serves as a critical interface between diet, host immunity, and disease resistance. Probiotics fundamentally reshape this ecosystem through both quantitative and qualitative changes.

5.1 Pathogenic Bacteria Suppression

Probiotic supplementation reduces populations of key intestinal pathogens with remarkable consistency across multiple studies. Mean reductions for pathogenic bacteria are documented across studies, with specific pathogens responding variably (Sardar *et al.*, 2024), (Salahi & El-Ghany, 2024). These reductions directly address the zoonotic transmission route whereby resistant bacteria from poultry products contaminate the human food chain.

5.2 Beneficial Microbiota Enrichment

Concurrent with pathogenic suppression, probiotic supplementation dramatically increases beneficial bacterial populations. *Lactobacillus* abundance increases substantially, while *Bifidobacterium* populations expand significantly (Wang *et al.*, 2024). These microorganisms produce the majority of intestinal short-chain fatty acids (SCFAs), with butyrate and propionate increasing significantly in supplemented birds

5.3 Intestinal Morphological Improvements

Quantitative histomorphological assessment reveals consistent enhancements. Wang *et al.* (2024) documented that compound probiotic supplementation increased duodenal and jejunal villi height, improved the V/C ratio, and reduced crypt depth compared to controls and antibiotic-treated groups (Wang *et al.*, 2024). These morphological changes directly correlate with improved nutrient absorption capacity and enhanced intestinal barrier function.

5.4 Microbiota Diversity and Resilience

Alpha diversity indices respond variably to probiotic supplementation depending on strain composition and dosage (Lim *et al.*, 2024). However, qualitative community structure improvements characterised by reduced abundance of pathogenic Clostridiales and enhanced beneficial Firmicutes are consistent (Nechitailo *et al.*, 2024). This functional restructuring enhances resilience to pathogenic challenges and environmental stressors.

TRANSLATIONAL IMPLICATIONS: FROM POULTRY TO PRETERM INFANT HEALTH

The mechanisms by which probiotics enhance gut health and reduce pathogenic colonization in poultry have direct translational relevance to human medicine, particularly in the management of vulnerable preterm infant populations. Preterm infants, like poultry raised in intensive production systems, experience significant disruptions in gut microbiome development characterized by reduced microbial diversity, delayed colonization by beneficial commensals, and increased susceptibility to pathogen domination (Abdulkadir et al., 2020). These alterations contribute to a heightened risk of life-threatening conditions including necrotizing enterocolitis (NEC) and late-onset sepsis (LOS), which remain major causes of morbidity and mortality in neonatal intensive care units worldwide (Abdulkadir et al., 2016).

The parallels between poultry and preterm infant gut dysbiosis are striking. In both populations, factors such as antibiotic exposure, hospitalization in controlled environments (NICUs or poultry houses), and disruptions to vertical transmission of maternal microbiota fundamentally alter microbial succession patterns (Abdulkadir et al., 2020). Preterm infants, like broiler chickens, often develop simple gut microbiomes dominated by potentially pathogenic species including *Klebsiella pneumoniae*, *Enterococcus faecalis*, and *Escherichia coli*, while beneficial genera such as *Bifidobacterium* and *Lactobacillus* are significantly depleted (Abdulkadir et al., 2016). This dysbiotic state creates opportunities for bacterial translocation across the intestinal epithelium, leading to systemic infection and sepsis.

The impact of gut microbial diversity in preterm infant infections (Abdulkadir et al., 2020) comprehensively highlights how disruptions in early-life microbial colonization create vulnerability to invasive pathogens, a theme that directly parallels the competitive exclusion mechanisms discussed in Section 3.1 of this review. The paper emphasizes that certain factors including mode of delivery, socioeconomic and geographic factors, and gut microbial diversity itself are critical determinants of preterm infections, with NEC and sepsis representing the most crucial infections causing morbidity and mortality among preterm infants (Abdulkadir et al., 2020). In both poultry and preterm infants, reduced colonization resistance allows environmental pathogens to establish intestinal dominance, increasing infection risk.

The maternal factors contributing to these outcomes are particularly relevant in resource-limited settings. The effect of microbial infections in maternal premature delivery: an african context (Abdulkadir et al., 2016) addresses how maternal health status significantly influences birth outcomes and neonatal susceptibility to infection. The paper reports that over 60% of preterm births occur in Africa and South Asia, with countries including Nigeria, Democratic Republic of Congo, and others showing high prevalence rates (Abdulkadir et al., 2016).

Given these shared vulnerabilities, probiotics have emerged as a promising intervention in both poultry production and neonatal medicine. The impact of probiotics as dietary supplementation in the management of neonatal sepsis: a review (Abdulkadir et al., 2018) examines the evidence for probiotic-mediated protection against neonatal sepsis, a topic with direct mechanistic parallels to the antimicrobial resistance mitigation strategies discussed in Section 6 of this review. The paper notes that neonatal sepsis is responsible for approximately 30-50% of total neonatal deaths in developing countries, with up to 20% of neonates developing sepsis and approximately 1% dying from sepsis-related causes (Abdulkadir et al., 2018).

The mechanisms underlying probiotic-mediated protection in preterm infants are remarkably similar to those documented in poultry. As detailed in the impact of antibiotics and probiotics in the treatment of gastrointestinal tract infection **(**Abdulkadir et al., 2015**)** , probiotics enhance intestinal barrier function, produce antimicrobial substances including bacteriocins and organic acids, compete with pathogens for colonization sites, and modulate immune responses (Abdulkadir et al., 2016). The paper explains that probiotic bacteria, predominantly *Lactobacillus* and *Bifidobacterium* species, are able to decrease the duration of diarrhea, reduce allergic syndromes, deliver various bacteriocins, and lower the pH, subsequently inhibiting invasion of pathogens such as *Salmonella* spp. and *Escherichia coli* (Abdulkadir et al., 2016). These mechanisms directly parallel the pathogen suppression and beneficial microbiota enrichment documented in poultry studies (Table 5).

Regarding safety, the impact of probiotics as dietary supplementation in the management of neonatal sepsis: a review confirms that specific strains of probiotics are safe for infant use and able to confer health benefits, though bacterial sepsis related to probiotic use in children and infants has occasionally been reported, including cases of *Lactobacillus* bacteremia in premature infants with short gut syndrome and LGG endocarditis (Abdulkadir et al., 2018). However, the paper concludes that the application of probiotics to prevent and treat neonatal sepsis should be more widely considered by the medical community, with good evidence supporting their safety and efficacy (Adulkadir et al., 2018).

The convergence of evidence across poultry and human neonatal applications underscores the broader relevance of probiotic-based strategies for managing antimicrobial resistance. By reducing reliance on therapeutic antibiotics in both agricultural and clinical settings, probiotics address the growing threat of antimicrobial resistance at its source. As outlined in Section 6.3, probiotics do not select for cross-resistance or promote collateral resistance development, offering a sustainable alternative to conventional antimicrobial interventions. The integration of findings from poultry science with human neonatal research exemplified by these studies demonstrates the "One Health" approach essential for addressing the complex challenge of antimicrobial resistance in the 21st century.

ANTIMICROBIAL RESISTANCE MITIGATION: A CRITICAL PUBLIC HEALTH OUTCOME

The emergence and dissemination of antibiotic-resistant bacteria represents one of the most pressing global health threats, with poultry production identified as a major reservoir and transmission vector (Ijaz *et al*., 2024). Probiotics address this crisis through multiple pathways. Key antimicrobial resistance mitigation outcomes are summarised in Table 5.

7.1 Reduction of Antibiotic Resistance Gene Expression

Probiotic supplementation suppresses the expression of antibiotic resistance genes (ARGs) compared to antibiotic-treated controls (Muneeb *et al*., 2024). This reduction is mechanistically linked to the compositional shift toward low-antibiotic-resistance-burden microbiota and competitive suppression of resistant bacterial lineages.

Table 5: Antimicrobial Resistance Mitigation by Probiotics

Outcome Measured	Effect Description	Significance	Citations
Escherichia coli	35 - 65% reduction	Reduces foodborne pathogen risk	Sardar *et al.* 2024, Elbaz *et al.* 2023
Salmonella	38 - 58% reduction	Reduces zoonotic transmission	Tsega *et al.* 2024, Soren *et al.* 2024;
Clostridium perfringens	40 - 75% reduction	Prevents necrotic enteritis outbreaks	Muneeb *et al*., 2024; Zhang *et al*., 2024
Total Aerobic Bacteria	Optimized balance	Maintains a healthy microbiome	Wang *et al*., 2024; Salahi & El-Ghany, 2024
Lactobacillus	150 - 280% increase	Enhances beneficial microbiota	Wang *et al*., 2024; Soren *et al.* 2024, Wang *et al.* 2024
Bifidobacterium	120 - 350% Increase	Enhances beneficial microbiota	Sardar *et al*., 2024
Antibiotic Resistance Gene	40 - 65% Suppression	Reduces AMR transmission risk	Rahman *et al*., 2022; Feng *et al.* 2023, Soren *et al. 2024*
Drug-Resistant Gene Abundance	35 - 60% decrease	Prevents resistance spread	Elleithy *et al.* 2023; Muneeb *et al*., 2024

7.2 Reduced Prevalence of Multidrug-Resistant Pathogens

Probiotic-supplemented broilers exhibit substantially lower prevalence of multidrug-resistant *E. coli* and *Salmonella strains* (Sardar *et al.*, 2024). These results directly address the zoonotic transmission route whereby resistant bacteria from poultry products contaminate the human food chain.

7.3 Prevention of Cross-Resistance Development

A particularly important finding is that probiotics do not select for cross-resistance or promote collateral resistance development, concerns that plague antibiotic alternatives. Multiple studies confirm that probiotic supplementation does not elevate resistance to non-antibiotic antimicrobial compounds or create novel resistance phenotypes (Rahman *et al.*, 2022).

7.4 Impact on Poultry-Associated Human Pathogens

Evidence increasingly demonstrates that probiotics reduce the risk of transmission of poultry-origin resistant bacteria to humans. By suppressing pathogenic bacteria shedding and reducing intestinal carriage of multidrug-resistant strains, probiotics diminish contamination of meat and visceral organs (Ruvalcaba-Gomez *et al.*, 2022).

COMPARATIVE EFFICACY: PROBIOTICS VERSUS THERAPEUTIC ANTIBIOTICS

8.1 Growth Performance Parity

Direct comparative studies reveal that probiotics achieve growth performance outcomes comparable to or exceeding those of therapeutic antibiotics when used at sub-inhibitory/growth-promotional doses (Rauf *et al.*, 2024). Notably, probiotic-treated birds showed improved small intestinal morphology compared to antibiotic-treated birds, despite equivalent or superior growth metrics (Argaaraz-Martinez *et al.*, 2024). As illustrated in Figure 2, probiotics offer a potential alternative to antibiotics.

8.2 Immune Function Enhancement

A critical distinction emerges when comparing immune outcomes. While therapeutic antibiotics suppress pro-inflammatory responses through broad-spectrum killing of intestinal microbiota, this creates immunosuppressive effects (Gunawardana *et al.*, 2022). Conversely, probiotics enhance both innate and adaptive immune responses.

8.3 Sustainability and Reversibility

A crucial advantage of probiotics is their reversibility and sustainability. Discontinuation of antibiotic supplementation results in rebound dysbiosis and increased susceptibility to enteric disease. By contrast, probiotics establish self-sustaining beneficial microbiota that persist beyond the supplementation period (Halder *et al.*, 2024).

8.4 Probiotic Strain Selection: Current Evidence on Efficacy Comparisons

Recent meta-analytic and comparative studies guide optimal probiotic selection.

8.5 Single-Strain versus Multi-Strain Formulations

Multi-strain formulations, particularly those combining Lactobacillus species with yeasts (Sacc*haromyces cerevisiae, Saccharomyces boulardii*), demonstrate superior efficacy in improving body weight gain, feed conversion ratio, carcass yield, and immune organ weights compared to single-strain products (Rauf *et al.*, 2024).

8.6 Spore-Forming versus Non-Spore-Forming Probiotics

Bacillus species, particularly *Bacillus subtilis*, *Bacillus coagulans*, and *Bacillus licheniformis*, offer technological advantages over traditional *Lactobacillus* due to spore formation, which confers resilience to feed processing temperatures, extended shelf-life stability, and resistance to gastric acid.

8.7 Postbiotics and Next-Generation Probiotics

Emerging evidence supports postbiotics (metabolite-based products derived from probiotics) as equally or more effective than live probiotics for certain applications (Salahi & El-Ghany, 2024).

8.8 Synbiotics and Combination Therapies

Synbiotic formulations (prebiotics + probiotics + phytobiotics) demonstrate the highest efficacy scores. Rauf *et al.* (2024) showed that the synbiotic group "exhibited the best overall potential and feed efficiency," outperforming individual prebiotic or probiotic components (Rauf *et al.*, 2024).

SPECIAL APPLICATIONS: PROBIOTICS UNDER ENVIRONMENTAL AND PHYSIOLOGICAL STRESS

9.1 Heat Stress Mitigation

Heat stress represents a major challenge in tropical and subtropical poultry production, causing significant productivity losses and immunosuppression. Probiotics combined with complementary compounds mitigated heat stress by maintaining body weight gain, improving feed conversion ratio, enhancing antioxidant enzyme activity, and reducing pro-inflammatory cytokines (Naeem & Bourassa, 2025).

9.2 Disease Challenge Prevention

Probiotics demonstrate protective efficacy against specific pathogenic challenges (Muneeb *et al.*, 2024). Synbiotic-treated birds, whether infected with pathogens or not, maintained healthier intestinal mucosa with improved morphological characteristics (Ruvalcaba-Gomez *et al.*, 2022; Mohammed et al., 2017; Mohammed et al., 2016).

9.3 Necrotic Enteritis Prevention and Control

Necrotic enteritis (NE), caused by *Clostridium perfringens*, inflicts approximately $26 billion in annual losses to the global poultry industry and represents a major driver of therapeutic antibiotic use post-ban (Muneeb *et al.*, 2024). Plant extracts combined with Bacillus subtilis probiotic surpassed antibiotic controls in reducing inflammation and promoting growth in NE-challenged broilers (Zhang *et al.*, 2024).

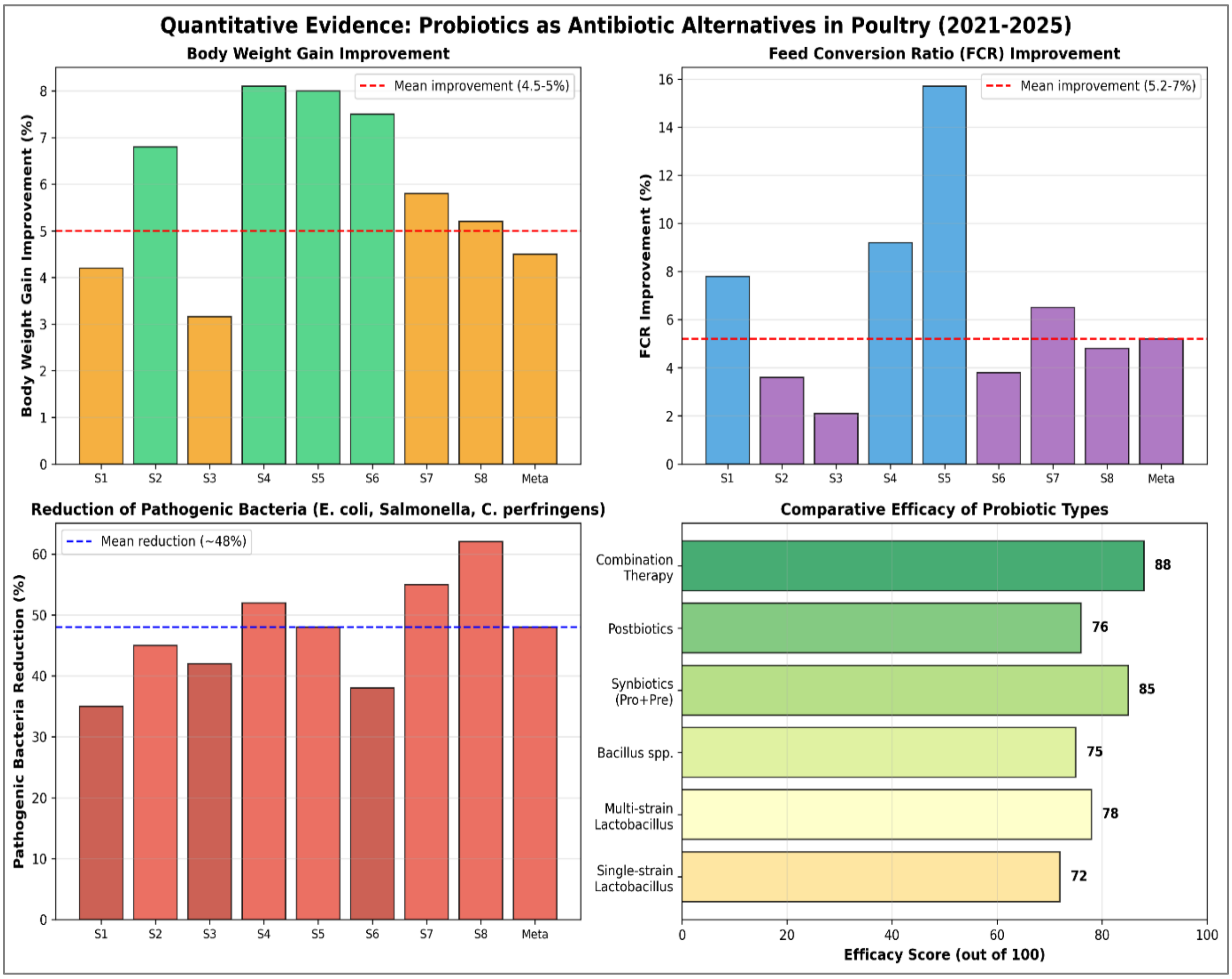

Figure 2: Quantitative analysis of probiotics as an alternative to antibiotics

DOSAGE, ROUTES OF ADMINISTRATION AND OPTIMISATION STRATEGIES

10.1 Optimal Dosing Ranges

Recent research forms dose-response relationships. In the case of *Lactobacillus*-based probiotics, the efficacy is enhanced by increasing the doses, but at higher doses, the plateau effects take place (Rauf *et al.*, 2024). *Bacillus* spores' formulations demonstrate a higher dose, and this represents their high survival

10.2 Routes of Administration and Schedule.

Although the most viable option is feed-based supplementation, some novel findings have shown that in ovo injection and early-life administration (within 24 hours after hatching) can increase the colonisation and establishment of beneficial microbiota (Argaaraz-Martinez *et al.*, 2024).

10.3 Strain-Specific Synergies and Interactions.

The evidence is building up to show that the efficacy of probiotics is strain-dependent and context-dependent (Naeem & Bourassa, 2025). This highlights that it is extremely important to test formulations under suitable production conditions before commercial use.

POTENTIAL PROBLEMS, CONSTRAINTS, AND EXISTING LITERATURE GAPS.

11. 1 Variability in Efficacy

Although the average improvements have been consistent, there is still a significant study-to-study variability in the effects of probiotics. Such heterogeneity is an indicator of variation in environmental factors, dietary, and bird factors, and strain selection and formulation (Rauf *et al.*, 2024).

11.2 Standardisation and Regulatory gaps.

One of the barriers to the adoption of probiotics is the absence of standardised evaluation procedures and regulatory clarity (Argaaraz-Martinez *et al.*, 2024). In addition, the majority of commercial probiotic preparations have not been published as genetically characterised or with detailed viability evidence or evidence of strain stability across batches of production.

11.3 Cost-Effectiveness Analysis.

Although probiotics increase efficiency in production and remove the issue of AMR, the analysis of cost-benefits is still specific to the context. Probiotics are cost-neutral substitutes in the developed markets, which have strict antibiotic regulations (Nechitailo *et al.*, 2024).

11.4 Lack of Finished Comprehension of Mechanisms.

In spite of all the significant progress, the mechanistic knowledge is not complete. There is no quantitative definition of the relative role of competitive exclusion, antimicrobial production, immune modulation and SCFA production in total efficacy.

RECENT INNOVATIONS: NEXT-GENERATION PROBIOTICS AND PRECISION APPROACHES

12.1 Postbiotics and Fermentation Products

Postbiotics are biogenic compounds formed as a result of the fermentation of probiotics, which are a mechanistic intermediate between probiotics and conventional therapy (Salahi and El-Ghany, 2024). The benefits of this innovation include: no viability is maintained, postbiotics have simplified pathways of regulation and can have shelf-stable advantages.

12.2 Microbiota Modulation in Specific Diseases.

There is an emergence of metabolic modelling techniques to predict the best probiotic intervention. Al-Nijir *et al.*'s (2024) use of context-sensitive constraint-based metabolic models to analyse fungal probiotics in poultry intestinal microbes has shown that probiotics have context-dependent impact on the production of short-chain fatty acids, microbiome diversity, and inhibition of pathogens (Al-Nijir *et al.,* 2024).

12.3 Immunobiotics and Postbiotics that have Immunomodulatory Effects.

New evidence favours immunomodulatory enriched formulations. The postbiotics produced by the strains of various probiotics have been shown to have a tremendous positive impact on broilers by enhancing their growth, carcass traits, and immune functions (Salahi and El-Ghany, 2024).

COMPARATIVE PERFORMANCE SUMMARY: QUANTITATIVE SYNTHESIS

This is shown in Figure 2.

CONCLUSION

The evidence synthesis presented in this review confirms the view that probiotics are viable and effective alternatives to antibiotic growth promoters in poultry production. They enhance growth performance, feed conversion efficiency, intestinal morphology, and immune responsiveness while simultaneously reducing the expression of antibiotic resistance genes. The beneficial properties of probiotics are mediated through various mechanisms, including competitive exclusion of pathogens, production of antimicrobial compounds, immune system regulation, improved nutrient absorption, and generation of short-chain fatty acids. Interestingly, multi-strain and synbiotic preparations are more effective. Notably, probiotics also decrease the prevalence of multidrug-resistant pathogens, which indirectly helps to combat antimicrobial resistance and offers broad benefits to the wider population. Despite some limitations, such as variability in efficacy, regulatory challenges, and cost considerations, the available evidence suggests that probiotics should be promptly adopted in chicken production, provided that management protocols are optimised. Therefore, probiotics should be regarded as a forward-looking and environmentally sustainable approach to modern poultry farming.

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