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SYSTEMATIC REVIEW

Countering Antimicrobial Resistance: Impacts of Natural Growth Promoters on Growth Performance, Immunity, Gut Microbiota and Morphology in Broiler Chicken: A Systematic Review

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Abstract

Background and Objective: Antibiotic Growth Promoters (AGPs) have been widely used to improve broiler performance. However, their use is declining due to concerns about antimicrobial resistance (AMR) and increasing consumer demand for antibiotic-free products. This systematic review aimed to evaluate the effects of alternative growth promoters on growth performance, immune response, gut microbiota, and intestinal morphology in broiler chickens.

Materials and Methods: Relevant studies published between 2015 and 2025 were collected from PubMed, ScienceDirect, Scopus, and Google Scholar. A total of 183 articles were identified, and 99 were included after a rigorous screening process. Data on growth performance, gut morphology, microbial modulation, and immune responses were synthesized qualitatively.

Results: The findings showed improved growth performance, indicated by better Feed Conversion Ratio (FCR) and increased Body Weight Gain (BWG). Enhanced immune function was observed through higher serum antibody levels and increased weights of immune organs. Natural alternatives also promoted beneficial microbes while reducing pathogenic populations. Positive changes in gut morphology were noted, including increased villus height and a higher villus height to crypt depth ratio. Dietary inclusion was the primary method of administering these growth promoters.

Conclusion: Alternative growth promoters have positive and often synergistic effects on growth performance, gut health, immune function, and intestinal microbiota in broiler chickens. However, existing challenges and knowledge gaps highlight the need for optimized formulations and standardized application protocols.

INTRODUCTION

The rapid growth of the global human population has increased the demand for animal products and by-products. This demand has intensified animal production systems. Although intensive farming reduces costs, increases yield, and improves efficiency, it can negatively affect human health, the environment, and animal welfare[1]. In such systems, limited space and poor hygiene promote the spread of infections. As a result, animals are more susceptible to disease, leading to frequent use of antibiotics. In poultry production, antibiotics have been widely used to treat and prevent infections and to promote growth. Their use reduces the incidence of subclinical diseases, lowers morbidity and mortality and modulates gut microbiota, thereby improving overall growth performance[2,3]. Consequently, Antibiotic Growth Promoters (AGPs) have historically played an important role in increasing poultry production to meet rising demand .

However, long-term use of antibiotics has contributed significantly to the development and global spread of Antimicrobial Resistance (AMR), with serious implications for both animal and human health[5]. Evidence shows that antimicrobial use in food animals, especially for growth promotion, selects for resistant bacteria. These resistant strains can spread from animals to the environment and to humans . Although some poultry and livestock diseases are zoonotic, most primarily affect animal health and productivity[7].

Globally, AMR-related infections currently cause more than 0.7 million deaths each year and are projected to exceed 10 million annually by 2050[8]. This growing threat has led to regulatory bans and restrictions in many regions, including the European Union, and has increased consumer demand for antibiotic-free poultry products. Therefore, there is a need to identify and validate natural alternatives that can maintain productivity without the risks associated with antibiotics.

Natural alternatives include a wide range of feed additives such as prebiotics, bacteriophages, organic acids, probiotics, phytogenics, enzymes, and antimicrobial peptides[2]. These alternatives have been reported to promote growth in a manner similar to antibiotics, without leaving harmful residues in animal products or contributing to antimicrobial resistance.

Probiotics: Probiotics are live microorganisms administered in adequate amounts to improve intestinal microbial balance by inhibiting pathogenic bacteria and providing health benefits to the host[2]. Common probiotic bacteria include *Lactobacillus plantarum*, *Bacillus subtilis*, *Bacillus licheniformis*, *Lactobacillus acidophilus* and *Enterococcus faecium* .

Organic acids: Organic acids are carboxylic acids that partially dissociate to form weak acids, typically with a pH range of 3 to 5. Many organic acids exhibit antibacterial activity. Examples used as growth promoters include formic, propionic, citric, succinic, butyric, benzoic, and fumaric acids, as well as their salts[10].

Phytogenic compounds: Phytogenic compounds, also known as phytobiotics, are plant-derived bioactive substances classified as herbs, spices, extracts, or essential oils[2]. They are widely used in poultry, aquaculture, and livestock production as alternatives to AGPs. Examples include essential oils such as carvacrol, thymol, eugenol and menthol, as well as saponins, flavonoids, and tannins[11].

Enzymes: Enzymes are protein catalysts that use vitamins and minerals as cofactors. They reduce activation energy and accelerate biological reactions[12]. Exogenous enzymes are dietary supplements, often derived from fungi or bacteria such as *Aspergillus niger* and *Bacillus subtilis*, and are used to improve nutrient digestibility and growth performance[12]. Common examples include amylases, pectinases, cellulases, xylanases, galactosidases, β-glucanases, phytases, lipases and proteases[13].

Prebiotics: Prebiotics are non-digestible carbohydrates that can be utilized by beneficial gut microorganisms[14]. They pass through the upper gastrointestinal tract with minimal digestion and interact with intestinal microbiota[15]. Common prebiotics used in poultry feed include inulin, mannan-oligosaccharides, fructooligosaccharides, pectin, xylooligosaccharides and galactans. Green algae are also considered prebiotics due to their content of water-soluble sulfated polysaccharides[15].

Antimicrobial peptides: Antimicrobial peptides are small, natural, non-toxic, cationic polypeptides with broad-spectrum activity against pathogens and a low risk of resistance development[16]. Examples include nisin, lactoferrin B, defensins, and cecropins[17].

Bacteriophages: Bacteriophages are viruses that specifically infect and lyse pathogenic bacteria, making them promising alternatives to antibiotic growth promoters[18]. Examples include Bafasal®, Biotector S1® and ProBe-Bac, which target specific bacterial pathogens[19].

Previous research has demonstrated that alternative growth promoters can support gut health, improve nutrient utilization, modulate microbiota composition, and enhance immune responses, with effects similar to those of AGPs in poultry[14]. This review aims to analyze the literature on their modes of administration and their effects on growth performance, gut morphology, immune function and microbiota modulation, as well as to identify research gaps and limitations of major natural alternatives used in poultry production.

MATERIALS AND METHODS

Search strategy: A systematic literature search was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines[20]. The PRISMA checklist is provided in Supplementary Material S1. The search was performed in PubMed, ScienceDirect, Scopus and Google Scholar for studies published between 2015 and 2025. The search terms included "broiler antibiotic growth promoter alternatives", "natural growth promoters", "probiotics", "prebiotics", "organic acids", "enzymes", "antimicrobial peptides", "phytogenics", and "bacteriophages". Boolean operators (AND/OR) were applied to refine the search. Only original research articles published in English were included.

Inclusion and exclusion criteria: Studies were included if they involved broiler chickens and reported in vivo experiments evaluating natural additives as alternatives to Antibiotic Growth Promoters (AGPs), with comparison to a control group. The control group could consist of either an AGP or a basal diet. Studies reporting outcomes related to growth performance, immune response, gut health, and microbiota modulation were considered.

Studies were excluded if they were abstracts without full text, *in vitro* studies, studies lacking a comparative control group, or case reports without a controlled experimental design.

Study selection: Study selection was carried out in two stages: Screening of titles and abstracts, followed by full-text assessment. All retrieved records were imported into reference management software and duplicates were removed. Two independent reviewers screened titles and abstracts based on the predefined inclusion and exclusion criteria. Relevant studies were then assessed in full text for final inclusion. Disagreements between reviewers were resolved through discussion and consensus. No automation tools were used during the selection process.

Risk of bias assessment: The risk of bias was evaluated using domains adapted from the SYRCLE risk-of-bias tool for animal studies[21]. The assessed domains included randomization, baseline characteristics, blinding of outcome assessment, completeness of outcome data and selective outcome reporting. Each domain was classified as low, high, or unclear risk of bias based on the reported information. The assessment was conducted independently by two reviewers without the use of automation tools.

Data extraction and analysis: Data were extracted using a predefined data extraction form. Two independent reviewers collected data from each included study to reduce errors and bias. Extracted information included study characteristics (author, year, and broiler type), type of alternative growth promoter, mode of administration, and measured outcomes. Outcomes included growth performance indicators such as Body Weight Gain (BWG) and Feed Conversion Ratio (FCR), microbiota modulation, immune response and gut morphology. Body weight gain was defined as the increase in live body weight over the experimental period, while FCR was calculated as the ratio of feed intake to body weight gain. Immune response parameters included antibody titers, immune organ weights, and cytokine expression. Intestinal morphology parameters included villus height, crypt depth, and the villus height to crypt depth ratio.

Any discrepancies in the extracted data were resolved through discussion and agreement between the reviewers. No automation tools were used. Confidence in the findings was assessed based on the risk of bias and the consistency of results across studies.

Due to variability in additive types, dosages and experimental designs, the findings were synthesized qualitatively using a narrative approach. Data were presented in tables and graphs, with graphs prepared using Microsoft Excel.

RESULTS

Overview of research trends: After screening, 99 of 183 retrieved articles met the inclusion criteria. Most studies used commercial broiler strains, mainly Ross 308 and Cobb 500. The experiments evaluated growth performance, immune response, gut microbiota, and intestinal health over periods ranging from 21 to 42 days. The dosage of natural growth promoters varied depending on the type of additive and its formulation.

Variation in reported outcomes was observed across studies. This heterogeneity may be due to differences in additive type, method of administration, dosage and broiler strain. Despite these differences, the overall consistency of findings suggests that the results are reliable and not strongly influenced by individual studies or specific methodological limitations. Most studies showed a low risk of bias across major domains. However, publication bias cannot be excluded, as studies with non-significant or negative results are less likely to be published.

Table 1: Effects of natural growth promoters on broiler chicken growth performance

Additive type	Administration route	Examples	BWG	FCR	Citations
Organic acids	Feed, drinking water	Propionic, lactic, acetic, formic, sorbic, ammonium formate, citric acid	↑	↓	60-64
Probiotics	Feed, drinking water	*Saccharomyces cerevisiae, Bacillus coagulans, L. acidophilus*	↑	↓,=	65-68
Phytogenics	Feed, drinking water	Capsicum, peppermint extracts, thymol essential oil	↑	↓,=	69-71
Prebiotics	*In ovo*, feed, drinking water	Mannan oligosaccharides, Fructo-oligosaccharides	↑,=	↓	59,72,73
AMP	Feed	cytotoxic peptide from Isometroides scorpions	↑,=	↓,=	74-78
Bacteriophage	Feed, drinking water	UPWr_S134, SPFM10/14, 88-kP1	↑	↓	79,80
Enzymes	Feed	Phytases, Proteases, xylanases, glucanases, galactosidases	↑	↓	81-84

↓- decrease, ↑- increase, = indicates similar outcomes to control

Table 2: Effects of Multi- component natural growth promoters on broiler chicken growth performance

Additive: Major blends	Administration route	Examples	BWG	FCR	Citations
Phytogenics+Organic acids	Feed	Acids (Fumaric, Sorbic, Malic, Citric) essential oils (Thymol, Vanillin and Eugenol.	↑	↓	85-87
Prebiotics+Probiotics	Feed	prebiotic (inulin) probiotic *Lactobacillus* and *Bifidobacterium* mix)	↑	↓	88-91
Probiotics-Phytogenics	Feed	Probiotics *(Bacillus* sp., *Clostridium butyricum*) phytogenics (Sanguinarine)	↑	↓	92,93
Probiotics+Prebiotics+Enzymes	Feed	Prebiotics (mannose oligosaccharides) probiotics (*Clostridium butyricum* and *Bacillus subtilis*) enzymes (glucose oxidase and α-galactosidase)	↑	↓	94-95

↓- decrease, ↑- increase, = indicates similar outcomes to control

Use of alternatives to AGPs: The review indicates that blended formulations of natural growth promoters were more commonly studied than single additives. Two main blending approaches were identified. The first involved combining additives within the same category, such as mixtures of organic acids (for example, formic, lactic, or butyric acid) or multiple probiotic strains. The second approach combined different categories of additives, such as phytogenics with probiotics, organic acids, bacteriophages, antimicrobial peptides, or enzymes.

Among single additives, organic acids, phytogenics and probiotics were the most frequently studied. Research on prebiotics, enzymes, antimicrobial peptides and bacteriophages is increasing, although fewer studies are available compared to the major categories. Some studies were excluded because full texts were not accessible[22,23].

Effects of non-antibiotic growth promoters on growth performance: Most studies reported that natural alternatives improved or maintained growth performance, including Body Weight Gain (BWG) and Feed Conversion Ratio (FCR), compared with control groups, as shown in Table 1. Combinations of different additives generally produced greater improvements in growth performance in broiler chickens, as presented in Table 2.

Effects on immunity, microbiota modulation and gut morphology: Most studies reported significant improvements in immune response, gut microbial balance and intestinal morphology following supplementation with alternative growth promoters, as shown in Table 3. Combinations of probiotics, organic acids, enzymes and phytogenics were particularly effective. These combinations enhanced gut health and immune function, as indicated by increased antibody titers and higher weights of immune organs.

Effects of mode of administration: Three main routes of administration were identified: Inclusion in feed, supplementation in drinking water and in ovo delivery. Feed supplementation was the most commonly used method, followed by water supplementation, while in ovo delivery was less frequently applied.

Feed-based administration consistently improved growth performance, gut morphology, microbiota balance and immune response. Water supplementation supported gut microbial balance and immune function, but its effects on growth performance were less consistent. In ovo delivery, applied during the late stage of incubation, was associated with improved early post-hatch growth, better intestinal development, and enhanced immune system development. Figure 1 presents examples of natural growth promoters. Figure 2 shows the flowchart outlining the literature search process used in the systematic review on alternatives to antibiotic growth promoters in broiler chickens. Figure 3 illustrates the main domains considered in the risk of bias assessment.

DISCUSSION

This review shows that alternative growth promoters consistently have positive effects on growth performance, immune function, gut microbiota, and intestinal morphology in broiler chickens, regardless of the mode of administration. Although, the magnitude of these effects varies with additive

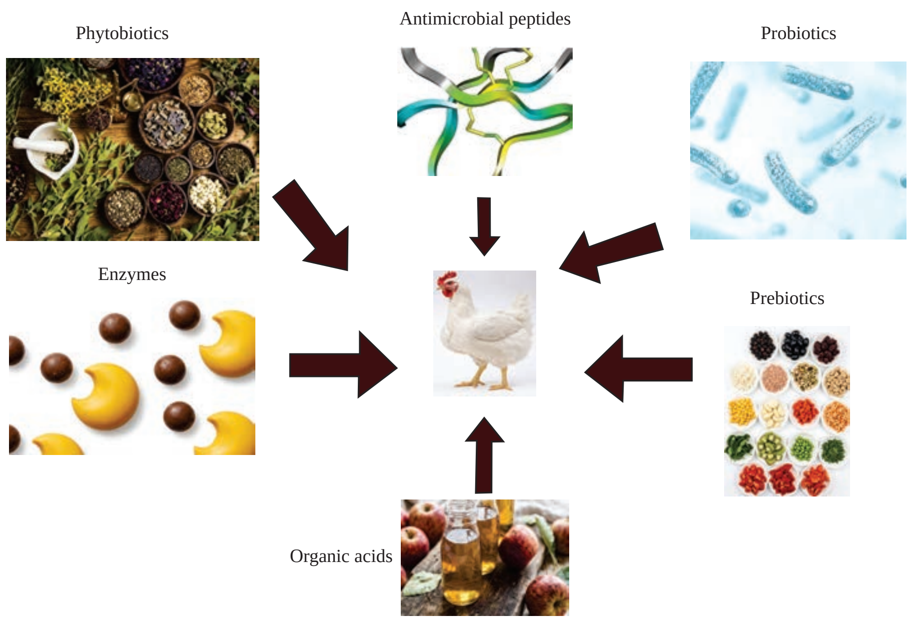

Fig 1: Examples of natural growth promoters. Source; Khan *et al.*[103]

Table 3: Effects of natural growth promoters on immunity, microbial shifts and gut morphology of broiler chicken

Type of additive	Gut morphology	Microbiota shifts	Immunity	Citations
Organic acids	Villi height ↑, Crypt depth ↓, VH:CD ↑ Goblet cells ↑	Pathogenic↓, Beneficial ↑	Serum antibodies ↑ Immune organs development↑, Antioxidant capacity ↑	10,61,96,97
Probiotics	Villi height ↑, VH:CD ↑ Goblet cells ↑	Beneficial ↑	Serum antibodies ↑, Strengthened intestinal barriers IL-10 ↑	66,68,98
Phytogenics	Villi height ↑, Crypt depth ↓ VH:CD ↑	Beneficial ↑, Pathogenic ↓	IL-10 ↑, Serum antibodies ↑, Antioxidant activity ↑, Immune organs development↑, TNF-α ↓	69,70,99,100
Prebiotics	Villi height ↑, Crypt depth ↓ VH:CD ↑++++	Beneficial↑, Pathogenic load)↓	Strengthened intestinal barriers, Serum antibodies ↑	72,73
Antimicrobial peptides	Villi height ↑, Crypt depth ↓	Beneficial ↑, Pathogenic load ↓	Serum antibodies ↑, Immune organs development↑ IL-6 ↓	74,75,76,101
Bacteriophage	Villi height ↑, VH:CD ↑	Favorable microbiome shifts, Pathogenic load↓	Immune organs development ↑, Serum antibodies ↑	79,80
Enzyme	Villi height ↑, Crypt depth ↓ VH:CD ↑	Altered Microbiota, Pathogenic ↓	Improved mucosal intergrity	81,102

↑ - increase compared with control; ↓ - decrease compared to control= indicates similar outcomes to control, VH- Villus height (jejunal and ileal), CD-Crypt depth (jejunal and ileal)

type, dosage and experimental conditions, most alternatives produce results that are comparable to or better than conventional antibiotic growth promoters.

Growth performance: Across the reviewed studies, probiotics, prebiotics, organic acids, enzymes, phytogenics, bacteriophages and Antimicrobial Peptides (AMPs) improved growth performance, particularly Feed Conversion Ratio (FCR) and Body Weight Gain (BWG). However, the responses differed depending on additive type, dosage, experimental conditions, and formulation (free or encapsulated).

Probiotics such as *Lactobacillus* and *Enterococcus* species improved nutrient utilization and growth by stabilizing gut microbiota, enhancing digestion and reducing

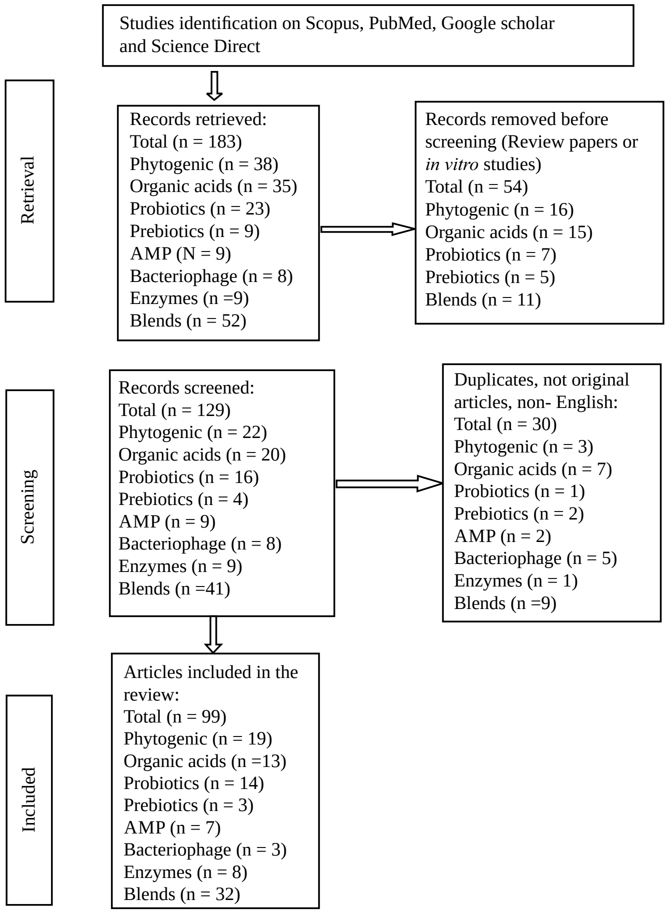

Fig. 2: A PRISMA flowchart illustrating the process of literature search of systematic review for alternatives to antibiotic growth promoters in broiler chickens

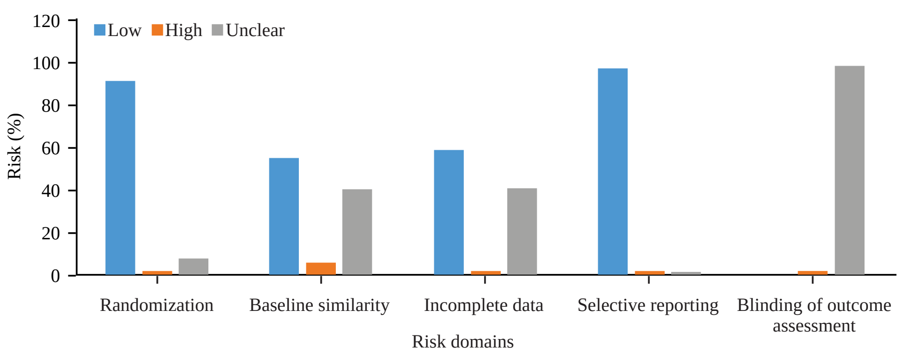

Fig. 3: Bar graph showing major domains of risk of bias assessment

pathogenic bacteria[24]. Similarly, prebiotics such as mannan-oligosaccharides and fructo-oligosaccharides promoted the growth of beneficial microbes and the production of Short-chain Fatty Acids (SCFAs), which indirectly enhanced nutrient absorption and growth[25].

Exogenous enzymes improved growth performance by reducing digesta viscosity, releasing nutrients from feed components and increasing metabolizable energy, especially in cereal-based diets[26]. Phytogenic additives improved feed intake and efficiency through antimicrobial and digestive- stimulating effects, leading to better growth performance[27].

Bacteriophages and AMPs enhanced growth indirectly by improving gut health. They controlled pathogenic bacteria, balanced gut microbiota, reduced inflammation and improved nutrient utilization, resulting in better BWG and FCR[28,29]. Organic acids improved growth by lowering gastrointestinal pH, enhancing mineral absorption, and inhibiting enteric pathogens, thereby increasing nutrient utilization efficiency[30].

Effects of antibiotic alternatives on immune modulation: A consistent finding across studies is that alternative growth promoters enhance immune function in broiler chickens rather than suppress it. This effect is mainly observed through increased development of immune organs, such as the spleen and bursa of Fabricius and higher immunoglobulin production, in contrast to antibiotics, which may delay immune development and cause suppression[31]. The bursa of Fabricius, spleen, and thymus play essential roles in both cellular and humoral immunity in poultry[32].

Cytokine modulation was also observed, with increased anti-inflammatory cytokines such as IL-10 and reduced pro-inflammatory markers, indicating improved immune balance[33]. Probiotics stimulated both innate and adaptive immune responses, as shown by increased immunoglobulin levels, improved mucosal barrier function, and enhanced lymphoid organ development[34].

Prebiotics supported immune system maturation by promoting microbial fermentation and SCFA production, which influence gut-associated lymphoid tissue (GALT) and improve mucosal integrity[35]. Organic acids contributed to immune modulation by lowering gut pH, suppressing pathogens, supporting beneficial microbiota and regulating inflammatory responses, leading to balanced cytokine production and improved immunity[36].

Phytogenic compounds showed antimicrobial, anti-inflammatory, antioxidant and gut-protective effects that support immune balance. They reduced pathogenic microbes, supported beneficial microbiota, and stabilized gut homeostasis[37]. Some phytogenics also directly regulated immune signaling pathways by decreasing pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α and increasing anti-inflammatory mediators such as IL-10[38].

Bacteriophages improved immune status indirectly by reducing pathogenic bacteria, which lowered inflammatory stress and chronic immune activation[18]. Antimicrobial peptides enhanced immunity by directly killing pathogens and regulating immune signaling pathways, thereby improving mucosal immunity without promoting resistance[39].

Effects of antibiotic alternatives on gut microbiota modulation: This review shows that modulation of gut microbiota is a key mechanism by which alternative growth promoters exert beneficial effects. Many studies reported an increase in beneficial bacteria, including lactic acid bacteria, *Lactobacillus*, and *Bifidobacterium*, along with a reduction in pathogenic bacteria such as *E. coli*, *Salmonella*, *Clostridium perfringens* and *Campylobacter*. These findings support competitive exclusion as an important mode of action for probiotics and prebiotics[35].

Organic acids have been shown to inhibit pH-sensitive pathogens such as *E. coli* and *Salmonella* by lowering the pH of the gastrointestinal tract and disrupting bacterial metabolism[40]. They also create an acidic and anaerobic environment that favors the growth of beneficial Short-chain Fatty Acid (SCFA)-producing bacteria.

Phytogenic additives modulate gut microbiota through selective antimicrobial activity and by shaping the microbial ecosystem, as well as through indirect interactions between the host and microbes[37]. Unlike antibiotics, they do not cause broad suppression of microbial populations, which helps maintain a stable and resilient gut microbiome. Their mechanisms include strengthening intestinal tight junctions and reducing oxidative stress[11].

Bacteriophages regulate gut microbiota through highly specific targeting of pathogenic bacteria while preserving beneficial microbial populations. Unlike antibiotic growth promoters, bacteriophages infect and lyse only their host bacteria, reducing pathogen load without disturbing overall microbial diversity[41].

Antimicrobial peptides (AMPs) influence gut microbiota by selectively inhibiting pathogens and supporting beneficial microbes, thereby maintaining microbial balance[42]. AMPs, such as defensins and cathelicidins, act rapidly by disrupting bacterial cell membranes, leading to cell lysis without causing the broad microbial imbalance commonly associated with antibiotics[43].

Enzyme supplementation, including xylanase, phytase, and protease, improves broiler performance by breaking down anti-nutritional components in feed[44]. This process reduces intestinal viscosity and enhances nutrient digestibility, which alters the gut environment in a way that supports beneficial microbial populations[45]. These microbial changes are similar to those observed with antibiotic growth promoters but do not contribute to antimicrobial resistance.

Effects of antibiotic alternatives on gut morphology and barrier function: This review found consistent improvements in intestinal morphology with the use of alternative growth promoters. Probiotics and prebiotics increased villus height, villus height to crypt depth ratio, and goblet cell numbers, indicating improved nutrient absorption and stronger mucosal protection[46]. A previous review[47] reported similar effects for enzyme supplementation and suggested that these improvements result from reduced intestinal irritation caused by undigested nutrients, leading to better epithelial integrity and nutrient absorption.

Several studies also showed that organic acids and phytogenics improved intestinal morphology by enhancing epithelial development and increasing absorptive surface area. These effects were reflected in greater villus height and villus height to crypt depth ratio in both the ileum and jejunum[48]. Such structural changes strengthen gut barrier integrity and support more efficient nutrient utilization[49].

Antimicrobial peptides have been reported to improve intestinal health by repairing epithelial damage, increasing villus height and enhancing mucosal defense[29]. Bacteriophages also support gut health indirectly by reducing pathogenic bacteria, which lowers toxin production and helps restore microbial balance[50].

Synergistic effects of combined additives (blends): A consistent finding across the reviewed studies is the synergistic potential of combining different alternative growth promoters. Increasing evidence shows that combinations of probiotics, prebiotics, enzymes, organic acids, phytogenics, bacteriophages, and antimicrobial peptides produce stronger effects than individual additives alone[51]. These synergistic effects result from complementary and sequential actions on digestion, gut microbial balance, immune regulation and intestinal structure, which together improve broiler performance in antibiotic-free production systems[33].

For example, the combination of probiotics and prebiotics has been reported to produce greater improvements in growth performance, immune response, and gut morphology in broiler chickens compared to their individual use[52]. Similarly, blends of organic acids are widely used because individual acids differ in their dissociation constants and antimicrobial activity. This variation allows combined formulations to act across different regions of the gastrointestinal tract[49].

The frequent use of blended additives in the reviewed studies highlights the complex nature of poultry gut health and growth performance[51]. Broiler productivity is influenced by multiple interacting factors, including nutrient absorption, intestinal integrity, gut microbiota, and immune function. Therefore, single additives may not be sufficient to address all these physiological processes[52].

Influence of administration route on performance and health: In-feed supplementation was the most commonly used method for delivering alternative growth promoters, likely due to its practicality and suitability for commercial poultry systems[53]. This approach ensures a steady and controlled intake of additives, allowing birds to receive relatively consistent doses throughout the production cycle[54]. In addition, many natural growth promoters, such as enzymes, organic acids, and antimicrobial peptides, act directly within the gastrointestinal tract, making feed an effective delivery medium[55]. The improvements in growth performance and feed efficiency observed in these studies are therefore associated with enhanced nutrient digestibility, improved intestinal structure, and modulation of gut microbiota[56].

Although, less frequently used than feed inclusion, supplementation through drinking water offers several practical advantages. It allows rapid delivery of additives and can be particularly useful during periods of stress, disease challenge, or environmental changes[57].

In ovo administration is a relatively recent method for delivering alternatives to antibiotic growth promoters, aiming to influence gastrointestinal and immune system development before hatching[58]. The reviewed studies suggest that *in ovo* injection of prebiotics or probiotics promotes early colonization of beneficial microorganisms. This early microbial establishment may improve intestinal health and enhance immune responses in newly hatched chicks[59]. However, the limited number of studies using this approach suggests that factors such as technical complexity, specialized equipment requirements, and potential risks to embryo viability may restrict its wider adoption.

CONCLUSION

The use of alternative growth promoters provides complementary and often synergistic benefits for growth performance, gut morphology, immune modulation and intestinal microbial composition in broiler chickens compared with antibiotics. Their mechanisms of action support their potential as sustainable substitutes for antibiotic growth promoters. However, further optimization and standardization are required to achieve consistent and cost-effective results. The increasing use of multi-component formulations reflects growing recognition that synergistic and complementary actions are important for effectively replacing antibiotic growth promoters in poultry production. Future research should focus on improving administration strategies and developing integrated approaches that combine early-life *in ovo* techniques with post-hatch supplementation through feed or water to enhance broiler health and productivity.

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