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Dr. B Dhanunjaya
Postgraduate Student, Department of Animal Nutrition, Rajiv Gandhi Institute of Veterinary Education and Research (RIVER), Puducherry, India

Corresponding Author:
Dr. B Dhanunjaya
Postgraduate Student, Department of Animal Nutrition, Rajiv Gandhi Institute of Veterinary Education and Research (RIVER), Puducherry, India

Post-biotics as emerging next-generation feed additives in poultry nutrition: A review of gut health, immunity, and production performance

B Dhanunjaya

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Abstract
Following the ban of antibiotic growth promoters from poultry production, there has been much research on safe nutritional alternatives that can be used to improve gut health and productive performance. Among the alternatives that have been investigated, post-biotics have attracted much attention due to their advantages over viable microorganisms. According to the International Scientific Association of Probiotics and Prebiotics (ISAPP), post-biotics are defined as preparations of inactivated microorganisms and/or their components that confer a health benefit on the host. The resistance to feed-processing conditions, longer storage stability, better safety profile, and convenience make post-biotics a functional feed additive in modern poultry production systems. The present review summarizes recent (2020-2025) developments in the manufacture, characterization, biological modes of action, and application of post-biotics in poultry nutrition. Postbiotic preparations obtained from lactic acid bacteria, *Bacillus* spp., yeasts, and other microbial sources contain functional bioactive compounds such as organic acids, short-chain fatty acids, bacteriocins, exopolysaccharides, enzymes, peptides, and cell wall components. They are bioactive compounds involved in stabilizing intestinal microbiotas, fortifying the epithelial barrier, and controlling immune responses, antioxidant activity, and nutrient absorption. Studies show that feeding post-biotics to broilers, layers, and breeder birds improves growth performance, feed conversion, intestinal morphology, disease resistance, meat quality, and other productive performance-related factors. However, responses to post-biotics can vary greatly depending on various factors such as microbial strains and production technologies, inactivation methods, dosages and feeding modes, genotypes, physiological condition, and environmental conditions of the birds.
Although evidence supports the potential of post-biotics as next-generation functional feed additives, there is little known about optimal dose selection, mechanisms of action, safety, and commercial-scale validation. Future studies using microbiome profiling, metabolomics, transcriptomics, and controlled challenges will help us better understand the host–microbe interactions and develop standard, evidence-based postbiotic products for sustainable poultry production.

Keywords: Post-biotics, poultry nutrition, gut microbiota, intestinal health, immune modulation, antioxidant activity, feed additives, sustainable poultry production

Introduction
Poultry has become one of the fastest-growing livestock industries worldwide because of its ability to produce high-quality meat and eggs. Genetic improvement, balanced nutrition, and management have enabled modern strains of poultry to grow rapidly, improve feed efficiency, and productivity. Such improvement has also improved the bird's ability to use energy, protein, vitamins, and minerals effectively. Despite these advances, poultry are still vulnerable to diseases, environmental stresses, poor management, and nutritional deficiencies. Changing diet, heat stress, high stocking density, pathogen exposure, and intestinal disorders can impact gut function and bird performance. Maintaining intestinal health has become an important concern of modern poultry nutrition. The gastrointestinal tract is not only crucial for the digestion and absorption of nutrients but is also a key defense against pathogens and regulates the function of the immune system. The gut microbiota is instrumental in the metabolism of nutrients, the development of intestinal epithelium after birth, the development of the immune system, and helps protect the host against colonization by harmful pathogens.

A balanced microbial community protects the intestinal wall from destruction and contributes to the efficient use of nutrients. Dysbiosis, or disruption of the normal gut microbiota, can result in symptoms. Dysbiosis has the potential to reduce digestibility of nutrients and feed efficiency, alter intestinal morphology, lead to wet litter production, degrade carcass quality, and increase the risk of food-borne illness, causing economic losses and food safety concerns (Hou *et al.*, 2022; Zhong *et al.*, 2022; Roque *et al.*, 2025) [33, 99, 70].

As efforts to curb antimicrobial resistance worldwide increase and restrictions on the use of antibiotic growth promoters tighten, more feed additives have been sought as safer, better alternatives for poultry. Among the new options being studied is biological feed additive. It has shown potential to help improve gut health and performance without increasing antibiotic resistance. Live feed additives are less effective because they lose viability during feed processing, especially pelleting, and in storage. Also, some probiotic strains have varying numbers of lives and may not be safe to be used. Post-biotics are a promising solution that is able to replicate the beneficial effects of microbial fermentation without the need for live microorganisms. Because of their stability in feed production and storage, ease of handling, and compatibility with existing feed production systems, post-biotics are especially useful to poultry. But, the biological activity of post-biotics is limited to the type of microbial strain, fermentation conditions, and inactivation process used in their production and not by the mere presence of inactive microbial cells (Urban *et al.*, 2024; Saeed *et al.*, 2023; Waqas *et al.*, 2024) [84, 72, 88].

This review summarizes recent advances in the production, mechanisms of action, and applications of post-biotics in poultry nutrition, with particular emphasis on their role in improving gut health, modulating immune function, controlling enteric pathogens, and enhancing growth performance, feed efficiency, meat quality, and reproductive performance. It also discusses current challenges, knowledge gaps, and future research directions to support the development of post-biotics as next-generation feed additives.

2. Literature Search and Review Methodology

A narrative and integrative review of the literature published between January 2020 and December 2025 was conducted to summarize current knowledge on the use of post-biotics in poultry nutrition. Relevant studies were identified through systematic searches of PubMed, Scopus, Web of Science, and Crossref-linked databases using combinations of Boolean operators and keywords including *postbiotic*, *paraprobiotic*, *inactivated microorganism*, *cell-free supernatant*, *fermented feed*, *microbial metabolite*, *broiler*, *layer*, *breeder*, *poultry*, *gut microbiota*, *intestinal barrier*, *immunity*, *antioxidant*, *Salmonella*, *Campylobacter*, *Escherichia coli*, *Clostridium perfringens*, *growth performance*, *feed conversion ratio*, *meat quality*, *carcass traits*, and *egg quality*.

Only peer-reviewed articles published in English between 2020 and 2025 were considered for inclusion. Eligible studies were required to have a valid DOI and focus on post-biotics, poultry nutrition, microbial metabolites, intestinal barrier function, gut microbiota, analytical characterization, or safety evaluation. Books, book chapters, conference abstracts, dissertations, preprints, and non-peer-reviewed online sources were excluded. Priority was given to studies conducted in poultry, including broilers, laying hens, and breeder flocks. However, research involving pigs, ruminants, fish, laboratory animals, cell culture systems, food matrices, or human subjects was included when it provided relevant information on biological mechanisms, analytical methods, product stability, or safety that could be translated to poultry production.

Because of substantial variation among postbiotic preparations, microbial strains, production methods, dosages, experimental conditions, disease challenge models, and outcome measures, a quantitative meta-analysis was not considered appropriate. Instead, the available evidence was synthesized using a thematic narrative approach. Bibliographic information, including publication year, journal, and DOI, was verified for each study, and articles were screened for duplication and retraction status before inclusion. This review was conducted as a narrative review rather than a PRISMA-based systematic review. Consequently, it did not follow a prospectively registered protocol, perform exhaustive study screening, or include quantitative evidence synthesis. Instead, the emphasis was placed on critically evaluating and integrating recent findings to provide a comprehensive overview of post-biotics as emerging feed additives in poultry nutrition.

3. What are post-biotics and how are they defined?

The International Scientific Association of Probiotics and Prebiotics (ISAPP) defines post-biotics as "prepared of inanimate microorganisms and/or parts that confer a health benefit on host" (Salminen *et al.*, 2021) [74]. Of these three points, the microorganisms must be well-characterized, the inactivation process must be intentional, and described, and the final preparation must have measurable health benefits. Although purified microbial metabolites may exhibit biological activity, they are not considered post-biotics unless they are present as part of the final inactivated microbial preparation. Post-biotics is distinct from other microbial products and is not a generic term for organic acids, antimicrobial peptides, cell-free supernatants, or other fermentation products that do not meet ISAPP criteria (Vinderola *et al.*, 2022; Scott *et al.*, 2022; Ma *et al.*, 2023) [86, 76, 54].

It is essential to use the correct language in scientific communication and product development. There are a number of similar terms commonly used in poultry nutrition. Probiotics are live organisms that are beneficial in sufficient amounts when administered as well as prebiotics, which are substances that provide specific nutrients to the beneficial bacteria in their host, either to grow or to function. Synbiotics are those with live probiotics in combination with prebiotic substrates for added bioavailability. Paraprobiotics are non-live microbial cells or fragments that might retain biological activity (No international standard exists for the term yet) (Swanson *et al.*, 2020; Siciliano *et al.*, 2021; Nataraj *et al.*, 2020; Zókiewicz *et al.*, 2020) [82, 78, 57, 100].

Fermented feed products often contain complex mixtures of viable microorganisms, inactivated cells, microbial metabolites, residual fermentation substrates, and cell-wall components. So, not every fermentation product should be considered a postbiotic. This term should only be used on those preparations that meet the ISAPP definition, that are properly characterised and that have a demonstrated health benefit. Consensus documents have stressed the need to clearly describe viability, composition of the product, manufacturing techniques, and identity of the final product for consistent terminology and easier comparisons between studies.Accurate nomenclature is particularly important for the commercial development of poultry feed additives. Heat-inactivated bacterial biomass, clarified fermentation supernatants, yeast fermentation products, and spray-dried fermentates differ substantially in their chemical composition, stability, mechanisms of action, and regulatory status. Consequently, products marketed as post-biotics cannot be assumed to produce similar biological responses simply because they share the same general classification. Their efficacy depends on several factors, including the microbial strain, fermentation substrate, culture conditions, inactivation method, processing technology, and the composition of the final preparation. Comprehensive characterization of these factors is therefore essential for ensuring product identity, reproducibility, and reliable evaluation of biological efficacy.

Table 1: Definitions and Distinguishing Characteristics of Major Microbiome-Related Feed Additives

Feed Additive	Definition	Viability	Major Components	Primary Action	Main Advantage	Main Limitation
Probiotic	Live microorganisms that confer a health benefit on the host	Required	Viable bacteria or yeast	Competitive exclusion, metabolite production, immune modulation	Supports a healthy gut microbiota	Reduced viability during processing and storage
Prebiotic	Substrate selectively utilized by beneficial microorganisms	Not applicable	Non-digestible carbohydrates and oligosaccharides	Stimulates beneficial gut bacteria	Stable during feed processing	Effect depends on existing microbiota
Synbiotic	Combination of probiotics and prebiotics	Required (probiotic)	Live microbes + prebiotic substrate	Synergistic microbial and metabolic effects	Enhanced gut health	Requires careful formulation
Postbiotic	Preparation of inanimate microorganisms and/or their components that confer a health benefit	Not required	Inactivated cells and microbial metabolites	Improves gut barrier, immunity, and antimicrobial activity	High thermal and storage stability	Product composition varies among manufacturers
Paraprobiotic	Non-viable microbial cells or cell components	Not required	Inactivated microbial cells	Immune modulation through cell components	Improved safety	Terminology not yet standardized
Fermented Feed	Feed produced through microbial fermentation	Variable	Fermented substrate, microbes, metabolites	Improves feed quality and gut health	Nutritional and microbial benefits	Not all fermented feeds are post-biotics

4. Sources, Components, and Production of Post-biotics

Post-biotics are produced from a variety of microorganisms, but most widely from lactic acid bacteria (LAB). The most common genera of LAB include Lactiplantibacillus, Lacticaseibacillus, Limosilactobacillus, Lactobacillus, Bifidobacterium, Bacillus, and Saccharomyces. The organisms produce a range of bioactive compounds, including organic acids, short-chain fatty acids, bacteriocins, enzymes, bioactive peptides, exopolysaccharides, vitamins, and other secondary metabolites. The yeast and fungal fermentations also produce -glucans, mannans, nucleotides, peptides, and antioxidants. The composition of the postbiotic is heavily dependent on the microbial strain, fermentation substrate, culture conditions, and growth phase. Even closely related microorganisms can differ significantly in their antimicrobial, antioxidant, and immunomodulatory activities (Chang *et al.*, 2020; Kiran *et al.*, 2023; Sigala-Robles *et al.*, 2024; Xie *et al.*, 2024) [13, 45, 79, 90].

The production of post-biotics is initiated by controlled microbial fermentation and the bacteria are then intentionally killed, while still maintaining their biological activity. Different inactivation methods have been employed, including heat treatment, high-pressure processing, ultrasonication, enzymatic lysis, irradiation, and chemical methods. Other processing steps may include centrifugation, filtration, freeze-drying, spray-drying, microencapsulation, and absorption onto carrier materials, to maintain product stability, store product, or separate microbial biomass from cell-free supernatant. Inactivation is not only an important preservation step, but also an important factor in product composition and function. Processing may remove heat-sensitive bacteriocins and enzymes as well as peptidoglycan, lipoteichoic acid, teichoic acids, nucleic acids, and intracellular components, while at the same time expose other proteins such as peptidoglycan, lipoteichoic acid, teichoic acids, nucleic acids, and intracellular metabolites. The method used to produce post-biotics may influence the receptor, biological activity, particle size, solubility, and shelf stability (Peluzio *et al.*, 2021; Park *et al.*, 2022; Pimentel *et al.*, 2023) [60, 58, 61].

Analyzing postbiotic products is critical to ensure product integrity and reproducibility. Mass spectrometry (MS) and nuclear magnetic resonance (NMR) analysis have been used to identify bioactive metabolites, along with high-throughput screening, metabolomics, and computational analyses, allowing the identification of chemical signatures that are associated with antimicrobial, antioxidant, immunomodulatory, and other biological activities (Chan *et al.*, 2021; Bauermeister *et al.*, 2022; Ye *et al.*, 2022; Ayon, 2023) [12, 6, 91, 4].

Post-biotics are composed of more than one active ingredient: organic acids, short-chain fatty acids, bacteriocins, exopolysaccharides, lipoteichoic acid, peptidoglycan fragments, phenyllactic acid, cell-free supernatant constituents, enzymes, peptides, nucleotides, -

glucans, mannans, and other microbial metabolites (Giordani *et al.*, 2023; Shin *et al.*, 2023; Vitale *et al.*, 2023) [29, 77, 87]. The relative concentration of these compounds varies depending on the strain and manufacturing process, highlighting the need to characterize chemical composition prior to measuring effectiveness.

Table 2: Major Sources, Components, and Functional Properties of Post-biotics Used in Poultry Nutrition

Microbial source	Postbiotic preparation	Major active components	Proposed biological activity	Poultry application	Supporting reference
Lactiplantibacillus / Lacticaseibacillus / Lactobacillus	Fermentation followed by heat inactivation, clarification or drying	Lactic and acetic acids, bacteriocins, peptides, exopolysaccharides, cell-wall ligands	Pathogen inhibition, barrier support, immune modulation, antioxidant effects	Broiler gut health and performance; challenge support	Chang *et al.*, 2020; Monika *et al.*, 2024 [13, 56]
Bacillus spp.	Fermentation, sporulation-derived products or inactivated biomass	Enzymes, peptides, lipopeptides, cell-wall components	Digestive support and pathogen restraint	Potential use in resilient pelleted feeds	Ramlucken *et al.*, 2020; Zhong *et al.*, 2022 [66, 99]
Saccharomyces cerevisiae	Yeast fermentation product or inactivated yeast fractions	Mannans, beta-glucans, nucleotides, metabolites	Pathogen binding, immune modulation, stress resilience	Layers, broilers and breeders	Gingerich *et al.*, 2021; Forder *et al.*, 2024 [28, 27]
Aspergillus oryzae/fermented fungi	Controlled fungal fermentation and drying	Peptides, enzymes, organic acids and secondary metabolites	Stress attenuation and digestive effects	Heat-stress and feed-efficiency applications	Kaufman *et al.*, 2021; Lin & Lee, 2021 [41, 52]
Enterococcus/Lactococcus	Whey or food-substrate bioconversion	Organic acids, peptides, bacteriocin-like metabolites	Antimicrobial and gut-health activity	Candidate mixed-culture preparations	Park *et al.*, 2021 [58]
Mixed fermented substrates	Co-fermentation, cell separation and stabilisation	Composite acids, enzymes, peptides, cell fragments	Multifactorial gut and antioxidant effects	Broiler diets and functional feed ingredients	Chuang *et al.*, 2021; Urban *et al.*, 2024 [18, 84]

5. Mechanisms of action of post-biotics in poultry health and production

The biological effects of post-biotics are mediated by several mechanisms, which act both at their site of action in the gastrointestinal tract and systemically by influencing host physiological and immune responses. Such complementary actions result in better intestinal health, increased immune function, nutrient utilization and overall poultry performance. The organic acid generation is one of the main antimicrobial activity mechanisms that help reduce the pH of the gut and thus make it less favorable for the growth of acid-sensitive pathogenic bacteria. Moreover, bacteriocins and antimicrobial peptides cause disruptions in the cell membrane of bacteria and halt the growth of harmful microorganisms. Other components secreted by microbes such as biosurfactants, and EVs (extracellular vesicles), modulate pathogen adhesion and biofilm formation to limit their interactions with the intestinal epithelium. Many studies have been carried out *in vitro* to show these antimicrobial and antibiofilm properties, however, the effectiveness of *in vivo* studies depends on feed composition, buffering capacity of digesta, proteolytic degradation, dosage, contact time, etc. (Khani *et al.*, 2023; Ebrahimi *et al.*, 2021; Vitale *et al.*, 2023; Giordani *et al.*, 2023) [43, 25, 87, 29].

Along with the benefits of improving epithelial integrity, promoting mucin production, and interacting with the intestinal microbiota, post-biotics also have many other functions to strengthen the function of intestinal barrier. Peptidoglycan, lipoteichoic acid, exopolysaccharides, nucleotides and microbial metabolites interact with pattern recognition receptors, including Toll-like receptors, which help to regulate immune responses and antioxidant signaling pathways. Some bioactive molecules have been identified with certain physiological attributes, such as polyphosphate (associated with epithelium regeneration) and lipoteichoic acid (anti-inflammatory property) (Isozaki *et al.*, 2021; Bae *et al.*, 2022; Pradhan *et al.*, 2023) [35, 5, 63].

The immunological activity, however, depends on the amount of the post-biotics, and an overstimulation of the receptors leading to immune activity might result in undesirable immune response. Thus the dosage and physiological status of host should be taken into consideration while assessing the efficacy of post-biotics. Another mechanism that plays a key role is modulating gut flora. Post-biotics are not introduced directly into the gastrointestinal tract, but rather work as modulators of the micro-ecology that lead to the inhibition of pathogenic microorganisms, favouring friendly bacteria cross-feeding, changing intestinal redox conditions, supplying metabolites of bacterial fermentation entailed to maintain the micro-ecological homeostasis of the gastrointestinal tract. animals, post-biotics can have effects on the composition of the microbial community, on the integrity of the intestinal barrier and on host metabolism (Zhang *et al.*, 2022b; Ryu *et al.*, 2022; Izuddin *et al.*, 2020; Rawling *et al.*, 2023) [96, 71, 36, 67]. However, future research is needed to further identify causal links between changes in the microbiome and health and production benefits in poultry, using combined microbiome, metabolomics and transcriptomics.

6. Effects of post-biotics on intestinal health and gut barrier function

Post-biotics are crucial to gut health as they normalize intestinal morphology, reinforce the integrity of the epithelial barrier and enhance the antioxidant capacity. Several experiments have shown that feeding post-biotics can enhance and support the growth and integrity of the digestive tract of poultry. A Lactobacillus acidophilus postbiotic enhanced growth performance and intestinal development in slow growing broilers; a Lactiplantibacillus plantarum postbiotic successfully reduced the use of ABP in slow growing broilers. Likewise, broiler chicken subjected to colisepticemia also gained benefits from Lactobacillus-based PBs for beneficial effects on gut health and minimizing intestinal damage (Chang *et al.,* 2022; Monika *et al.,* 2024) [14, 56].

But these responses differ with strain of the microbe, formulation, dose and bird genotype as well as under challenge conditions. There is a good correlation between the improvement of intestinal function and the improvement of villus architecture, villus height to crypt depth ratio and maintenance of the TJP (*e.g*. occludin, claudins and zonula occludens), mucus secretion and decreased intestinal permeability. The changes observed enhance the intestinal barrier, the nutrient absorption and the lower potential translocation of pathogenic microorganisms. Another study also found that fermented fungus extract and cell wall extract from yeast enhanced the numbers of goblet cells and improved intestinal morphology as a supportive agent for the protection of mucous membranes (Lin and Lee, 2021; Pascual *et al.,* 2020) [52, 59]. Favorable intestinal morphology is far from a sure sign of increased body weight, or feed efficiency, however pointing to other factors besides gut morphology.

However, recent studies have indicated that the efficacy of post-biotics is also affected by the composition of the food that they are fed. In broiler production research, fiber modification, protein digestibility, buffering capacity, and antinutrient factor caused bird reactions to pro- or post-biotics to vary with changes in the fiber content of the diet. While alterations of gut microbial community was consistently detected, improvements of immune responses were less apparent (Jansseune *et al.,* 2024a; Jansseune *et al.,* 2024b) [37, 38]. Based on these results, it seems that the composition of the diet may be important in assessing the efficacy of the postbiotic in commercial production.

The health-promoting properties of post-biotics on the intestinal barrier function, inflammatory responses and microbial balance are also validated in studies conducted on pigs, ruminants, fish and laboratory animals (Feng *et al.,* 2022; Li *et al.,* 2023; Zhang *et al.,* 2023a; Jin *et al.,* 2024) [26, 50, 97, 40]. These results support its biological justification for post-biotics supplementation though more poultry targeted studies are needed. Future research needs to include additional outcomes to assess the mechanisms by which post-biotics enhance intestinal health in poultry, such as assess intestinal permeability along with expression of the tight junction proteins, production of short-chain fatty acids, function of gut microbiota, digestibility of nutrients, and lesion score.

7. Role of post-biotics in pathogen control and disease resistance

In poultry, post-biotics are used to prevent disease through various mechanisms such as lowering the population of pathogenic microorganisms, supporting the intestinal barrier, and regulating host immune response. The mechanisms of action of post-biotics are complementary to antibiotics, such as the production of antimicrobial metabolites, blocking the colonization of pathogens, use of antiadhesive properties, and strengthening the intestinal microbiota, and strengthening the intestinal barriers. These synergistic interactions favor intestinal homeostasis and prevent intestinal infections.

There are several studies that have proved the efficacy of post-biotics against the economically important poultry pathogens of the world. A layer pullet trial using a Saccharomyces cerevisiae-founded publish biarithmetic system lowered enteric Salmonella colonization of the cecal tissue which suggests it might be used as a preharvest intervention to enhance flock health and food safety (Gingerich *et al.,* 2021) [28]. The same for Lactobacillus helveticus postbiotic in commercial layer chicks which inhibited the presence of Salmonella Gallinarum in the guts (Ribeiro *et al.,* 2023) [68]. Control of these pathogens in the intestine and/or in the faeces may help limit the spread of these pathogens in flocks and the risk of faecal contamination of poultry products in the food chain.

Additionally, intestinal health and the prevention of pathogen-induced damage to intestinal tissues may help protect against other enteric pathogens, as the case of post-biotics. In broilers challenged with enteric pathogens, beneficial effects on intestinal morphology, gut permeability, microbial composition of the cecum, and biochemical responses have been reported. Many studies that currently exist, however, have compared the effects of post-biotics with other feed supplements, together with prebiotics or probiotics and it is challenging to obtain information on the effect of post-biotics alone. Therefore, more controlled experiments from various programs are required to identify their independent effects in commercial poultry settings.

Results for control of Clostridium perfringens and *Campylobacter* spp. and pathogens responsible for coccidiosis are promising but are limited. There are various reports in the literature for reduced lesions, integrity of the intestine and microbial balance, but these responses are varying depending on the type of microbial strain and composition of the post-biotics, the disease challenge and the strain of diet (Ramlucken *et al.,* 2020) [66]. Standardised methods for investigating the efficacy of post-biotics against these pathogens need to be developed.

In vitro studies have also shown that post-biotics exhibit antimicrobial properties against S. aureus, as well as against fungi producers of A. flavus, microbial biofilms, and some models of viral infections (Ebrahimi *et al.,* 2021; Vitale *et al.,* 2023; Khani *et al.,* 2023; Kumar *et al.,* 2023) [25, 87, 43, 46]. The results are significant in terms of the mechanisms and need to confirm through well-designed poultry challenge studies prior to advice being given in practice.

The use of standard pathogen challenge models using more than one outcome measure, such as pathogen shedding, tissue invasion, lesion severity, mortality, AMR, persistence during poultry processing, and overall production performance, should be followed in future investigations. Erica's work through such in-depth studies will give better proof of post-biotics disease control and food safety.

8. Immunomodulatory effects of post-biotics in poultry

The avian immune system is central to combating infectiousdiseases in birds and is essential for tolerance to gut-not-damaging microbes and antigens in food. The intestinal immune system plays a critical and always active role in interacting with the gut microbiota and sustaining intestinal homeostasis as an important part of gut-associated lymphoid tissue (GALT). A growing body of evidence indicates that post-biotics play a role in both innate and adaptive immune regulation, which involves regulatory metabolites of the microbes and structural components of the microbial cells. Some bioactive components in post-biotics, such as peptidoglycan, lipoteichoic acid, exopolysaccharides and microbial metabolites, engage with immune cells including heterophils, macrophages, dendritic cells, and lymphocytes. All these interactions play a crucial role in controlling the production of cytokines, synthesis of immunoglobulin and activity of immune organs, which leads to better defense against pathogenic organisms. Experimental results have demonstrated that application of heat-inactivated Lactiplantibacillus has positive effects on immune responses and enhances intestinal health and growth performance of poultry as well (Bae *et al.*, 2022; Chuang *et al.*, 2021; Abd El-Ghany *et al.*, 2022) [5, 18, 1].

The goal of postbiotic supplementation should be maintaining immune homeostasis rather than activating immune system. A well-regulated immune response will allow birds to mount an appropriate immune response against pathogenic microorganisms and not overreact and respond to create excessive inflammation that may lead to poor growth and nutrient absorption. In situations of low inflammation, nutrients are free to promote growth and production instead of stimulating the immune system. For instance, some postbiotic products developed from yeast have been reported to improve goblet cell function and modulate inflammatory responses, but not necessarily all the improvements in terms of productive performance have been consistent (Pascual *et al.*, 2020) [59]. Likewise, it was reported that a significant modulation was possible by improving broiler performance (Ward *et al.*, 2020), although immune parameters have been changed only slightly. Another recent study with broilers demonstrated that while the gut microbiota was significantly affected, immune responses were measured with only minor changes (Jansseune *et al.*, 2024b) [38].

Additionally, studies in poultry and other animal species have shown that heat-strengthened cultures of Bifidobacterium, Saccharomyces, or other microbes can dampen overactive inflammatory signals, maintain gut barrier integrity and boost gut immune function (Choi *et al.*, 2021; Zhang *et al.*, 2022a; Feng *et al.*, 2022; Jin *et al.*, 2024) [17, 95, 26, 40]. Increases in individual cytokines, especially without an increase in resistance to the infection and disease or improvements in production, should not be taken as a sign of improved immunity. Immune responses should be assessed in the future in the context of a full complement of immune system biomarkers including cytokine profiles, secretory immunoglobulin A (sIgA), systemic antibody responses, and responses to vaccination and/or pathogen challenge. Furthermore, the effects of age, health status, diet composition, postbiotic dose, and economics need to be taken into account for assessing the potential of post-biotics in commodity poultry feed.

Table 3: Effects of post-biotics on poultry gut health, immunity, and pathogen control

Author and year	Poultry type	Postbiotic source	Dose or administration	Study duration	Main outcome	DOI
Monika *et al.* (2024) [56]	Broilers	*L. acidophilus*-derived postbiotic	0.2%, 0.4%, or 0.6% in feed	42 days	Improved growth-related indices, intestinal morphology, antioxidant status and caecal microbial profile; response was dose-dependent	https://doi.org/10.1038/s41598-024-74078-0
Abd El-Ghany *et al.* (2022) [1]	Colisepticaemic broilers	Stabilised non-viable *Lactobacilli*	Dietary supplementation	Study-defined trial period	Supported health, immunity, growth and gut status under E. coli challenge	https://doi.org/10.1007/s11250-022-03300-w
Chang *et al.* (2022) [14]	Broilers	*L. plantarum* post-biotics	Dietary inclusion	Study-defined broiler cycle	Demonstrated gut-health potential as an antibiotic-growth-promoter alternative	https://doi.org/10.3389/fvets.2022.883324
de Souza *et al.* (2024) [22]	Broilers	Probiotic, paraprobiotic and postbiotic combination	Oral/dietary study protocol	Challenge period	Modulated intestinal changes from C. perfringens and deoxynivalenol; independent postbiotic effect cannot be isolated	https://doi.org/10.3390/toxins16010046
Gingerich *et al.* (2021) [28]	Layer pullets	*S. cerevisiae* fermentation-derived postbiotic	Dietary feeding	Preharvest challenge study	Reduced caecal Salmonella Enteritidis concern as a food-safety hurdle	https://doi.org/10.4315/JFP-20-330
Ribeiro *et al.* (2023) [68]	Commercial layer chicks	*L. helveticus* ATCC 15009-derived postbiotic	Study-defined administration	Early-life challenge study	Mitigated Salmonella Gallinarum colonisation	https://doi.org/10.1016/j.psj.2023.103095
Jansseune *et al.* (2024b) [38]	Broilers	*Lactobacilli* pro- and postbiotic	Diet × additive factorial design	35 days	Microbiota effects were associated with diet–additive interaction without clear immune modulation	https://doi.org/10.1016/j.psj.2024.104184

9. Antioxidant effects of post-biotics in poultry

Oxidative stress is a significant problem in today's poultry industry, and frequently is caused by heat stress, high stocking density, mycotoxin exposure, pathogenic infection, etc. Oxidative stress is a big problem in the modern day with poultry production and is often triggered by heat stress, overcrowding, exposure to mycotoxins, pathogenic infections and other environmental stressors. These factors generate greater amounts of Reactive Oxygen species (ROS) which leads to elevated levels of lipid peroxidation, resulting in damage to the cells, poor immunity and diminished growth. Post-biotics have been shown to exert direct antioxidant activity, as well as influence the body's own endogenous antioxidant system, which further indicates their role in reducing oxidative stress. The products of biotransformation are diverse antioxidants metabolites and bioactive compounds with the ability to neutralize free radicals and reduce oxidative damages as a result of which post biotin products have been prepared. Additionally, post-biotics may act by increasing the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) along with activation of the nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway. The use of diet supplementation with post-biotics was demonstrated to have beneficial effects on antioxidant enzyme activity, decreased lipid peroxidation, reduced physiological stress, serum lipid profile and meat quality in heat-stressed broilers (Humam *et al.,* 2020) [34]. Post-biotics from Lactobacillus acidophilus and fermented fungal formulations have also been reported to have antioxidant and gut health benefits, respectively (Lin & Lee, 2021; Monika *et al.,* 2024) [52, 56].

The antioxidant activity of a postbiotic varies in intensity depending on the strain of the microorganism, its fermentation substrate, and the fermentation process. Many different postbiotic preparations have been shown to exhibit differences in concentrations of organic acids, phenols, peptides with antioxidant activities, and other molecules that inhibit free radicals, in metabolomic and chemical profiling studies. The antioxidant activity and various bioactive metabolite contents of post-biotics produced by Lactiplantibacillus plantarum, *Limosilactobacillus fermentum* and *Weissella* spp. are different (Chang *et al.,* 2020; Kiran *et al.,* 2023; Grishina *et al.,* 2023) [13, 45, 30]. Hence, the *in vitro* antioxidant activity is not necessarily correlated with the *in vivo* biological effect as it is modified by intestinal environment, metabolism and digestion.

Other studies in other livestock species also corroborate the antioxidant properties of post-biotics. Lactiplantibacillus preparations have increased the antioxidant capacity and integrity of the intestinal barrier in lambs, and Aspergillus oryzae post-biotics have decreased the effects of heat stress in various animal models (Izuddin *et al.,* 2020; Kaufman *et al.,* 2021) [36, 41]. These results do support the biological rationale for the use of post-biotics in poultry nutrition but further research is required to validate their efficacy in commercial poultry production conditions.

Future research needs to consider multiple parameters of oxidative stress, such as malondialdehyde (MDA), antioxidant enzyme activity (catalase, CAT; superoxide dismutase, SOD; and glutathione peroxidase, GPx), inflammatory cytokines, intestinal tissue damage, growth performance, and oxidative stability of poultry product. This integrated approaches will equip a more holistic knowledge of the interactive mechanisms of post-biotics action as antioxidants and its application in the poultry nutrition.

10. Effects of post-biotics on growth performance and production efficiency

One of the essential measures for determining the effectiveness of poultry feeds is their growth performance. Many studies have shown that supplementation of the diet based on post-biotics can increase feed intake, feed conversion ratio (FCR), production efficiency, and improve the body weight gain. These responses are greatly affected, however, by the nutritional status, bird genotype, management conditions and liposome preparation.

Lactobacillus-derived post-biotics, stabilized non-viable lactobacilli, microbial product-based fermentation, and yeast-based preparations have yielded promising results in various studies assessing the effects on growth performance, feed efficiency, and physiological health of fish (Monika *et al.* 2024; Abd El-Ghany *et al.* 2022; Chang *et al.* 2022; Chuang *et al.* 2021) [56, 1, 18, 14]. Conversely, certain studies have reported that intestinal tract health and immune system function were enhanced, while body weight and feed conversion ratio were unaffected. Likewise, feed conversion ratio was enhanced by yeast-cell wall related preparation during particular developmental stages of broilers, but these supplements yielded relatively poor results in terms of body weight and carcass traits (Pascual *et al.,* 2020) [59]. These results suggest gut health may not have a direct impact on production performance.

The composition of the diet can also influence the postbiotic supplementation. In recent studies on broilers there are large variation among the findings regarding the impact of post-biotics on growth performance, intestinal physiology and metabolism whether broils are fed nutritionally balanced diets or challenged by dietary or environmental stressors (Jansseune *et al.,* 2024a) [37]. Microbial cross-feeding and nutrient utilization are functions of microbial ecosystem, saltability, nutrient density, and nutritive value of dietary fiber, which can change the efficacy of post-biotics. A number of biological and management factors influence the responses evoked by post-biotics, such as: bird age, genetic strain, health status, environmental temperature, stocking density, hygiene, dose, period of the supplementation and type of microbial inactivation as well as the carrier material. Variableness of outcome reported across different studies points to the need for standardized assessment protocols, and these variables account for the different results reported.

Overall, available current evidence indicates that post-biotics have a significant potential to enhance nutrient utilization and production efficiency, especially when stressed by environmental or diseases (Urban *et al.,* 2024; Saeed *et al.,* 2023; Waqas *et al.,* 2024; Zhong *et al.,* 2022 [99]) [84, 72, 88, 99]. But under all practices there is not any proof to the improvement of growth rate consistently. Thus, assessment of postbiotic supplementation needs to be done beyond feed conversion ratio. This should be complemented by a comprehensive economic evaluation of the product, encompassing feed costs per kg of marketable product, mortality, medication costs, the processing loss, carcass value and overall flock profitability.

11. Effects of post-biotics on carcass traits and meat quality

Post-biotics may influence carcass characteristics and meat quality by improving nutrient utilization, modulating lipid metabolism, reducing oxidative stress, and enhancing intestinal health. Improved gut function and nutrient absorption can promote lean tissue deposition while reducing fat accumulation, thereby contributing to better carcass composition. In addition, the antimicrobial and antioxidant properties of post-biotics may improve the microbial safety and shelf life of poultry meat. Several studies have reported beneficial effects of postbiotic supplementation on carcass yield, meat quality, and oxidative stability, particularly under conditions of heat stress. Improvements have been observed in lipid peroxidation, antioxidant status, and meat quality parameters following dietary supplementation with post-biotics. Enhanced intestinal health has also been associated with increased breast muscle yield and reduced abdominal fat deposition. However, the effects of post-biotics on carcass traits are generally less consistent than their effects on gut health and feed efficiency. For example, supplementation with a yeast-derived cell wall preparation improved feed conversion and intestinal health but produced inconsistent responses in carcass characteristics (Pascual *et al.,* 2020) [59].

The effects of post-biotics on meat quality should be evaluated using a comprehensive range of carcass and physicochemical parameters, including dressing percentage, breast and thigh yield, abdominal fat percentage, muscle pH, drip loss, cooking loss, water-holding capacity, tenderness, color, lipid oxidation, fatty acid composition, sensory characteristics, and microbial shelf life. Standardized evaluation of these parameters will improve the comparability of studies and help establish the relationship between postbiotic composition and meat quality outcomes (Urban *et al.,* 2024; Waqas *et al.,* 2024) [84, 88].

Beyond their effects during poultry production, postbiotic metabolites also show potential for improving food preservation. Studies have demonstrated that antimicrobial compounds produced during microbial fermentation can inhibit spoilage microorganisms and foodborne pathogens in packaging systems and food matrices, suggesting potential applications as natural preservatives in poultry products (Mohammadi *et al.,* 2022; Anvar *et al.,* 2023) [55, 3]. Nevertheless, improvements in carcass characteristics resulting from dietary supplementation should not be interpreted as evidence of enhanced post-harvest meat preservation. Separate investigations are required to evaluate the direct effects of postbiotic compounds on meat storage stability, microbial safety, and shelf life.

12. Effects of post-biotics on laying performance and egg quality

Comparatively to broiler chickens, the research on the use of post-biotics in laying hens is less specific, but growing evidence suggests that post-biotics may help maintain intestinal health, reduce environmental and metabolic stress, and increase longevity throughout the laying cycle. This is important because commercial laying hens can be in a production environment for extended periods of time during which gut health is paramount to egg production and overall well-being. Post-biotics have been evaluated to control enteric pathogens in laying hens. A postbiotic from fermentation of Saccharomyces cerevisiae was used as a preharvest strategy to reduce cecal colonization by Salmonella Enteritidis in layer pullets (Gingerich *et al.,* 2021) [28]. A Lactobacillus helveticus postbiotic reduced intestinal colonization by Salmonella Gallinarum in commercial layer chicks (Ribeiro *et al.,* 2023) [68].

This study suggests that post-biotics may provide improved flock health and decrease the risk of foodborne pathogen transmission through the egg production chain. But, the available evidence does not provide consistent improvement in egg production or laying throughout the production process. Consider evaluating the efficacy of post-biotics in laying hens by assessing production, reproductive ability and egg quality. Egg production on hen day, egg mass, feed ratio per kilogram of egg mass, egg weight, eggshell strength, shell thickness, Haugh unit, albumen height, yolk color, yolk lipid profile, cholesterol concentration, oxidative status, mortality, and bone mineralization are measures that will help determine the beneficial and economic value of postbiotic supplementation. Even breeder chickens have also been sparsely studied. A fungicide derived from yeast Saccharomyces cerevisiae has been reported to enhance reproductive performance without impairing the intestinal barrier (Forder *et al.,* 2024) [27].

However, reproductive performance, intestinal health, and immune responses should be evaluated independently to accurately determine the specific effects of post-biotics. Future studies should focus on long-term experiments involving commercial laying hens and breeder flocks under practical production conditions. Multi-cycle studies that integrate productive performance, egg quality, gut health, immune function, and economic analyses will provide stronger evidence for the successful application of post-biotics in the poultry industry (Roque *et al.,* 2025; Urban *et al.,* 2024; Saeed *et al.,* 2023) [70, 84, 72].

Table 4: Effects of post-biotics on poultry production performance and product quality

Author and year	Bird type	Postbiotic product	Performance variables	Major findings	Study limitation	DOI
Monika *et al.* (2024) [56]	Broilers	*L. acidophilus*-derived preparation	Body weight, feed conversion, antioxidant and intestinal indices	Favourable performance and gut responses, with the lowest tested inclusion showing strong overall efficacy	Single breed and controlled setting	https://doi.org/10.1038/s41598-024-74078-0
Humam *et al.* (2020) [34]	Heat-stressed broilers	*Lactobacillus*-derived post-biotics	Stress, antioxidant enzymes, lipid oxidation, lipid profile and meat quality	Mitigated several adverse heat-stress responses	Results are stress-model and product specific	https://doi.org/10.3390/ani10060982
Pascual *et al.* (2020) [59]	Ross 308 broilers	Yeast cell-wall extracts	Growth, feed conversion, carcass and gut response	Improved feed conversion during parts of production and altered gut indices; final performance/carcass effects were limited	Yeast fraction is compositionally broader than a defined single postbiotic	https://doi.org/10.1186/s40104-020-00448-z
Lin & Lee (2021) [52]	Broilers	*Laetiporus sulphureus* fermented product	Antioxidant status, tight junctions and intestinal morphology	Enhanced antioxidant and barrier-related outcomes	Fermented product contains multiple constituents	https://doi.org/10.3390/ani11010149
Jansseune *et al.* (2024a)	Broilers	*Lactobacilli postbiotic*	Growth and physiological indices under	Diet composition modified postbiotic effects	Factorial findings may not transfer to other diets	https://doi.org/10.1016/j.psj.2024.103650

[37]			standard/challenge diets			
Forder *et al.* (2024) [27]	Chicken meat breeders	*S. cerevisiae* metabolite	Reproductive performance and intestinal permeability	Improved reproductive performance without altered permeability	Two breeder lines; mechanism unresolved	https://doi.org/10.1016/j.psj.2024.103595
Chuang *et al.* (2021) [18]	Broilers	*S. cerevisiae*/phytase co-fermented wheat bran	Growth, antioxidation, immunity and morphology	Supported multiple productive and physiological endpoints	Co-fermented matrix prevents attribution to one component	https://doi.org/10.5713/ajas.20.0399

13. Post-biotics as potential alternatives to antibiotic growth promoters

The global ban on antibiotic growth promoters (AGP) has made it all the more urgent to look for safe and effective substitute feed additives that can ensure poultry health and productivity without contributing to antimicrobial resistance (AMR). Due to their greater stability and safety and biological efficacy, post-biotics are regarded as promising feed additives. Post-biotics do not require the living and active microbial cells and do not rely on colonizing the intestine and are therefore more resilient to feed processing, such as pelleting, and to storage and transportation. Additionally, post-biotics do not contain viable microorganisms, which means that there is a lower theoretical risk for microbial translocation or horizontal transfer of antimicrobial resistance genes (Salminen *et al.*, 2021; Vinderola *et al.*, 2022) [74, 86]. After their synthesis, post-biotics exert their biological effects via a combination of bioactive compounds such as organic acids, bacteriocins, antimicrobial peptides, enzymes, exopolysaccharides and microbial cell components. Further comparative studies are required to assess the relative contribution of each metabolite of the purified preparations and the full postbiotic preparations and the mechanisms responsible for their beneficial effects. A standardized manufacturing process and comprehensive chemical characterization is crucial to guarantee the uniformity of product quality, reproducibility, and biological efficacy, (Salminen *et al.*, 2021; Vinderola *et al.*, 2022; Nataraj *et al.*, 2020) [74, 86, 57].

Although the potential of post-biotics is great, they cannot be considered as a general or all-purpose replacement for antibiotics in all production scenarios. These are typically multi-factorial, strain specific and depend on diet composition, environment, pathogen challenge, bird genotype, and management practices. Where product stability and safety are well controlled, viable microbial products like Bacillus-based probiotics and synbiotics are still effective alternatives in certain cases (Ramlucken *et al.*, 2020; Slawson *et al.*, 2020) [66]. Thus post-biotics should be viewed as a piece of a puzzle aimed at reducing antibiotics in combination with other measures, not a silver bullet in itself. Achieving successful antibiotic free production must be through a multi-faceted approach that integrates effective biosecurity, vaccination, litter and water management, nutritionally balanced and digestible diets, scientifically proven microbial feed additives (MFAs), and organic acids. Combination of these complementary approaches offers increased resistance to enteric diseases with production efficiency and food safety.

There are still a number of limitations to the use of post-biotics. These involve differences in formulations, lack of full characterization of active ingredients, variation in the manufacturing method, uncertainty over the optimum dose, differences in regulation across countries and lack of validation in commercial production. Standardized manufacturing procedures, product characterization, dose-response studies and large-scale field trials should be the priorities of future research. The product composition, stability, safety, efficacy and economic performance should be assessed in comparative evaluations with negative controls, conventional AGPs, probiotics and other feed additives. Importantly, health and performance claims, if used, must be based on a solid scientific foundation and be specific to the product of the postbiotic evaluated.

14. Practical applications, safety considerations, and regulatory perspectives

Apart from the biological activity, standardized production, product safety, regulatory considerations, and product quality, successful commercialization of post-biotics is dependent on other factors. Commercial development should focus on complete strain characterization, including whole genome sequencing to validate taxonomic identity, assess genetic stability, and to search for antimicrobial resistance genes, virulence factors and toxin-producing capacity. Fermentation processes must be carried out in controlled conditions, followed by process validated microbial inactivation processes. Non-viability claims should be backed up with suitable microbiological testing conducted to see that there are no viable microorganisms in the final product (Zdouc *et al.*, 2021; Thomas *et al.*, 2022) [93, 83].

Postbiotic preparations should be subject to quantitative analysis of characteristic bioactive marker compounds, analysis of potential contaminants and/or residual toxins, and product stability during storage and feed processing (including pelleting). New analytical techniques like chromatography, metabolomics, proteomics and compositional fingerprinting can help achieve batch-to-batch consistency and set objective release criteria. The addition of information regarding the microbial strain, fermentation substrate, processing conditions, chemical composition and biological activity will help in the production of standardized and reproducible postbiotic products (Li *et al.*, 2022; Sahayasheela *et al.*, 2022) [49, 73].

The biological effectiveness is also affected by product formulation. Encapsulation technologies can enhance storage stability and targeted delivery of bioactive compounds, but the properties of the bioactive compound may be affected by the process of encapsulation and the material used. The use of microencapsulated butyrate provides a proven case in which the site of release in the gastrointestinal tract plays an important role in the biological activity (Lewandowski *et al.*, 2022) [48]. These factors are likely to also be relevant for other formulations of post-biotics. Food, clinical and animal studies have demonstrated that the use of microbial preparations, fermented food and matrices have generally demonstrated favourable safety profiles and are well tolerated (Vandenplas *et al.,* 2020; Béghin *et al.,* 2021; Bellaiche *et al.,* 2021; Bellaiche *et al.,* 2023) [85, 11, 8, 9]. However, other species safety evaluations may not be a substitute for target-species safety evaluation in poultry.

Evaluation of the final commercial formulations should be done directly, as formulation components, processing techniques and carrier materials can affect biological activity and safety. Heat-treated and fermented microbial preparations have been shown to have beneficial effects on the integrity of the intestinal barrier, inflammatory responses, metabolic markers, and overall physiological condition in several studies (Choi *et al.,* 2021; Zhang *et al.,* 2022a; Bednarska *et al.,* 2022; Srivastava *et al.,* 2024) [17, 95, 7, 81]. These effects are product- and formulation-specific, however, and cannot be translated to other products for use as a postbiotic.

Regulatory approval for postbiotic feed additives must be based on comprehensive safety data for the target species, data showing that there are no harmful residues and/or toxins in the product, control of product consistency during manufacture, product stability and stability of the product in use, efficacy data under conditions of use, no interactions of the product with other feed additives and unexpected adverse data from post-market monitoring. The regulatory dossier should be well described to cover the microbial source, genomic safety, fermentation substrate, inactivation process, compositional fingerprint, analytical specifications, shelf life, intended conditions of use and efficacy data. The successful commercialization of post-biotics in the poultry sector will require scientific advances as well as the reliability of product performance, cost, application and proof of return on investment in commercial production systems.

15. Future perspectives and research priorities

While the research and promising results on post-biotics' application in poultry nutrition extend their use to commercial production, there are still some scientific and practical issues to be addressed for wider adoption. The standardisation of terms, production methods, analytical characterization and experimental procedures are among the most important. Further research is required which should include detailed data on microbial strain accession numbers, culture media, fermentation pH and time, inactivation methods, confirmation of non-viability, biomass/ supernatant ratio, drying process, carrier material, chemically equivalent marker compounds and biologically equivalent doses. This will enhance the reproducibility of studies, make it easier to compare between different studies and aid regulatory approval. In addition, sophisticated analytical methods, such as high-resolution metabolomics, proteomics, lipidomics, extracellular vesicle analysis and functional bioassays, are needed to elucidate the bioactive molecules involved in the beneficial actions of post-biotics and to identify those that survive feed processing and reach their target tissues in the gastrointestinal tract (Qiu *et al.,* 2023; Xie *et al.,* 2024; Sigala-Robles *et al.,* 2024; Ye *et al.,* 2022) [64, 90, 79, 91]. Large dose-response trials and multi-site trials under both conventional and antibiotic-reduced production systems are needed for efficacy research to go beyond the small, single-site trials.

Experimental model should include nutritionally balanced and challenge diets, heat stress, coccidiosis, necrotic enteritis, bacterial infection and various genetic lines of broilers to more closely mimic commercial production conditions. Similarly, long-term trials should be conducted for commercial laying hens and breeder flocks in full production cycles to assess the long-term impacts of post-biotics on productivity, health, and reproductive performance. Multigenomic analysis such as microbiome sequencing should be coupled with metagenomics, metabolomics, transcriptomics, and other multi-omics studies to gain a better overall understanding of host–microbiome interactions. While research on fish, pigs, ruminants, and other species offers important mechanistic insights, they should be used mainly to formulate biological hypotheses to predict the size of the responses in poultry (Quintanilla-Pineda *et al.,* 2024; Li *et al.,* 2023; Ryu *et al.,* 2022; Izuddin *et al.,* 2020) [65, 50, 71, 36].

The future challenge is to move away from an empirical formulation and towards mechanism based design for future product development. Novel opportunities for discovery of new bioactive metabolites and post-biotics-producing microbial strains are emerging, such as advances in microbiomics and microbial cultivation of previously unculturable microorganisms, high throughput screening technologies, and next generation microbial therapeutic platforms (Bodor *et al.,* 2020; Lee *et al.,* 2022; Ayon, 2023; Cruz *et al.,* 2022) [10, 47, 4, 21]. These strategies could enable the creation of precision post-biotics solutions to specific production challenges, such as heat stress, necrotic enteritis, controlling Salmonella, preserving laying persistence and enhancing meat oxidative stability. Reliable biomarkers linked to responsive flocks would further aid in targeted nutritional interventions. This is always a crucial balance to achieve safety and efficacy, which will be a key consideration for the success of post-biotics on the market.

The biological activity of fermented products, heat treated microorganisms and post-biotics preparations are consistently different from the biological activity of live microbial counterparts. Several factors, such as formulation characteristics, delivery matrix, route of administration, physiological status of the host and production environment, affect these responses (D'Auria *et al.,* 2021; Jeong *et al.,* 2020; Heydari *et al.,* 2023; Chen *et al.,* 2023) [24, 39, 32, 15].

Consequently, mechanistic evidence obtained from *in vitro* systems or alternative animal models should always be complemented by well-designed target-species studies conducted under practical poultry production conditions. Microecological and nutritional research further emphasizes the importance of distinguishing mechanistic plausibility from demonstrated efficacy in poultry, highlighting the need for formulation-specific validation under commercial conditions (Yu *et al.,* 2020; Companys *et al.,* 2022; Zhang *et al.,* 2023b; Xavier-Santos *et al.,* 2022) [92, 20, 98, 89]. Therefore, future recommendations and regulatory approval of postbiotic products should be based on robust, reproducible evidence generated for each specific formulation.

Emerging research also suggests that traditional fermented food microorganisms, co-culture fermentation systems, and microbiome-informed approaches that consider microbial interactions and metabolic cross-talk represent promising strategies for discovering novel postbiotic candidates (Dobreva *et al.,* 2023; Kaur *et al.,* 2021; Kienesberger *et al.,* 2022; Chung *et al.,* 2022 [18a]) [23, 42, 44, 19]. In addition, increasing evidence indicates that host responses to post-biotics are influenced by formulation characteristics, developmental stage, nutritional status, metabolic context, and the biological compartment being evaluated (Plaza-Díaz *et al.,* 2023; Rodriguez-Herrera *et al.,* 2022; Yu *et al.,* 2023) [62, 69, 93]. Future research should integrate these factors into precision nutrition strategies to maximize the effectiveness of postbiotic supplementation. Ultimately, the successful adoption of post-biotics in poultry production will depend on formulation-specific, scientifically validated, and reproducible evidence demonstrating consistent improvements in bird health, production efficiency, food safety, and economic return.

16. Conclusion

Post-biotics have become an exciting new category of feed ingredients that could provide a safe, stable and biologically active means to enhance poultry health and production. However, the current evidence suggests that post-biotics can positively impact gut microbiota composition, integrity of the intestinal barrier, pathogen control, antioxidant defense, immune homeostasis, nutrient utilization, feed efficiency, stress resilience, meat quality and, to a lesser extent, reproductive performance. Post-biotics have a variety of effects, making them potential addition to sustainable poultry production systems and antibiotic-reduction programs. The evidence is strongest for broiler chickens; there are numerous studies showing broiler chickens have improved intestinal health, growth performance, antioxidant activity and production efficiency. Research on laying hens has mainly been on pathogen control, intestinal health and productive performance, whereas there is comparatively less evidence on egg quality, laying persistence, and performance of breeders. Therefore, further research is needed on postbiotic supplementation in commercial laying hens and breeder flocks during entire production cycles to determine the practical effects of this supplementation. Though there are promising results, post-biotics cannot be viewed as a uniform class of feed additives. The biological activity they exert is dependent upon a variety of interacting factors such as microbial strain, fermentation substrate, manufacturing, inactivation methods, separation and drying methods, carrier material, dietary inclusion level, bird genotype, age, nutritional status, environmental conditions and disease challenge. Variability in these areas can add to the findings differences seen from one study to another, and thus underscores the importance of product-specific evaluation. More consistent manufacturing procedures, in-depth compositional characterization, validated identity fingerprints, optimized dosage recommendations, target-species safety assessments, and multi-site field validation on a large scale in commercial production will be important to future progress. Just as crucial are strong economic assessments which show improvements that can be repeated in terms of production efficiency and profitability. As the microbial biotechnology sector continues to develop, along with the analytical characterization and precision nutrition, post-biotics have a great potential to be a part of the antibiotic-free poultry production process. However, the successful adoption of their use should be based on formulation specific evidence, scientifically validated and reproducible evidence, and not on general claims across all postbiotic products.

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