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Probiotics in Poultry Feed: Mechanisms, Applications, and Implications for Antibiotic-Free Production

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The global poultry industry has long relied on antibiotic growth promoters (AGPs) to sustain bird health and optimise production efficiency. However, growing evidence linking AGP use to antimicrobial resistance and the tightening of regulatory frameworks has driven demand for safe, efficacious alternatives. Probiotics, defined as live microorganisms that confer health benefits on the host when administered in adequate quantities, have emerged as among the most scientifically credible candidates. This review synthesises current knowledge on the mechanisms by which probiotics exert their effects in the gastrointestinal tract (GIT) of poultry, including competitive exclusion of pathogens, modulation of intestinal morphology, organic acid production, and immune system stimulation. Evidence from experimental studies is presented across key topic areas: enzyme production, gut microbiota dynamics, mucin biosynthesis, intestinal immune responses, competitive exclusion, foodborne pathogen control, and heat stress mitigation.

Keywords: Probiotics; poultry; gastrointestinal tract; competitive exclusion; gut health; antimicrobial resistance; *Bacillus subtilis*; Lactobacillus; heat stress; feed additives

Introduction

The global poultry industry has undergone substantial transformation over the past five decades, driven by advances in genetics, nutritional science, and management practices (Gado *et al*., 2019). Central to this progress has been the use of feed additives substances that enhance nutrient effectiveness and exert positive effects on performance. These include antibiotics, probiotics, oligosaccharides, enzymes, and organic acids, each serving distinct functional roles in the diets of commercial birds.

Among these additives, AGPs held a dominant position for more than 50 years, providing commercially significant improvements in weight gain and feed conversion efficiency at subtherapeutic doses. However, concerns over the transmission of antibiotic resistance genes from food animals to humans emerged as early as the 1950s, when resistance was documented for streptomycin in turkeys and tetracycline in broilers (Vieco-Saiz *et al*., 2019). Regulatory responses have since accelerated: the U.S. Food and Drug Administration has progressively restricted the use of medically important antimicrobials in food-animal production (FDA, 2012, 2016, 2017), while consumer pressure, including commitments by major fast-food retailers to source antibiotic-free poultry, has reinforced the commercial imperative to find alternatives.

Probiotics were first conceptualised by Elie Metchnikoff at the Pasteur Institute, who observed an association between fermented dairy consumption and enhanced longevity in Bulgarian peasants (Forkus *et al*., 2017). The term *probiotika* was formally introduced by Werner Kollath in 1953 to describe live organisms essential for healthy gut development, and the concept was subsequently refined by Lilley and Stillwell (1965) and Morelli & Capurso (2012) to its current FAO/WHO definition: live microorganisms that, when administered in adequate quantities, confer a health benefit on the host (FAO/WHO, 2001). Importantly, probiotic properties, benefits, and purposes are strain-specific, unique strains may induce different systemic effects, and efficacy is contingent on both the microbial species employed and the form of supplementation used (Morelli & Capurso, 2012).

Historical Development And Taxonomy Of Probiotics

The etymology of the term *probiotic* reflects its conceptual foundation: the Latin root *pro* (in favour of) combined with the Greek *bios* (life) conveys the conceptual opposite of antibiotic (Morelli & Capurso, 2012). In 1965, Lilley and Stillwell redefined probiotics as microorganisms that promote the growth of other beneficial gut inhabitants (Vila *et al*., 2010). This definition gained traction partly because it contrasted with Waksman's (1947) definition of antibiotics as chemical substances that inhibit bacterial growth.

Probiotic sources vary widely but include milk, fermented foods, faeces, and the gut microbiota of various animals. The two most commercially important groups are species of lactic acid bacteria (LAB) and *Bifidobacteria*. LAB have been associated with improved lactose digestion, prevention of certain cancers, reduced intestinal infections, and lower serum cholesterol levels, though several of these benefits remain incompletely substantiated (Vieco-Saiz *et al*., 2019). *Bifidobacteria* produce metabolic end-products including acetate and lactate, which have been shown to reduce both gram-positive and gram-negative pathogenic microorganisms, and are associated with additional benefits including reduced blood ammonia levels and production of B vitamins.

Spore-forming bacteria of the genus *Bacillus* have gained particular commercial importance in the animal feed sector. Their spores confer resistance to the high-temperature pelleting conditions encountered at feed mills, and their longer shelf life relative to vegetative LAB strains makes them practically advantageous (Chaiyawan *et al*., 2010). *Bacillus licheniformis*, for instance, produces the natural polypeptide antibiotic bacitracin under both aerobic and anaerobic conditions, adding an antimicrobial dimension to its probiotic effects (Pattnaik *et al*., 2001).

Mechanisms of probiotic action

Competitive Exclusion

Competitive exclusion (CE), also termed bacterial antagonism or bacterial interference, refers to the capacity of probiotic bacteria to prevent pathogenic colonisation of the GIT through superior competition for adhesion sites on the intestinal mucosa, nutrients, and physical space. The conceptual basis for CE in poultry was formalised through the Nurmi concept, which demonstrated that early inoculation of newly hatched chicks with normal intestinal microflora could establish pathogen-resistant microbial communities, a strategy that later evolved into the broader CE concept (Mead, 2000).

Organic Acid Production and pH Modulation

Bacterial probiotics resident in the gut produce organic acids, including lactic and acetic acids, as metabolic byproducts, thereby lowering luminal pH and creating an environment favourable to acid-tolerant beneficial microflora while suppressing acid-sensitive pathogens. Organic acids can also diffuse passively into bacterial cells and dissociate within the cytoplasm (approximate pH 7.0), where they inhibit key bacterial enzymes including decarboxylases and catalases, causing intracellular acidification incompatible with pathogen survival.

Enzymatic Activity

Several probiotic strains contribute directly to the digestive enzyme pool of the GIT. *Bacillus licheniformis* strains have been extensively characterised for their production of amylase, alkaline protease, keratinase, and β-mannanase. These enzyme contributions supplement the host's endogenous enzymatic output and have been associated with measurable improvements in nutrient digestibility (Abd El-Moneim *et al*., 2020).

Effects on Intestinal Morphology And Mucin Dynamics

The surface area available for nutrient absorption in the small intestine is principally determined by villus height (VH) and crypt depth (CD). Increased VH expands absorptive surface area and enhances nutrient uptake, whilst excessive CD may reduce digestive enzyme secretion and ultimately lower growth performance. Numerous investigations have confirmed that dietary probiotic administration increases VH in the ileum without corresponding increases in CD, a beneficial morphological profile attributed to probiotic-induced stimulation of mitotic cell division in gut epithelial cells.

Probiotics and Intestinal Immune Response

The GIT constitutes one of the largest immune structures in the body, and the microbial communities residing within it exert a profound influence on both local and systemic immune competence (Sherman *et al*., 2009). A desirable characteristic of a direct-fed microbial (DFM) is that it is non-pathogenic while increasing the number of beneficial colonies in the host, thereby creating commensal or symbiotic host–microbe relationships. Probiotic supplementation with *B. subtilis* strains has been associated with significantly increased duodenal secretory immunoglobulin A (IgA), a key mediator of mucosal immunity. Amerah *et al*. (2013) demonstrated that broilers receiving three *B. subtilis* strains with pelleting at 85°C or 90°C showed IgA increases of 61% and 51%, respectively, over controls at day 21, and a 2.3% improvement in feed conversion ratio (FCR) by day 42. These gains were attributed to the capacity of *B. subtilis* to secrete exogenous enzymes that augment the host's endogenous digestive activity

Probiotics and Heat Stress

Heat stress (HS) is among the most economically consequential environmental challenges in poultry production. The physiological consequences of sustained elevated ambient temperature cascade across multiple systems: the hypothalamo pituitary adrenal (HPA) axis is activated, corticosterone release increases, and general immune competence is suppressed, increasing susceptibility to pathogens including coccidia and *C. perfringens*. Sohail *et al*. (2010) demonstrated that cyclic HS broilers supplemented with a synbiotic combination (mannan-oligosaccharides plus *Lactobacillus*-based probiotic) exhibited numerically higher paraoxonase enzyme activity and significantly reduced oxidative stress markers ($p < 0.05$) relative to unsupplemented HS controls, suggesting that probiotics can partially offset the metabolic disruptions induced by elevated temperature. These findings align with the broader evidence that probiotic feeding strategies are applicable to mitigating HS-associated performance losses, particularly in fast-growing commercial broiler lines that exhibit disproportionately severe HS responses due to their elevated basal metabolic heat output.

Probiotic-Mediated Reduction of Foodborne Pathogens

Salmonella contamination originating in poultry production constitutes a major global public health burden. The World Health Organization (2006) estimates that 10% of consumers annually become ill from *Salmonella*, and that 25% of all global diarrhoeal diseases are attributable to this pathogen. The emergence of multidrug-resistant serotypes places particular importance on farm-level interventions capable of reducing carriage in live birds before slaughter. Several investigations have further confirmed the protective roles of LAB against *Salmonella enterica* serovar enteritidis and *Escherichia coli* O78:K80 infections in chicken (Carey *et al*., 2008; Maragkoudakis *et al*., 2009).

Conclusion

The collective body of evidence reviewed here supports characterising probiotics as multi-functional, evidence-based alternatives to AGPs in commercial poultry production. Rather than acting through a single pathway, probiotics confer their benefits through a network of overlapping mechanisms such as direct competition with pathogens for intestinal adhesion sites and nutrients production of organic acids that acidify the gut environment and disrupt bacterial enzymatic function; secretion of digestive enzymes that supplement the host's own activity; modulation of intestinal morphology to maximise nutrient absorption; reinforcement of mucosal barrier integrity; and stimulation of both innate and adaptive immune responses. The efficiency and food-safety gains achievable through probiotic supplementation, particularly in antibiotic-restricted or antibiotic-free production systems, represent both a commercial and a public health priority. As regulatory and market pressures on AGP use continue to intensify globally, probiotics will occupy an increasingly central position in evidence-based poultry nutrition.

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