



REVIEW ARTICLE

Synergistic Effects of Postbiotics and Non-Nutritive Feed Additives on Poultry Immune Modulation and Gut Microbiome Stability

Zhengtian Li¹, Nisar Ahmad Koka², Atique Ahmed Behan³, and Shuang Yang^{1*}

¹Qujing Normal University, College of Biological and Food Engineering, 655011 Yunnan, China; ²Department of English, College of Languages and Translation, King Khalid University Abha, Kingdom of Saudi Arabia; ³Department of Animal and Veterinary Sciences, College of Agricultural and Marine Sciences, Sultan Qaboos University, Muscat, Oman
*Corresponding author: 101237@mail.qjnu.edu.cn

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ABSTRACT

The increasing concern over antibiotic growth promoters (AGPs) and the global rise in antimicrobial resistance have prompted the poultry industry to explore safer and more sustainable alternatives. Among these, postbiotics are non-viable microbial cells and/or their metabolic by-products along with non-nutritive feed additives such as organic acids, prebiotics, phytochemicals, and enzymes. These additives contribute to improved gut health by modulating the intestinal microbiome, enhancing immune function, and supporting overall physiological performance. This review critically evaluates the individual roles of postbiotics and non-nutritive feed additives in modulating poultry physiology and gut microbial ecology, while also integrating current evidence on their synergistic interactions. Particular emphasis is placed on combinatorial feeding strategies and their effects on microbial diversity, intestinal barrier integrity, pathogen suppression, and immune modulation. Recent advances in omics-based research are also discussed to provide mechanistic insights and highlight their implications for precision dietary formulation. Furthermore, current limitations and future research directions are outlined, including the need for standardized formulations, dose optimization, and large-scale field validation. Overall, postbiotics and non-nutritive feed additives represent promising next-generation tools for sustainable poultry production and for reducing antibiotic dependence in modern animal agriculture.

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INTRODUCTION

The poultry industry has expanded rapidly since World War II, driven by a steadily increasing demand for poultry products (FAO, 2020). Over the past five decades, intensive genetic selection has resulted in more than a 300% increase in the growth rate of broiler chickens. This remarkable improvement in productivity has contributed to the expansion of the global poultry industry, with approximately 23 billion broilers produced worldwide in 2016 alone (Alsudani 2018; FAO 2018). The growth of the poultry industry follows global trends of increased consumption of dietary meat protein, which result from societal changes, urbanization, and rising incomes (Seto and Ramankutty, 2016). The quantity of poultry products, particularly meat and eggs produced each year, has increased significantly worldwide to meet rising consumer demand (Ricke, 2018). Other significant social benefits of

the poultry industry include the provision of livelihoods (in particular rural dwellers), as well as food security and the production of high-quality proteins for human consumption (Gaggia Mattarelli and Biavati, 2010). It is estimated that the global population will exceed 9 billion by 2050 (UN 2015a), global economic growth and urbanization are also projected to increase (Herrero *et al.*, 2015). Consequently, the demand for poultry products such as meat and egg has been rising. This places considerable pressure on the poultry industry to meet global production demands despite limited resources (FAO, 2016). It is evident that there is a strong need to develop globally sustainable poultry production systems that utilize scarce resources efficiently and are environmentally friendly. Such continuous growth in the poultry sector and the demand for poultry products will require scientific advancements in poultry nutrition, health, genetic development, food safety, and more

advanced processing methods (Ricke, 2017; Abdullah *et al.*, 2024).

Rising consumer demand for safe, natural, and drug residue-free foods has significantly accelerated interest in nutraceutical-based strategies for livestock farming (Park *et al.*, 2016; Pinheiro *et al.*, 2020). For more than six decades, in-feed antibiotics (IFAs), also known as antibiotic growth promoters (AGPs), have been widely used to control diseases and improve growth performance and feed efficiency by approximately 3–5% (Dahiya *et al.*, 2006; Gadde *et al.*, 2018). However, their prolonged and indiscriminate use has raised serious concerns, including the accumulation of drug residues in poultry products, the emergence of antibiotic-resistant bacteria, disruption of gut microbial balance (intestinal dysbiosis), and an increased risk of hypersensitivity reactions. These challenges have led to strict regulatory actions, including bans and restrictions in regions such as the European Union (EPC, 2005; Founou *et al.*, 2016). Consequently, the poultry industry now faces additional challenges, including inconsistent production performance and increased production costs, highlighting the urgent need for safer and more sustainable alternatives (Park *et al.*, 2016; Alsudani, 2018). Poultry growth performance, immune competence and gut resilience to enteric diseases depend on gut health. The poultry gastrointestinal tract is a highly complex and dynamic microbial ecosystem that is vital for nutrient digestion, pathogen exclusion, as well as the development of the immune system and overall host physiology (Attia *et al.*, 2025; Deng *et al.*, 2025). Traditionally, AGPs have been widely used in poultry diets to increase feed efficiency and prevent subclinical infections. However, increasing concern regarding antimicrobial resistance (AMR) and antibiotic residues in animal products led to highly restrictive regulations on AGPs, culminating in a total ban on AGPs in the European Union in 2006 (Li *et al.*, 2025; Fatima *et al.*, 2026). The changes in these regulations have led to heightened efforts to find safe, sustainable, and effective options that can maintain gut health and production without causing AMR (Idowu *et al.*, 2025; Oni & Oke 2025).

The use of microbiome-targeted feed additives is an emerging strategy that has become very popular. The beneficial impact of probiotics and prebiotics has already been reported, but they can be less efficient because of the instability of these functional ingredients in the process of feed processing and storage, as well as the ability to survive in the harsh environment of the gastrointestinal system (Markowiak and Slizewska 2018; Kumari *et al.*, 2024). In poultry nutrition, synergistic effects refer to interactions in which the combined impact of two or more feed additives exceeds the sum of their individual effects, resulting in enhanced biological performance beyond simple addition. This differs from additive effects, where the overall response equals the sum of independent actions without biological interaction, and from complementary effects, where components act independently on different but functionally related targets to collectively improve outcomes (Berenbaum, 1989). Synergistic interactions in poultry systems often arise through microbiome-mediated and host-metabolite interface mechanisms, including microbial cross-feeding, pathway convergence, and amplification of immune and metabolic signaling networks. For example, prebiotics enhance microbial fermentation

and short-chain fatty acid (SCFA) production, while postbiotics directly supply bioactive metabolites; together, they intensify SCFA–G-protein-coupled receptor (GPR) signaling and downstream immune modulation, producing effects greater than either intervention alone. Additive interactions, by contrast, reflect parallel but non-interacting biological effects, whereas complementary interactions involve distinct mechanisms that converge functionally, such as one additive improving gut barrier integrity while another modulates microbiota composition (Hemaiswarya and Doble, 2010). Clearly distinguishing these interaction types is essential in poultry nutrition research to avoid overinterpretation of “synergy,” which should be supported by mechanistic validation or quantitative interaction modeling rather than descriptive inference alone (Sarsan *et al.*, 2022). This review aims to critically evaluate the mechanisms of action of postbiotics and non-nutritive feed additives, analyze their synergistic interactions in poultry, and identify current research gaps and future directions for optimizing antibiotic-free production systems.

Postbiotics and Synergistic Interactions in Poultry Nutrition: Postbiotics defined as preparations of inanimate microorganisms and/or their metabolic components that confer health benefits to the host; have emerged as a promising alternative in poultry nutrition (Salminen *et al.*, 2021). They comprise a diverse range of bioactive compounds, including short-chain fatty acids (SCFAs), organic acids, bacteriocins, enzymes, polysaccharides, cell wall fragments (e.g., peptidoglycans and lipoteichoic acids), and bioactive peptides (Aguilar-Toala *et al.*, 2018; Abd El-Ghany, 2020; Waqas *et al.*, 2024). These components directly interact with host epithelial and immune cells, thereby modulating gut barrier integrity, microbial balance, and immune signaling pathways.

Unlike probiotics, postbiotics do not depend on microbial viability to exert their effects. While probiotic efficacy is often associated with the survival of live microbial strains (Sanders, 2009), emerging evidence indicates that microbial viability is not essential for conferring health benefits (Aguilar-Toalá *et al.*, 2018). This has led to the development of related terms such as parabiotics, ghost probiotics, inactivated probiotics, and non-viable probiotics, all describing microbial-derived preparations that retain biological activity without live cells.

The non-viable nature of postbiotics provides several advantages, including enhanced stability during feed processing, storage, and gastrointestinal transit, as well as improved safety by eliminating risks such as microbial translocation, horizontal gene transfer, and infection in immunocompromised hosts (Zolkiewicz *et al.*, 2020). Functionally, postbiotics have been shown to improve gut morphology, inhibit pathogenic bacteria, enhance antioxidant capacity, and modulate immune responses, making them effective alternatives to antibiotic growth promoters in poultry diets (Zanoni *et al.*, 2008).

In poultry nutrition, postbiotics demonstrate enhanced efficacy when combined with non-nutritive feed additives such as prebiotics, organic acids, and phytochemical compounds. These combinations can exert synergistic effects by promoting beneficial microbial populations, increasing microbial metabolite production, and

amplifying host immune signaling pathways (Gadde *et al.*, 2017). Such interactions contribute to improved gut health, enhanced disease resistance, and greater microbial stability, supporting sustainable poultry production in the post-antibiotic era.

Synergistic effects in this context refer to interactions in which the combined impact of two or more feed additives exceeds the sum of their individual effects. This differs from additive effects, where responses are merely cumulative, and complementary effects, where agents act independently but supportively. Synergy may arise through microbial cross-feeding, convergence of metabolic pathways such as SCFA-mediated signaling, and amplification of host-microbiome interactions. For example, prebiotics enhance fermentation-derived SCFA production, while postbiotics directly supply bioactive metabolites, collectively strengthening signaling pathways such as G-protein-coupled receptor (GPR)-mediated immune modulation as summarized in Fig. 1.

Overall, postbiotics—alone or in combination with complementary feed additives—represent a promising next-generation strategy for gut health management in poultry. However, further research is required to elucidate their precise mechanisms of action, optimize formulations, and assess long-term effects on performance, immunity, and microbiome functionality under commercial production conditions.

Composition and Classification of Postbiotics:

Postbiotics are a heterogeneous group of soluble bioactive compounds released by microorganisms, including enzymes, short-chain fatty acids (SCFAs), peptides, organic acids, exopolysaccharides, teichoic acids, vitamins, cell surface proteins, and other metabolites such as hydrogen peroxide and diacetyl (Rai *et al.*, 2018). Rather than being defined only by individual components,

postbiotics are commonly classified based on their biochemical nature into major groups such as proteins and peptides, carbohydrates (e.g., polysaccharides and teichoic acids), lipids and lipid-derived molecules, organic acids, and vitamins or cofactors.

Beyond their composition, postbiotics are distinguished by their structural stability, long shelf-life, and safety when used at appropriate concentrations (Tomar *et al.*, 2015). Their favorable physicochemical properties also support efficient absorption and systemic bioactivity, enabling them to interact with multiple host tissues and modulate diverse physiological functions (Shenderov, 2013). Collectively, these characteristics underpin their functional versatility and growing interest as bioactive feed and health modulators.

Sources of Postbiotics: Postbiotics can be derived from a wide range of microorganisms, with lactic acid bacteria representing the most extensively studied sources. Among these, *Lactobacillus* and *Bifidobacterium* species are the primary contributors due to their well-established safety profiles and extensive use in food and feed applications. These genera produce a variety of bioactive metabolites, including organic acids, bacteriocins, exopolysaccharides, and enzymes, which are commonly associated with beneficial effects on host health.

In addition to traditional probiotic strains, emerging evidence has highlighted other microbial sources with promising postbiotic potential. For instance, *Faecalibacterium* and *Streptococcus* species have been identified as important producers of metabolites with immunomodulatory and gut health-promoting properties (Aguilar-Toalá *et al.*, 2018). These findings broaden the spectrum of microorganisms considered relevant for postbiotic development and application.

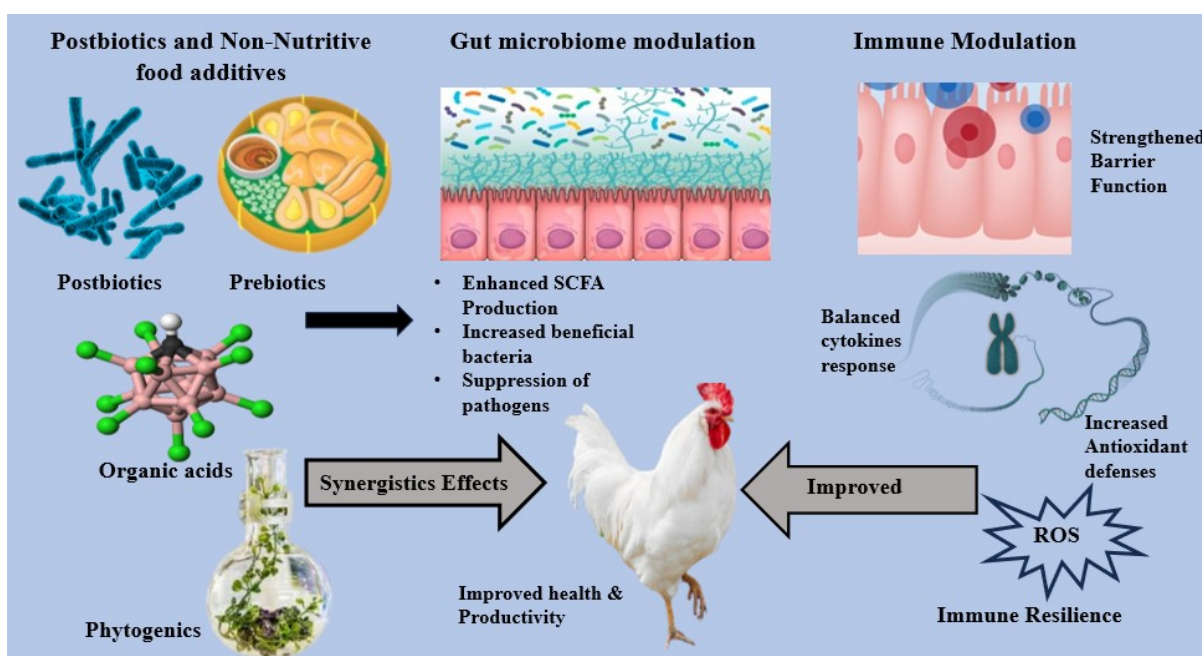


Fig. 1: Schematic representation of the synergistic effects of postbiotics and non-nutritive feed additives (prebiotics, organic acids, and phytochemicals) on poultry gut health. These additives modulate the gut microbiome by enhancing short-chain fatty acid (SCFA) production, promoting beneficial bacteria, and suppressing pathogens. They also improve immune function by strengthening the intestinal barrier, balancing cytokine responses, and increasing antioxidant defenses, ultimately leading to enhanced health, productivity, and immune resilience in poultry.

Importantly, the biological activity of postbiotics is not only determined by the microbial source but also by strain specificity, culture conditions, and the nature of the metabolites produced. Variations in fermentation environment, substrate availability, and processing methods can significantly influence the composition and functional properties of the resulting postbiotic preparations (de Almada *et al.*, 2016). Therefore, careful selection of microbial strains and controlled production conditions are essential to ensure consistency, efficacy, and reproducibility of postbiotic-based applications.

Non-Nutritive Feed Additives: Non-nutritive feed additives are dietary components that do not directly contribute to energy or nutritional value but exert functional effects on gut health, microbial ecology, and host physiology. These include organic acids, prebiotics, phytochemicals, and exogenous enzymes (Markowiak & Śliżewska, 2018).

Organic acids (e.g., formic, lactic, and butyric acids) enhance gut health primarily by lowering intestinal pH, thereby inhibiting pathogenic bacteria and favoring beneficial microbial populations. In particular, butyrate serves as an important energy source for intestinal epithelial cells and plays a key role in maintaining gut barrier integrity and regulating inflammatory responses. Prebiotics such as fructooligosaccharides (FOS) and mannan oligosaccharides (MOS) selectively stimulate the growth and metabolic activity of beneficial microbiota, indirectly promoting the production of health-enhancing metabolites, including short-chain fatty acids (SCFAs).

Phytochemical additives, including essential oils, flavonoids, and polyphenols, contribute through antimicrobial, antioxidant, and immunomodulatory activities, thereby supporting gut microbial balance and host defense mechanisms. Exogenous enzymes improve nutrient digestibility by breaking down complex feed components, which reduces the availability of undigested substrates that could otherwise support pathogenic bacterial growth (Gadde *et al.*, 2017).

Collectively, these non-nutritive feed additives act through complementary mechanisms to stabilize the gut ecosystem, enhance nutrient utilization, and improve overall intestinal health in poultry.

Gut Microbiome and Host Immune System in Poultry:

The poultry gut microbiome is a dynamic and complex ecosystem, mainly composed of bacteria belonging to the phyla Firmicutes, Bacteroidetes, and Proteobacteria. Its composition is strongly influenced by age, diet, environment, and overall health status (Oakley *et al.*, 2014). This microbial community plays a central role in nutrient metabolism, vitamin synthesis, pathogen exclusion, and immune system development. Disruption of this balance, known as dysbiosis, is frequently associated with reduced growth performance, increased disease susceptibility, and intestinal inflammation (Olayiwola & Adedokun, 2025).

Beneficial bacterial genera such as *Lactobacillus* and *Bifidobacterium* are key contributors to gut homeostasis. They promote the production of short-chain fatty acids (SCFAs), produce antimicrobial substances, and support competitive exclusion of pathogenic microbes, thereby

limiting pathogen colonization (Yue *et al.*, 2025). These microbes also enhance intestinal barrier integrity by regulating tight junction proteins and stimulating mucin production.

Gut microbiota also plays a crucial role in modulating the host immune system through its interaction with gut-associated lymphoid tissue (GALT). Microbial signals are recognized by pattern recognition receptors, including Toll-like receptors (TLRs) and NOD-like receptors (NLRs), which activate downstream signaling pathways involved in cytokine production, antimicrobial peptide expression, and immune cell differentiation (Kogut, 2019). These coordinated interactions help maintain immune balance, promote secretory immunoglobulin A (sIgA) production, and prevent excessive inflammation. As a result, intestinal homeostasis and disease resistance in poultry are significantly enhanced.

Biological Activities and Functional Effects of Postbiotics:

Postbiotics exhibit multiple biological activities in poultry by interacting with intestinal epithelial cells, immune cells, and resident microbiota through biochemical and molecular signaling pathways. Unlike live microorganisms, their effects are mediated by microbial metabolites, structural components, and bioactive molecules, making them particularly suitable for intensive poultry production systems.

Immunomodulatory Effects: One of the most important functions of postbiotics is the regulation of host immune responses in a balanced manner. This is primarily mediated through pattern recognition receptors (PRRs), including Toll-like receptors (TLR2, TLR4, and TLR9), which are expressed on intestinal epithelial cells, macrophages, and dendritic cells. These receptors recognize microbial-derived components such as peptidoglycans, lipoteichoic acids, and microbial DNA fragments (Kogut, 2019).

Activation of these receptors triggers intracellular signaling pathways, mainly nuclear factor kappa B (NF- κ B) and mitogen-activated protein kinase (MAPK), which regulate the expression of cytokine genes. Postbiotics have been shown to reduce excessive production of pro-inflammatory cytokines, including tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), and interleukin-6 (IL-6), while enhancing anti-inflammatory mediators such as interleukin-10 (IL-10) and transforming growth factor- β (TGF- β) (Zolkiewicz *et al.*, 2020).

This balanced immune regulation helps maintain immune homeostasis by supporting tolerance toward commensal microbiota and dietary antigens while preserving the ability to mount effective responses against pathogens. Additionally, postbiotics promote the development and activity of regulatory T cells (Tregs), contributing to long-term immune stability within the gut-associated lymphoid tissue (GALT) (Aguilar-Toala *et al.*, 2018).

Experimental evidence further supports these immunomodulatory effects. For example, postbiotic preparations derived from *Lactobacillus reuteri* 17938 have been shown to stimulate IL-10 production through retinoic acid-dependent pathways in mucosal dendritic cells, enhancing regulatory T cell responses (Haileselassie

et al., 2016). Similarly, cell-free supernatants of *Faecalibacterium prausnitzii* increased IL-8 production in Caco-2 cells and demonstrated protective effects against colitis in mouse models by elevating IL-10 levels (Sokol *et al.*, 2008). In addition, supernatants from *Lactobacillus paracasei* B21060 reduced inflammatory responses induced by *Salmonella* in intestinal mucosa (Tsilingiri *et al.*, 2012).

Clinical evidence also supports these findings. In human studies, *Lactobacillus casei* DG supernatant reduced inflammatory responses in the colonic and ileal mucosa of patients with post-infectious bowel syndrome (Compare *et al.*, 2017). Furthermore, postbiotics derived from *Lactobacillus gasserii* TMC0356 induced higher IL-12 production in macrophages compared to live bacteria, highlighting that microbial viability is not essential for immunomodulatory activity (Miyazawa *et al.*, 2011).

Mechanisms of Antimicrobial and Pathogen-Exclusion Effects: Postbiotics exert broad-spectrum antimicrobial activity through both direct pathogen inhibition and indirect competitive exclusion mechanisms. Their antimicrobial components include organic acids (e.g., lactic, acetic, and butyric acids), bacteriocins, hydrogen peroxide, and antimicrobial peptides (Aguilar-Toalá *et al.*, 2018).

Direct antimicrobial effects are primarily mediated by organic acids and bacteriocins. Organic acids penetrate bacterial cells in their undissociated form and dissociate in the cytoplasm, leading to intracellular acidification. This disrupts pH homeostasis, enzyme activity, and DNA replication, ultimately inhibiting pathogen growth. In addition, bacteriocins—ribosomally synthesized antimicrobial peptides—exert bactericidal effects by forming pores in bacterial membranes or interfering with cell wall biosynthesis. These mechanisms are particularly effective against major poultry pathogens, including *Salmonella enterica*, *Campylobacter jejuni*, *Escherichia coli*, and *Clostridium perfringens*, which are responsible for enteric diseases and food safety concerns in poultry production (Kogut, 2019; Yaseen *et al.*, 2025).

Indirect antimicrobial effects occur through competitive exclusion and modulation of the gut ecosystem. Postbiotics promote the growth and metabolic activity of beneficial microbiota, which reduces nutrient availability and adhesion sites for pathogens. This ecological shift limits pathogen colonization and supports microbial homeostasis. For example, cell-free supernatants derived from *Lactobacillus plantarum* strains (RI11, RG11, RG14, RS5, TL1, and UL4) have demonstrated inhibitory activity against pathogens such as *Salmonella enterica*, *Listeria monocytogenes*, vancomycin-resistant enterococci, and *Escherichia coli* (Kareem *et al.*, 2014). Overall, bacteriocins and SCFAs are considered key contributors to these antimicrobial effects (Aguilar-Toalá *et al.*, 2018).

Beyond antimicrobial activity, postbiotics also exert antioxidant and cytoprotective effects, which further support intestinal health. Oxidative stress in poultry, commonly induced by heat stress, high stocking density, and infections, leads to intestinal damage and impaired immune function. Postbiotics enhance endogenous antioxidant defense systems by upregulating enzymes such as superoxide dismutase (SOD), catalase (CAT), and

glutathione peroxidase (GPx), while reducing reactive oxygen species (ROS) accumulation and lipid peroxidation (Aguilar-Toalá *et al.*, 2018).

These antioxidant actions help maintain membrane integrity, preserve mitochondrial function, and prevent apoptosis of intestinal epithelial cells. In addition, postbiotics suppress oxidative stress-induced inflammatory signaling pathways, particularly NF- κ B activation, thereby reinforcing their combined antioxidant and anti-inflammatory effects (Zolkiewicz *et al.*, 2020).

Collectively, postbiotics support gut health not only by directly inhibiting pathogens but also by strengthening beneficial microbial populations and host cellular defenses, offering a more stable and controlled approach compared to supplementation with live and variable probiotic strains (Lebeer *et al.*, 2012; Iweala & Nagler, 2019).

Improvement in Intestinal Barrier Function: The intestinal barrier is an extremely important interface between the outside and the internal systems of the host. Postbiotics can increase barrier integrity through the stimulation of goblet cell function and the production of mucin (MUC2) that covers the epithelium with a protective mucus layer. At the molecular level, postbiotics increase the expression of tight-junction proteins like occludin, claudin-1, claudin-4, and zonula occludens-1 (ZO-1) and, as a result, minimize the intestinal permeability and translocation of pathogens and endotoxins, including lipopolysaccharides (LPS) into the systemic circulation (Zolkiewicz *et al.*, 2020). Increased barrier integrity is directly associated with an increase in nutrient absorption, a decrease in systemic inflammation, and an increase in growth performance in poultry.

Metabolic and Nutritional Modulation: Postbiotics also influence host metabolism by modulating energy utilization, lipid metabolism, and nutrient absorption. Short-chain fatty acids (SCFAs) produced or supplied by postbiotic preparations serve as key energy substrates for enterocytes, with butyrate being the primary fuel supporting intestinal epithelial development and regulating the expression of nutrient transporter genes (Markowiak and Śliżewska, 2018).

Beyond their role as energy sources, SCFAs activate G-protein-coupled receptors (GPR41 and GPR43), which are involved in the regulation of glucose homeostasis, lipid metabolism, and immune signaling pathways. Through these mechanisms, postbiotics enhance nutrient utilization efficiency and support improved growth performance in poultry.

From a production perspective, these metabolic effects translate into better feed efficiency, as more dietary nutrients are converted into usable energy and biomass rather than being lost through inefficient digestion or microbial competition. Improved gut integrity and nutrient transporter activity further enhance feed conversion ratio (FCR), which is a key economic parameter in commercial poultry systems. Collectively, these benefits can reduce production costs by improving weight gain per unit of feed intake, lowering feed wastage, and increasing overall flock productivity, making postbiotics a promising tool for economically sustainable poultry production.

Non-Nutritive Feed Additives: Non-nutritive feed additives are central to the process of maintaining intestinal homeostasis to benefit poultry health by altering intestinal microbial composition, regulating host immune system response, and altering intestinal morphology and function. They act through biochemical and signaling processes instead of specific nutritional contribution, so they are especially useful in the production system free of antibiotics (Celi *et al.*, 2017; Rahman *et al.*, 2022).

Regulation of Gastrointestinal pH and Microbial Ecology:

The organic acids like formic, lactic, acetic, propionic, and butyric acids lower the gastrointestinal pH, which inhibits the growth of acid-sensitive pathogenic bacteria, including *Salmonella spp.*, *Escherichia coli*, and *Clostridium perfringens* (Dittoe *et al.*, 2018; Ali *et al.*, 2024). This acidification of the gut lumen interferes with the integrity of bacterial membranes, intracellular pH, and prevents enzymatic processes required by the pathogen to survive.

In addition to having anti-pathogenic properties, organic acids have been shown to increase the digestibility of nutrients by augmenting the solubility of mineral nutrients like calcium and phosphorus, as well as the induction of endogenous digestive enzymes. Specifically, butyric acid acts as a prime source of energy for the colonocytes and stimulates the differentiation of intestinal epithelial cells, the depth of the crypts, and the height of the villi, enhancing absorptive capacity (Bedford & Gong, 2018).

Fermentation and Short-Chain Fatty Acid Signaling by Prebiotics:

Prebiotics (fructooligosaccharides (FOS), mannan-oligosaccharides (MOS), inulin, and galactooligosaccharides (GOS)) can selectively promote the beneficial microbial populations, including *Lactobacillus*, *Bifidobacterium*, and *Faecalibacterium* species (Markowiak & Slizewska, 2018). The microorganisms decompose prebiotics to generate short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate that have pleiotropic effects on the gut physiology (Guo *et al.*, 2021).

SCFAs are important signaling molecules as activators of G-protein-coupled receptors (GPR41 and GPR43) on epithelial and immune cells, resulting in increased epithelial barrier integrity and inhibition of inflammatory signaling pathways. Butyrate is also a histone deacetylase inhibitor, which controls gene expression that is involved in immune tolerance and epithelial repair.

Moreover, prebiotics also aid in competitive displacement of pathogens by providing limited adhesion sites on the intestinal epithelium and depriving the pathogens of nutrients. In particular, type-1 fimbriae of gram-negative pathogens are bound by mannan-oligosaccharides and are unable to latch on to intestinal cells and get cleared of the gut (Markowiak & Slizewska, 2018).

Phytogenic Feed Additives and Host Immune Modulation:

Phytogenic feed additives are herbs, spices, plant extracts, which are converted to essential oils, flavonoids, tannins, saponins, and phenolic compounds. These bioactive molecules have a wide-spectrum antimicrobial effect because they disrupt bacterial

membranes, block quorum sensing, and reduce the expression of virulence genes.

The major constituents of essential oils like thymol, carvacrol, cinnamaldehyde, and eugenol have been revealed to decrease the number of intestinal pathogen load whilst boosting good microbes. Besides antimicrobial properties, phytochemicals are also good antioxidants, that is, they scavenge reactive oxygen species and increase endogenous antioxidant enzymes, including superoxide dismutase, catalase, and glutathione peroxidase (Windisch *et al.*, 2008).

Phytochemical compounds affect immune signaling pathways as well, altering nuclear factor- κ B (NF- κ B) and mitogen-activated protein kinase (MAPK) pathways with resulting down-regulation of pro-inflammatory cytokines (e.g. IL-1 β , TNF- α) and increased synthesis of immunoregulatory mediators. The effects lead to an increase in immune competence and resistance to enteric infections and environmental stressors.

Effects on Intestinal Morphology and Barrier Function:

Overall, non-nutritive additives enhance the intestinal morphology through an increase in the villus height, villus-to-crypt ratio, and goblet cell density, resulting in augmented mucin secretion and enhanced barriers to the mucosa (Bedford & Gong, 2018). Enhanced tight-junction integrity lowers intestinal permeability and restricts the translocation of the pathogen and endotoxins into the systemic circulation. Table 1 summarizes the mechanisms and functional effects of postbiotics and non-nutritive feed additives in poultry, highlighting their roles in modulating the gut microbiome, enhancing short-chain fatty acid production, promoting beneficial bacteria, suppressing pathogens, and improving immune function, health, and productivity.

These structural advancements are especially needed in the early stages of life, as the poultry gut is highly pliant and is prone to dysbiosis. Improved barrier activity decreases immune hyperactivity and instead devotes metabolic energy to growth and production performance.

Mitigation of Stress and Performance Enhancement:

Non-nutritive feed supplements counteract the adverse effects of environmental stress conditions like heat stress, overcrowding, and dietary change by stabilizing intestinal microbial flora and decreasing oxidative and inflammatory tissue harm. In particular, organic acids and phytochemicals have been proven to reduce corticosterone responses to stress and enhance the intake of feed and gain of weight in adverse environments.

These additives are part of a better feed conversion ratio (FCR), carcass yield, and general flock health when used strategically, which helps to sustain poultry production systems without using AGPs.

Synergistic Effects in Poultry Nutrition:

The combined use of postbiotics and non-nutritive feed additives offers an integrated approach to gut health management in poultry, particularly in antibiotic-free production systems. These combinations act at multiple levels, including microbial, epithelial, metabolic, and immune pathways, resulting in coordinated responses that exceed the effects of individual additives.

Modulation of Gut Microbial Ecology and Functional Stability: The poultry gut microbiome is highly dynamic and sensitive to diet, environmental stress, and pathogen exposure. The interaction between postbiotics and prebiotics enhances microbial stability by increasing diversity and functional redundancy, thereby supporting a resilient ecosystem capable of maintaining homeostasis under stress conditions (Fig. 2) (Markowiak & Śliżewska, 2018; Saeed *et al.*, 2023).

Prebiotics selectively stimulate beneficial microbial groups such as *Lactobacillus*, *Bifidobacterium*, and butyrate-producing *Clostridia*. In contrast, postbiotics provide bioactive metabolites, structural components, and signaling molecules that directly support microbial activity and host–microbe interactions. Together, these mechanisms enhance cross-feeding among microbial populations and sustain the production of health-promoting metabolites, even under dietary or environmental stress.

Table 1: Mechanisms and Functional Effects of Postbiotics and Non-Nutritive Feed Additives in Poultry

Additive Type	Bioactive Components	Primary Mechanisms of Action	Effects on Gut Health	Immunomodulatory Effects	Performance Outcomes	Key References
Postbiotics	SCFAs (acetate, propionate, butyrate), bacteriocins, cell wall fragments, enzymes, peptides	Modulation of gut microbiota; activation of GPR41/43; inhibition of NF- κ B signaling; enhancement of tight junction proteins	Improved villus height and crypt depth; enhanced epithelial barrier integrity; reduced pathogen colonization	Increased IgA secretion; modulation of cytokines (\uparrow IL-10, \downarrow TNF- α , IL-6); enhanced innate immunity	Improved feed efficiency, weight gain, and stress resilience	Abd El-Ghany (2020); Olayiwola & Adedokun (2025)
Organic Acids	Formic, acetic, propionic, butyric acids	Lowering gut pH; disruption of bacterial membrane potential; improved nutrient digestibility	Reduced pathogenic load (<i>Salmonella</i> , <i>E. coli</i>); improved mucosal morphology	Indirect immune modulation via microbiota stabilization	Better FCR; enhanced nutrient absorption	Markowiak & Śliżewska (2018); Olayiwola & Adedokun (2025)
Prebiotics	MOS, FOS, inulin, β -glucans	Selective stimulation of beneficial bacteria (<i>Lactobacillus</i> , <i>Bifidobacterium</i>); increased SCFA production	Enhanced microbial diversity; improved gut barrier	Enhanced macrophage and lymphocyte activity; improved antibody titers	Improved growth performance and disease resistance	Markowiak & Śliżewska (2018)
Phytogenics	Essential oils (thymol, carvacrol), flavonoids, tannins	Antioxidant activity; antimicrobial effects; modulation of digestive enzyme secretion	Improved intestinal morphology; reduced oxidative stress	Downregulation of pro-inflammatory cytokines; enhanced antioxidant enzyme activity	Improved weight gain and carcass quality	Oni & Oke (2025)
Synbiotic Combinations	Prebiotics + probiotics/postbiotics	Synergistic microbiota modulation; enhanced SCFA synthesis; improved colonization resistance	Increased microbial stability; improved barrier function	Balanced Th1/Th2 responses; improved systemic immunity	Enhanced growth performance under heat stress	Olayiwola & Adedokun (2025); Markowiak & Śliżewska (2018)

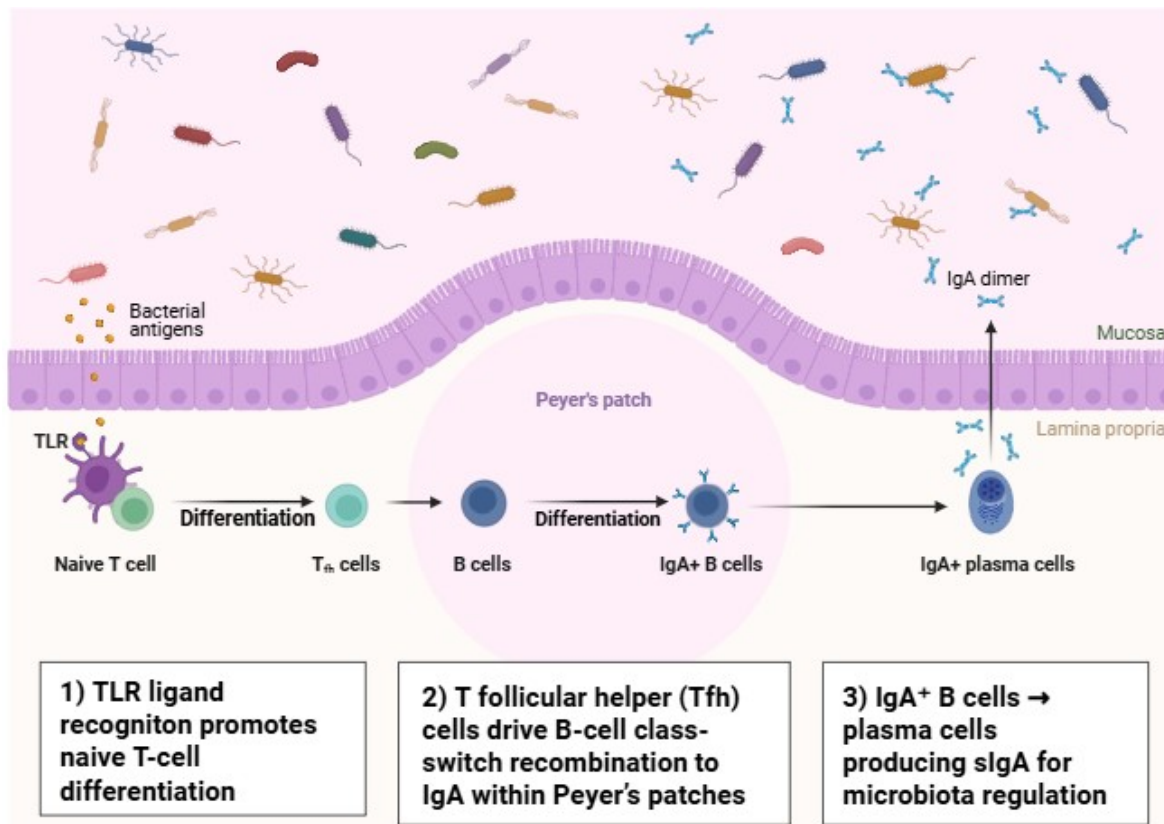


Fig. 2: Gut–Immune–Performance Axis Mediated by Postbiotics in Poultry.

Postbiotic-derived bioactive molecules interact with intestinal epithelial cells and antigen-presenting cells (APCs), activating TLR4-dependent signaling and promoting T helper (Th) cell polarization. T follicular helper (Tfh) cells support B-cell differentiation within Peyer's patches, leading to IgA-producing plasma cells that regulate gut microbiota composition. Concurrently, microbial metabolites enhance barrier integrity, modulate cytokine production, and suppress pathogenic colonization. These coordinated immune and metabolic responses contribute to improved gut homeostasis, enhanced nutrient utilization, and overall growth performance in poultry.

Increased Production of Short-Chain Fatty Acids and Host Signaling: Among the most important effects of synergistic supplementation is the increased and persistent production of short-chain fatty acids (SCFA), in this case, butyrate. Prebiotics are fermentable substances, and postbiotics provide fermentation end-products and metabolic intermediates that hasten and stabilize fermentation by microbes (Markowiak & Śliżewska, 2018).

SCFAs further function as energy source for enterocytes, signal activation of G-protein-coupled receptors (GPR41, GPR43), and histone deacetylases inhibition, which cause epigenetic regulation of genes that associate with barrier integrity, inflammation, and immune tolerance. These mechanisms allow the synergistic strategies to have a direct connection of microbial metabolism with host gene expression and intestinal health.

The synergy between postbiotics and non-nutritive additives is governed by a complex mechanistic model involving microbial cross-feeding and synchronized host-signaling pathways. Interaction models indicate that prebiotics function as substrates for primary fermenters, leading to the production of intermediate metabolites such as acetate, which are subsequently utilized by secondary butyrate-producing bacteria—an example of microbial cross-feeding that can be further stabilized by postbiotic-derived metabolic intermediates (Markowiak & Śliżewska, 2018; Aguilar-Toalá *et al.*, 2018). This interaction often follows a non-linear dose–response relationship, where optimal inclusion levels of both additives are required to maximize activation of G-protein-coupled receptors (GPR41/43) without inducing metabolic exhaustion or excessive luminal acidification (Kogut, 2019). In addition, temporal dynamics play a critical role, as early priming of the gut-associated lymphoid tissue (GALT) by postbiotic cell-wall components, such as peptidoglycans, enhances the host's responsiveness to subsequent short-chain fatty acid (SCFA)-mediated signaling (Salminen *et al.*, 2021). This “prime-and-modulate” sequence contributes to the downregulation of pro-inflammatory pathways such as NF- κ B and the upregulation of anti-inflammatory cytokines like IL-10, thereby redirecting metabolic energy from immune activation toward growth performance and muscle deposition (Kogut, 2019; Zolkiewicz *et al.*, 2020). Collectively, these findings highlight that synergistic effects are not merely additive but arise from coordinated microbial and host interactions that are dose-dependent, temporally regulated, and mechanistically integrated.

Reinforcement of Intestinal Barrier Integrity: The intestinal barrier plays a critical role in determining the absorption of nutrients, activation of immunity, and resistance to diseases. Postbiotics and organic acids used in synergy have shown considerable positive effects to improve the functionality of the barrier by promoting the assembly of tight-junctions, enhancing mucin secretion, and enhancing the turnover of epithelial cell function.

The postbiotic-derived peptides and metabolites promote the tight-junction protein expression of occludin, claudins, and zonula occludens-1, whereas organic acids lower luminal pH and inhibit the destruction of the epithelial tissue by pathogenic bacteria and their toxins. Enhanced barrier integrity has reduced intestinal permeability, translocation of endotoxins, and chronic low-grade inflammation, which adversely affects growth performance.

Coordinated Immune Modulation and Immune Homeostasis: The balance of immunomodulatory activity of synergistic combinations increases the level of protection against the development of inflammation. Postbiotics also interplay with the pattern recognition receptors, Toll-like receptors, and nucleotide-binding oligomerization domain receptors, which prime the innate immune cells and increase antigen presentation.

At the same time, phytochemical compounds and organic acids inhibit overactivation of pro-inflammatory signaling pathways, including NF- κ B and MAPK, which leads to a decrease in the production of TNF- α , IL-1 β , and IL-6 and an increase in the production of anti-inflammatory cytokines, including IL-10. Such a well-coordinated immune response helps to defend against the pathogens efficiently with a minimum of immune tissue damage and energy consumption.

Better Pathogen Management and Competitive Exclusion: The benefits of synergistic feed strategies are to complement pathogen control in a variety of ways. The pathogenic bacteria are directly inhibited by organic acids and phytochemicals through disrupting cell membranes, metabolic processes, and postbiotics, which enhances the competitive exclusion by boosting beneficial microbial communities and promoting the production of antimicrobial peptides (Markowiak & Śliżewska, 2018).

This combined antimicrobial effect will lead to a decrease in colonization of enteric pathogens (*Salmonella spp.*, *Campylobacter jejuni*, and *Clostridium perfringens*), thereby decreasing the number of diseases and preventing the use of therapeutic measures. Notably, this pathogen suppression is achieved without destabilizing any of the beneficial microbial communities, which is a major benefit over the traditional antimicrobial techniques.

Enhanced Nutrient Utilization and Growth Performance: Through microbial efficiency, epithelial functioning, and immune equilibrium, synergistic supplementation leads to increased nutrient digestibility and metabolic efficiency. Enhanced villus structure, enzyme activity, and efficiency of microbial fermentation also lead to an improved FCR (Markowiak & Śliżewska, 2018).

Less inflammatory burden also spares metabolic energy and nutrients used to defend against the immune

system become redirected to growth and production instead of defense. These effects are especially noticeable at the critical stages, such as in early life, heat stress, vaccination, or pathogen challenge.

Stress Resilience and Production Stability over a Long Period: Synergistic measures also increase the resistance to environmental and physiological stressors. The antioxidant compounds and stress-response metabolites are provided by postbiotics, and the mitochondrial activity is supported with the help of phyto-genic additives and organic acids.

The combination of these effects stabilizes the composition of the gut microbiota, decreases the level of oxidative stress, and can preserve immune competence during unfavorable conditions, leading to an increase in survivability, stable performance, and overall flock consistency.

Performance and Health Outcomes: Addition of postbiotics and non-nutritive feed additives to the diet of poultry has been widely associated with better growth performance, gut functioning, immune competence, and overall resilience of a flock. These effects are coordinated to work together to modulate the ecology of gut microbes, nutrient metabolism, epithelial integrity, and host immune response, to make these additives viable and sustainable alternatives to AGP.

Growth Performance, Feed Efficiency, and Nutrient Utilization: Postbiotics enhance nutrient utilization by increasing the activity of digestive enzymes such as amylase, protease, and lipase, improving microbial fermentation efficiency, and reducing intestinal inflammation that would otherwise divert nutrients away from growth. Specifically, the addition of postbiotics derived from *Lactobacillus* species has been shown to improve body weight gain (BWG) by up to 4.5% and lower feed conversion ratio (FCR) by 3.2% compared to control groups (Kareem *et al.*, 2014; Abd El-Ghany, 2020). In addition, organic acids improve mineral bioavailability by lowering gut pH and enhancing the solubility of minerals such as calcium and phosphorus, while prebiotic fermentation produces short-chain fatty acids (SCFAs) that serve as an additional energy source for enterocytes (Markowiak & Ślizewska, 2018; Olayiwola & Adedokun, 2025).

Intestinal Morphology and Maturation of Functions: Gut morphology is a key determinant of nutrient absorption efficiency. Dietary inclusion of postbiotics and non-nutritive feed additives has been associated with increased villus height, reduced crypt depth, and an improved villus height to crypt depth ratio, all of which indicate enhanced intestinal maturation and greater absorptive capacity.

These structural changes are accompanied by functional improvements, including increased expression of tight junction proteins such as occludin, claudins, and zonula occludens-1 (ZO-1), as well as goblet cell hyperplasia and increased mucus secretion. Collectively, these adaptations reduce intestinal permeability and limit the translocation of endotoxins.

Overall, these effects strengthen gut barrier integrity, reduce pathogen invasion, and prevent chronic low-grade inflammation, thereby supporting improved growth performance and feed efficiency in poultry.

Microbial Richness, Stability, and Pathogen Suppression: One of the consistent effects of supplementation with postbiotics and non-nutritive feed additives is the enhancement of beneficial microbial communities and overall microbial diversity, both of which are key indicators of a healthy and stable gut microbiome. Beneficial taxa such as *Lactobacillus*, *Bifidobacterium*, and butyrate-producing *Clostridiales* are promoted, while pathogenic organisms including *Salmonella* spp., *Escherichia coli*, and *Clostridium perfringens* are inhibited through improved competitive exclusion and increased production of antimicrobial metabolites (Olayiwola & Adedokun, 2025).

The reduction in pathogen colonization contributes to lower disease incidence and mortality, decreased fecal shedding of harmful microorganisms, and reduced environmental contamination. This also improves food safety and hygiene by minimizing the risk of zoonotic disease transmission. Notably, postbiotics achieve these benefits without requiring live microorganisms, making their effects more stable, predictable, and consistent under varying production conditions.

Immune Modulation and Disease Resistance: Non-nutritive additives and postbiotics play an important role in regulating immune responses and protecting against excessive inflammatory activity (Fig. 3). Research indicates a shift toward a balanced cytokine profile characterized by reduced expression of pro-inflammatory cytokines such as tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β), and interleukin-6 (IL-6), along with increased production of anti-inflammatory mediators such as interleukin-10 (IL-10), as well as enhanced secretion of mucosal immunoglobulin A (sIgA) (Olayiwola & Adedokun, 2025).

This immunomodulation improves resistance to enteric pathogens and enhances vaccine responsiveness, particularly during early life stages and under environmental stress conditions. By preventing immune overactivation, these additives reduce unnecessary metabolic energy expenditure, allowing more nutrients and energy to be directed toward growth and productivity in poultry (Oni & Oke, 2025).

Stress Resilience and Welfare Outcomes: The present-day poultry production systems expose birds to multiple stressors, including heat stress, high stocking density, dietary changes, and pathogen pressure. Under these conditions, postbiotics and non-nutritive feed additives have been shown to enhance physiological resilience by reducing oxidative stress through their antioxidant activity, regulating gut microbiota under thermal and nutritional challenges, and preventing stress-induced dysbiosis and immune suppression. Improved gut stability and immune modulation further contribute to greater flock uniformity, reduced mortality, and decreased reliance on therapeutic interventions. Collectively, these effects enhance overall bird welfare and support more ethical and sustainable poultry production systems (Zanoni *et al.*, 2008).

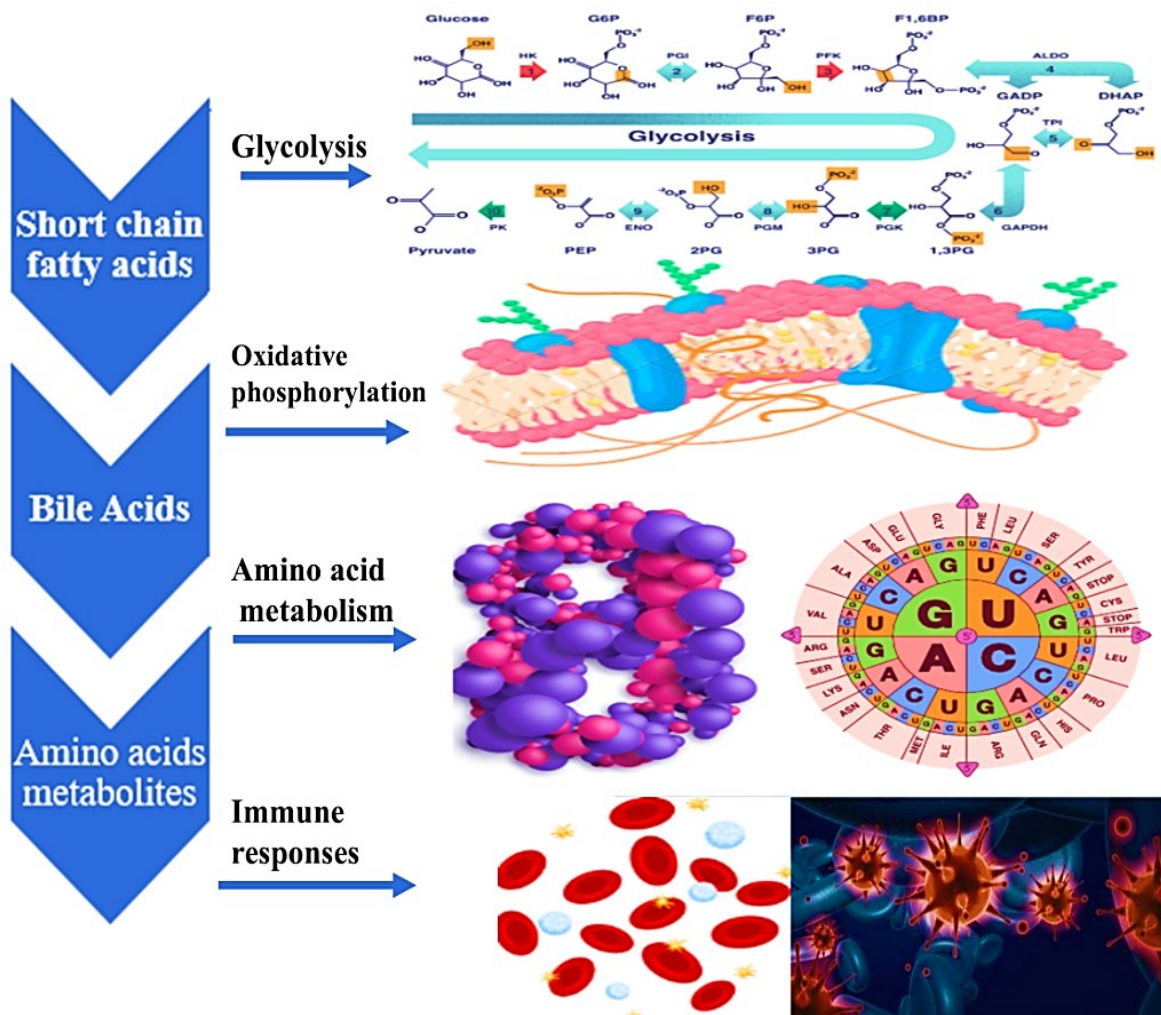


Fig. 3: Modulation of Host Metabolic Pathways by Microbial Metabolites.

The Implications of Antibiotic-Free and Sustainable Poultry Production: The existing body of evidence indicates that postbiotics and non-nutritive feed additives hold strong potential as alternatives to antibiotic growth promoters (AGPs) without compromising production efficiency. Their adoption aligns with international regulations restricting antibiotic use, as well as growing consumer demand for safe, residue-free animal products. These strategies support the development of antibiotic-free production systems, contribute to reducing the selective pressure driving antimicrobial resistance, and promote more environmentally sustainable poultry production practices (Castanon, 2007).

Obstacles and Future Projections: Although there is strong evidence on the use of postbiotics and non-nutritive feed additives, there are a number of biological, technical, and practical challenges that need to be overcome in order to maximize the potential of these feed additives in poultry production.

Postbiotics Standardization and Characterization; Another major problem is the non-uniformity of postbiotic preparations. The microbial source, fermentation conditions, and processing technique, as well as the

metabolite profile of postbiotics, can vary, and this may have a direct effect on bioactivity. Devoid of uniform protocols, batch-to-batch variability can be realized and result in different performance results across trials and farms. Such advanced techniques in analysis as liquid chromatography-mass spectrometry (LC-MS), nuclear magnetic resonance (NMR), and metabolomic profiling can be used to correctly describe bioactive components, including short-chain fatty acids (SCFAs), bacteriocins, exopolysaccharides, and peptides. Standardization is essential to reproducibility as well as regulatory approval, quality control, and commercialization.

Dose Optimization and Formulation Strategies: The use of non-nutritive additives with postbiotics and the determination of the best dosage, timing, and combination is tricky. Additive efficacy may be greatly determined by poultry species, age, gut microbial composition, and type of diet (Markowiak and Slizewska, 2018). An example is that broilers can be given increased postbiotic supplementation at early stages to help bolster gut barrier formation, while layers can be put on customized formulations to help them produce eggs and resist immune attack. Additionally, the synergistic associations (e.g., postbiotic + prebiotic, postbiotic + organic acid,

postbiotic + phytogetic compounds) should be optimally adjusted because high levels of them can adversely impact feed consumption, nutrient digestion rate, or microbiota composition.

Multi-Omics Mechanistic Understanding: One of the greatest limitations of existing studies is the poor knowledge of the molecular pathway of host-microbe-additive interactions. Multi-omics tools such as metagenomics, metatranscriptomics, metabolomics, and proteomics can be used to investigate the role of additives in remodelling the microbial community in detail, its functional potential, and its metabolic outputs (Olayiwola & Adedokun, 2025). Combining these datasets to host transcriptomic and immunological reactions may reveal mechanistic pathways of immune regulation, nutrient uptake, and stress resilience, to enable the development of targeted interventions.

Precision Nutrition and Microbiome Engineering: The future of poultry feed additive studies is in the emergent models of precision nutrition and microbiome engineering. Using information about genomics, microbiome, and environment, nutritionists can create specific additive approaches to the diet that enhance the growth, immune performance, and resistance to pathogens on an individual basis or in a flock. Microbiome engineering can involve selective manipulation of microbial consortia by the use of postbiotic and prebiotic mixes, specific taxa to stimulate production of short-chain fatty acids, competitive displacement of pathogens, or resistance to heat, crowding, or disease challenge conditions.

Dilemmas of Translation and Regulation: Experimental research proves to be effective in controlled settings, but it is hard to replicate this research in real-world scenarios involving commercial poultry businesses. Additive performance depends on factors like processing of feeds, storage stability, environmental stressors, load of pathogens, and the farm management practices (Markowiak and Slizewska, 2018). Besides, feed additive regulations demand that they prove safety, uniformity, and quantifiable benefits. These are the key areas that need to be dealt with to be used extensively and integrated into the market.

Limitations: Despite the growing interest in postbiotics and non-nutritive feed additives, several important limitations currently restrict their widespread application in poultry production. One of the major challenges is the lack of standardized formulations, as postbiotic preparations vary widely in composition depending on microbial strains, culture conditions, and processing methods. This variability leads to inconsistent results across different studies, making direct comparison and generalization difficult. In addition, most available evidence is derived from controlled laboratories or small-scale experimental trials, with limited validation under commercial field conditions. Another key limitation is the absence of well-defined dose-response relationships, as optimal inclusion levels for consistent biological and productive responses have not yet been clearly

established. Collectively, these gaps highlight the need for standardized protocols, large-scale validation studies, and systematic dose optimization to support reliable and practical application in poultry nutrition.

Future Research Priorities: Future research should focus on generating a deeper mechanistic and application-oriented understanding of postbiotics and their synergistic use with non-nutritive feed additives. A key priority is comprehensive compositional characterization of postbiotic preparations, including strain-specific metabolite profiling and identification of bioactive compounds responsible for functional effects. In parallel, studies should investigate synergy mechanisms using quantitative dose-response models and interaction frameworks across different poultry breeds, production stages, and environmental conditions.

A major advancement is expected through multi-omics integration, combining metagenomics, transcriptomics, proteomics, and metabolomics to unravel host-microbiome-diet interactions at a systems biology level. This should be complemented by precision nutrition approaches, enabling diet formulations tailored to microbiome composition, bird physiology, and production goals. In addition, microbiome engineering strategies, including targeted modulation of microbial communities and metabolic pathways, should be explored to enhance functional stability and resilience of the gut ecosystem.

Another critical area is the validation of laboratory findings under real-world conditions through large-scale, long-term field trials across commercial poultry production systems. These studies are essential to evaluate consistency, scalability, and practical effectiveness under variable management and environmental stresses. Furthermore, research should address the economic feasibility of postbiotic production by optimizing low-cost fermentation technologies, valorizing agro-industrial by-products, and improving downstream processing efficiency.

Addressing these research priorities will facilitate the development of robust postbiotic-based feed strategies capable of replacing antibiotic growth promoters, while enhancing poultry health, productivity, and sustainability within modern precision livestock systems.

Conclusions: Postbiotics and non-nutritive feed additives represent promising and sustainable alternatives to antibiotic growth promoters in poultry nutrition. Their primary value lies in their ability to modulate gut microbial balance, enhance intestinal barrier integrity, and regulate immune function, collectively leading to improved growth performance, FCR, and overall bird health. When used in combination, these additives may exert complementary and synergistic effects by supporting beneficial microbial populations, enhancing short-chain fatty acid production, and maintaining immune homeostasis.

A key advantage of these strategies is their alignment with global demands for antibiotic-free poultry production, improved food safety, and more sustainable livestock systems. However, their practical application still requires standardization of formulations, optimization of dosage, and validation across different poultry

genotypes and production environments to ensure consistency and reliability.

Future progress will depend on integrating multi-omics approaches to better understand host–microbiome–additive interactions and to support the development of precision nutrition strategies. In addition, large-scale field trials and microbiome-informed interventions, including microbiome engineering, are essential to translate laboratory findings into commercially viable solutions. Overall, postbiotics and non-nutritive feed additives offer a scientifically grounded pathway toward improving poultry productivity while reducing reliance on antibiotics, supporting the transition toward more resilient, efficient, and sustainable poultry production systems.

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