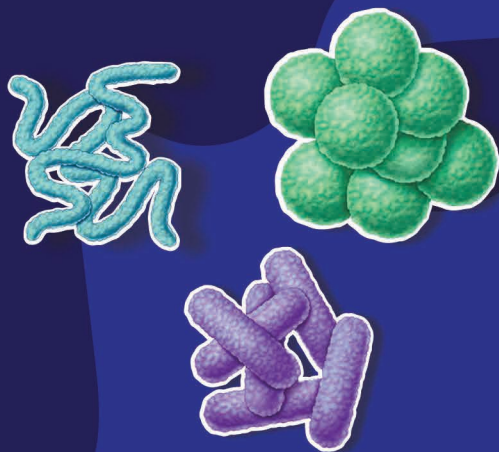




AviPod

An Update for Poultry Professionals

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Non-Antibiotic Growth Promoters

Since the beginning of 1950s, antibiotic growth promoters (AGPs) have been used in poultry production globally to improve gut health, feed efficiency, growth and reducing sub-clinical and clinical disease challenges, and cost of production. However, there is increasing concerns about applying antibiotics in feed

Non-Antibiotic Growth Promoters

Dear Readers,

Poultry meat and eggs are considered as major sources of food for rapidly growing human population across the globe. During the rearing of poultry, different antibiotics have been included in poultry diets in sub-therapeutic doses to attain improvements in growth performance. Use of these antibiotics in poultry production has been considered as one of the major reasons behind the emergence of antibiotic resistance in human and poultry pathogens. The pressing issue of antibiotic resistance led to complete or partial bans on the use of antibiotics as growth promoters in many parts of the world. Moreover, there have been increased concerns among the consumers about the antibiotic residues in poultry products.

These administrative bans led to an increased incidence of bacterial disease outbreaks thus compromising the poultry performance, welfare, and economic outcomes of poultry producers. This ultimately attracted the attention to find alternative strategies that could replace the antibiotics and bring production, health, and food safety benefits to the poultry production systems. These non-antibiotic growth promoters mainly include probiotics, prebiotics, synbiotics, phytochemical substances, organic acids, antimicrobial peptides, enzymes, bacteriophages, and egg yolk antibodies. Inclusion of these non-antibiotic solutions in poultry diets demonstrates promising results in terms of production performance and birds' health.

These promising results are demonstrated through improvements in nutrient absorption, proliferation of beneficial bacteria, reduction in pathogenic bacterial species, production of bacterial metabolites that serve as positive modulators of immune responses. Apart from reducing the colonization of bacterial species that are pathogenic for poultry, these alternative solutions have also exhibited satisfactory efficacy in reducing the colonization of foodborne pathogens like *Salmonella* and *Campylobacter jejuni*.

This issue of AviPod reviews the comparative effects of non-antibiotic growth promoters on poultry. You all also have a vast experience in this field and it would be worthwhile if you could share your valuable experiences and feedback or suggestions by scanning the below QR code or else you can reach out to us via email at aviglo@intaspharma.com.

Regards

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as growth promoters as use of antibiotics is increasing antimicrobial resistance (AMR) at a faster rate than the rate at which newer antibiotics are being developed. On July, 2019, the Union Ministry of Health and Family Welfare (MoHFW), Govt notified the prohibition of sale, manufacture and distribution of colistin and its formulations in food-producing animals, poultry, aqua farming and animal feed supplements. The move is expected to help regulate antibiotic misuse in these animals and contain antimicrobial resistance (AMR).

Prolonged use of low doses of antibiotics has been shown to favor the selection of resistance to more than one class of antimicrobials in bacterial community residing in gut. Bacteria can acquire resistance through different mechanisms such as development of antibiotic efflux, modification of antibiotic targets through genetic mutations or post-translational modifications of the target protein, synthesis of antibiotic hydrolyzing enzymes and reduced expression of porin proteins. Non-dividing microorganisms in stationary phase exposed to sub-lethal concentrations of antibiotics were shown to develop antibiotic resistance through adaptive mutation, directed mutation or selection-induced mutation. Thus, prolonged use of AGPs may promote the emergence and dissemination of antimicrobial resistance genes (ARGs) to microbial communities in humans or the environment besides generating drug residues in poultry products. Consequently, the development of new non-antibiotic feed additives as an alternative to AGPs has emerged as a priority area in poultry production.

To support this movement, researchers are continuously developing alternative strategies to replace AGPs.

Non-Antibiotics

These are any substance that can serve as a substitute for therapeutic drugs that are increasingly become ineffective against pathogenic bacteria, viruses or parasites.

Categories

All identified non-antibiotics from background literatures are categorized into 11 categories such as phytochemicals, probiotics, prebiotics, synbiotics, antimicrobial peptides (AMPs), bacteriophages etc. (Fig. 1).

These are intended to preserve a healthy gut microbiota (Fig. 2), hindering pathogenic organisms from attaching in early stages of life. The roles of gut microbiota in maintaining homeostasis and health, and in dysbiosis leading to various diseases have been increasingly recognized. Manipulation of gut microbiome composition can help achieve higher FCR efficiencies and good quality poultry products.

Phytochemicals

Also referred to as botanicals or plant-based additives, include herbs, spices and essential oils. Bioactive components of phytochemicals are secondary metabolites being polyphenols the main group. Other bioactive compounds include terpenoids (monoterpenes, steroids), phenolics (tannins), glycosides and alkaloids.

• Mode of Action

Phytochemicals exert multiple bioactive effects (Fig. 3) including, immunomodulation, antimicrobial, antioxidant, anti-inflammatory and digestive-stimulating properties. Phytochemicals have also been shown to modulate gut microbiota favorably, improving the ratio of beneficial to pathogenic microbes. They stimulate digestive enzyme activity and enhance nutrient absorption, thereby promoting growth. Their synergistic antimicrobial activity relies on their lipophilic properties and abilities to bind or damage membranes or to minimize cell division by inhibition of DNA synthesis.

In recent years, phytochemical feed additives have attracted an increasing attention as natural alternative to AGPs which can be included in feeds as dried, solid and ground form, or as extracts (crude, concentrated and purified). A wide variety of herbs and spices (thyme, oregano, cinnamon, rosemary, marjoram, yarrow, garlic, ginger, green tea, black cumin, coriander, among others) as well as essential oils (from thymol, carvacrol, cinnamaldehyde, garlic, anise, rosemary, citrus, clove,

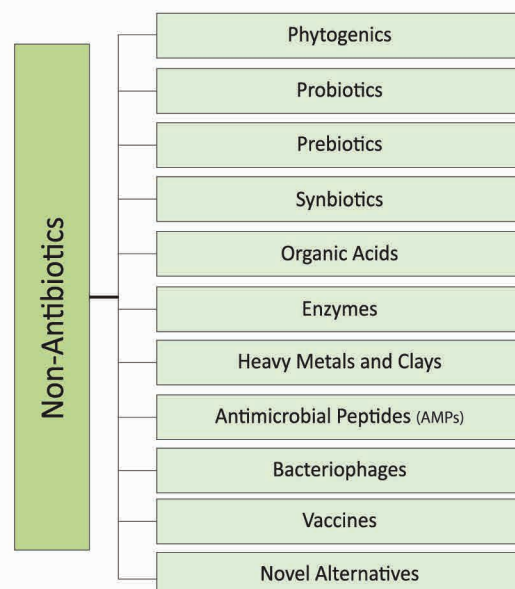


Fig. 1: Non-antibiotic categories

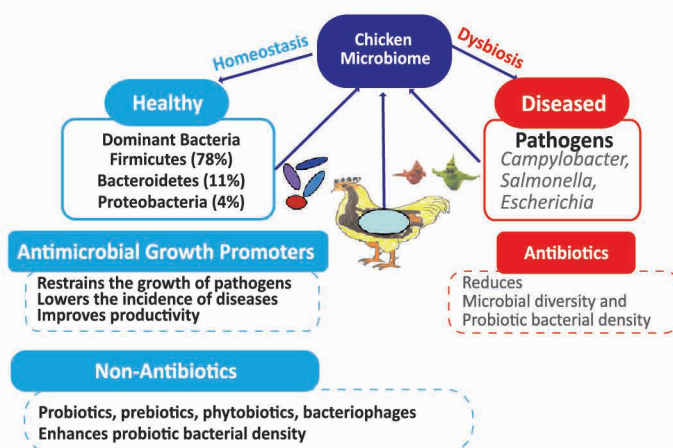


Fig. 2: Impacts of antibiotics and non-antibiotics on chicken microbiome

Phytochemicals

- ⊕ Stimulate innate immune cells
- ⬇ Reduce oxidative and nitrosative stress biomarkers
- ✓ Help maintain gut integrity
- ⬇ Mitigate the negative effects of enteric infection-induced inflammation

Fig. 3: Mode of action of phytochemicals

ginger) have been used individually or mixed. Although the repertoire mechanisms of action is not fully elucidated, one of their primary mode of action is related to their antimicrobial effects, which allow controlling potential pathogens. Essential oils from plants are highly volatile. Recent advances in microencapsulation technology have allowed for targeted delivery and improved stability of the volatile compounds.

Probiotics

Probiotics are live microorganisms that confer health benefits to birds by maintaining gut microbial balance when administered in adequate amounts. Commonly used probiotics include *Lactobacillus* spp., *Bacillus* spp., *Bifidobacterium* spp., *Enterococcus* and *Yeast* spp. These beneficial microbes compete with pathogens for nutrients and adhesion sites, secrete antimicrobial substances and stimulate immune response. *Bacillus licheniformis* and *Bacillus subtilis* have been widely regarded as an important growth promoter in recent years.

- **Mode of Action**

Use of probiotics in feed increases population of beneficial microorganisms such as *Lactobacillus* spp. and *Bifidobacterium* spp. through production of lactic acid and short-chain fatty acids (SCFA) and reduction of pH and inhibit growth of harmful microorganisms such as *Campylobacter*, *E. coli*, *Salmonella* spp. and *Clostridium* spp. through the release of inhibiting substances like organic acids and/or bacteriocins. Another key effect is the modification and regulation of gut immune responses through reduction in pro-inflammatory cytokines and an increase of IgA production, and promotion of specific and non-specific immune responses to pathogens (activation of macrophages, cytokine production by intraepithelial lymphocytes).

Some probiotics can improve nutrient digestion and absorption by increasing structure of crypts and villus height in intestines, e.g., *Bacillus subtilis* is a widely utilized bacteria that has been shown to increase intestinal villus height. Primary mode of action of *B. subtilis* spores is related to their ability to create an anaerobic environment in intestine after germination. It stimulates growth and proliferation of native lactobacilli, which leads to competitive colonization and production of lactic acid. Ultimately, this results in restrictions for developing pathogenic bacteria in intestines. Studies concluded that *B. subtilis* is an effective growth-promoting alternative to BMD that modulates microbiota and intestinal architecture.

Some probiotic can also improve digestive capacities by producing enzymes, e.g., *Bacillus licheniformis* strains have been used because of their abilities to produce amylase, alkaline proteases, mannanase and keratinase (besides producing bacteriocins) which are effective for bird's growth. *Bacillus subtilis* also produces amylase. Other mechanisms involves with *B. licheniformis* include competitive exclusion, immune enhancement and increase antioxidant functions.

Probiotics have shown very promising results as alternatives to antibiotics. Most studies have demonstrated that administering probiotic strains alone or in combination greatly boosts average daily feed intake (ADFI), average daily gain (ADG), FCR and reduces diarrhea.

Prebiotics

Compounds that act like food components or fertilizer for beneficial microorganisms in gut by stimulating their growth. Prebiotics include a wide range of non-starch polysaccharides or oligosaccharides such as mannan oligosaccharide (MOS), fructans (fructooligosaccharide or FOS and inulin), galactans (galactooligosaccharide), maltooligosaccharide, lactulose, lactitol, and glucooligosaccharides. Among these, MOS, FOS and inulin are most commonly used. These non-digestible oligosaccharides are fermented in large intestine by beneficial bacteria and provide energy for microbiota.

- **Mode of Action**

Prebiotics are neither digested nor absorbed in upper intestines as enzymes can not degrade it, but serve as a food supply for beneficial bacteria found in lower intestines, such as *Lactobacillus* and *Bifidobacterium*. They act *via* inhibiting pathogen adhesion, immunomodulation, fermentation-based synthesis of antimicrobial chemicals and alteration of gut morphology and pH.

Synbiotics

Together, probiotics and prebiotics can be used in synergy as synbiotics. These are developed to circumvent some of the challenges associated with probiotic survival in intestines. Taking advantage of probiotics and prebiotics characteristics, synbiotics have been shown to have a greater effect on microbiota than probiotics or prebiotics used separately, with enhanced production of lactic acid and short-chain fatty acids (SCFAs), and a reduction in branched chain fatty acids (BCFAs) concentration. Besides improved growth and microbiota health, synbiotics also limit antibiotic resistance development.

- **Mode of Action**

Mechanisms by which synbiotics effect the host include the prebiotic that encourages growth of probiotic or the prebiotic and probiotic that function independently in intestines. To increase and modulate intestinal microbiota, prebiotics are fermented in intestines by probiotic bacteria that colonize intestinal space. It has been shown that synbiotics can increase count of beneficial bacteria and restrict growth of potential pathogens in intestines. Synbiotics can also effect immune system. These additives help stabilize the intestinal environment, improve nutrient utilization and reduce incidences of enteric diseases. This translates to better growth performance and reduced mortality.

Organic acids

These are acidifiers such as butyric acid, benzoic, citric, formic, fumaric, lactic, acetic and propionic acid or their salt counterparts such as calcium, potassium, or sodium formate or sodium fumarate. Most organic acidifiers bear one or several carboxyl (COOH) functional groups that play an important role in their activity and can also be found on amino acids and fatty acids. Acidifiers have been used in poultry diets and drinking water for decades with positive responses on growth performance.

- **Mode of Action**

Organic acids have antibacterial effects. Three types of modes of action have been identified (Fig. 4). They can manipulate immune response. Can promote proper crypt cellular proliferation, increasing tissue regeneration and maintenance. They reduce microbial intestinal colonization and infectious processes in addition to having an inhibitory effect on inflammatory processes at intestinal mucosa. Overall, this led to improved villus width, height, and area of duodenum, jejunum and ileum, as well as secretion, digestion, nutrient absorption and FCR. Now a days, acidifiers have been encapsulated by fatty acids or other molecules to allow their controlled release in specific compartments of intestines. Furthermore, use of organic acids in combination with essential oils (such as cinnamaldehyde) has shown synergistic effects.

Acidifiers

- ↓ Reduce coliform and pathogenic bacteria along the GIT
- Modulate pancreatic secretions and intestinal mucosal morphology
- Inhibition of inflammatory processes

Fig. 4: Mode of action of acidifiers

Enzymes

These are biologically active proteins, which enable breakdown of specific chemical bonds of nutrients into smaller compounds for further digestion and absorption. Phytase, carbohydrases, xylanase, galactosidase, mannanase, amylase, glucanase, proteases, lipases and pectinase are some of the most commonly used feed enzymes. Enzymes used in feed are commonly produced by bacteria, fungi and yeast such as *Bacillus subtilis*, *Bacillus licheniformis* for amylase, *Trichoderma reesei* for cellulase, *Aspergillus niger* for glucanase and *Saccharomyces cerevisiae* for invertase. Exogenous enzymes can increase gut stability by reducing substrates for putrefactive organisms, increasing substrates for beneficial fermentative organisms, and improving intestine's ability to protect itself against unwanted bacterial condensation.

- **Mode of Action**

High substrate specificity adds a specific feature to an enzyme. Each enzyme recognizes specific substrates and performs their modification at specific reaction sites. Three types of modes of action have been identified (Fig. 5). However, the observed bird response to food enzymes is influenced by factors like feed humidity levels, dietary pH, length of time required for enzymes to interact with the substrate etc. Besides, enzymes can have direct antimicrobial effects by hydrolyzing bacterial cell walls or compromising the glycocalyx's integrity. Lysozymes are a group of most well-known antimicrobial enzymes that can hydrolyze the peptidoglycan in bacterial cell walls and cause cell death.

Enzymes

- 🔍 Breakdown of antinutrient substances that obstruct nutrient digestion
- ↑ Increase availability of nutrients following the removal of encapsulating barriers
- 📈 Improve digestive capacities of birds at very young age

Heavy Metals and Clays

Clays, especially when modified with metals (e.g., copper-exchanged clays), can adsorb microbial toxins and even directly bind to pathogens, limiting their growth. Copper-exchanged clays (CeC) have been studied as alternatives to AGPs. However, further research is needed to fully understand the mechanisms of action of CeC and to optimize its use in poultry production.

Antimicrobial Peptides (AMPs)

AMPs also known as host defense peptides (HDPs) are short amphipathic proteins (less than 100 amino acids) which constitute part of the innate immune defense existing in nearly all classes of organisms. Most of them are cationic (positively charged) and broad-spectrum inhibitors of Gram-positive or Gram-negative bacteria. Most AMPs are innate and adaptive immune effector molecules that can modulate pro- and anti-inflammatory responses and chemotactic activity.

With an 80-year application history, AMPs are considered strong candidates to replace antibiotics and have been widely studied, e.g., broiler feed supplemented with bacteriocin Microcin J25 improved performance, reduced systemic inflammation, improved fecal microbiota (lower population of total anaerobic bacteria), and decreased Salmonella infection rate. Results show that AMPs can be powerful antibiotic substitutes, especially under infection conditions. However, another study concluded dietary supplementation of AMP (Cecropin II) significantly increased body weight and could replace antibiotics for eliciting optimum performance.

Fig. 5: Mode of action of enzymes

- **Mode of Action**

Antibacterial activity of most AMPs is based primarily on the interaction of positively charged peptides with negatively charged components of bacterial membrane such as phospholipids and teichoic acids of Gram-positive or lipopolysaccharide of Gram-negative bacteria, which leads to pore formation, membrane permeabilization and cell lysis. Membrane permeabilization may also result in translocation of AMPs into cytoplasm, where they inhibit main cellular processes such as DNA and protein functions or synthesis. AMPs also modulate immune response. It has been shown that supplementation of feed with **Microcin J25**, a bacteriocin (from *E. coli*) active against several *E. coli* and *Salmonella* strains, can promote growth performance, improve intestinal morphology, influence fecal microbiota composition and reduce the secretion of pro-inflammatory factors (IL-1 β , TNF- α and IL-6) in broilers. Also observed that microcin J25 was able to attenuate intestinal inflammation diseases caused by enteric pathogens.

Bacteriophages

These are viruses that can infect only bacterial cells and kill their host by causing cell lysis, and have attracted a lot of attention in recent years due to their excellent specificity, non-toxicity, and natural abundance. Bacteriophages are composed of proteins that form a capsid (head) and a tail and of DNA or RNA as the viral genome. Capsid encapsulates and protects the genetic material. Tail is a complex multiprotein structure that plays a critical role in bacterial host recognition, attachment, digestion, cell wall penetration and genome ejection. Initially, phages bind to bacteria and deposit their genome inside the host to eventually replicate in the cytoplasm until the infected cell is lysed. Afterward, the released virions can infect other bacteria in the environment. As a result, bacteriophages have a direct impact on bacterial populations. Cocktail of two or more bacteriophages can be used. Many studies supporting their great potential as alternatives to antibiotics are reported. It is showed that bacteriophages infecting *Salmonella gallinarum* could be a promising alternative to antibiotics for control of fowl typhoid.

- **Mode of Action**

They act mainly *via* their antimicrobial activity. Bacteriophages have been shown to influence innate and adaptive immunity through phagocytosis and cytokine responses. By affecting stability of intestinal microbiota, bacteriophages can modulate intestine's immunological and metabolic capabilities.

Vaccines

Vaccines are promising alternatives to antibiotics. Vaccination strengthens bird's immune system, leading to healthier flocks and potentially improves growth performance. Studies have demonstrated that the use of various bacterial as well as viral vaccines can result in significant reduction in antibiotic consumption. However, many currently available vaccines have limitations that reduce their usefulness for preventing diseases and decreasing the need for antibiotics. In fact, many current vaccines have a number of shortcomings with regard to safety, efficacy and/or user-friendliness that limit their ability to replace antibiotic use. Developing an effective vaccine against colibacillosis in chickens is complex due to the high heterogeneity of *E. coli*, elusive disease mechanisms, and absence of definitive markers for pathogenic isolates.

Novel Alternatives

Among the various non-antibiotic growth promoters, probiotics, phytogenics, and prebiotics emerge as the most extensively covered categories in the published literature (constituting over 65% of the total) underscoring the popularity of these alternatives (Fig. 6). However, probiotics and phytogenics are popular as functional supplements when compared to other feed additives.

Other alternatives like antimicrobial peptides, bacteriophages, vaccines, and novel approaches exhibit a lower number of publications, each accounting for less than 5%. It is inferred that the development and testing of these alternative categories, such as poultry vaccines necessitate higher funding, substantial time, and specialized techniques and technology. As a result, only a few research groups are dedicated to undertaking such projects involving poultry. Enhanced legislative and funding support is imperative to bolster research efforts in the domains of **vaccines, antimicrobial peptides, and other innovative alternatives.**

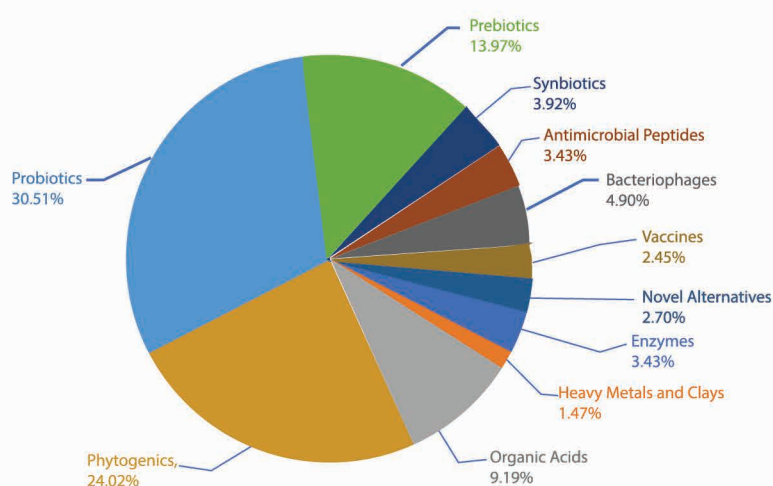


Fig. 6: Proportion of publications under 11 categories of identified non-antibiotic growth promoters

Among the developments regarding antimicrobial peptides research, Microcin J25 (**AVITIDE**) offers a novel, effective and safe alternative to AGPs for combating microbial infection especially Gram-negative bacteria (e.g. *E. coli* and *Salmonella*) challenge. However, Cecropin II (**SUBTIDE**) is a proven AGP alternative strategy to improve intestinal health and performance, which is having broad-spectrum antimicrobial activity and highly effective against Gram-positive bacteria (e.g. *Clostridium perfringens*).

Microcin J25 (MccJ25) is a potent antimicrobial peptide from *E. coli* containing 21 amino acids and has a strong inhibition on *E. coli* and *Salmonella*. It belongs to the class of lasso peptides meaning its structure includes a looped “lasso” configuration that contributes to its remarkable stability and activity. MccJ25 is known for its strong antibacterial activity and stability (pH, temperature and enzymatic conditions). It inhibits bacterial growth, particularly Gram-negative bacteria, and has acquired significant interest as non-antibiotic growth promoter. Microcin J25 has dual and independent mechanisms of action in *E. coli* - RNA polymerase inhibition and overproduction of reactive oxygen species (ROS), such as superoxide anions in bacterial cells. MccJ25 was found to disrupt bacterial cell membrane potential inhibiting oxygen consumption in *Salmonella*. Studies showed that MccJ25 promoted growth performance, improved intestinal morphology, and influenced fecal microbiota composition. Hence is an effective growth promoting alternative to Colistin.

Cecropin II, a highly potent antimicrobial peptide from *B. subtilis* that consists of 37 amino acids with a molecular weight of 38 kDa. It destroys cell membrane of Gram-positive (e.g. *Clostridium*) and Gram-negative bacteria. Cecropin binds to negatively charged bacterial membrane lipid with its strongly positively charged N-terminus, and then induces pore formation by its hydrophobic C-terminus, which then renders membrane-permeable and release of intracellular contents eventually leading to bacterial death. Another bactericidal mechanism is that it penetrates into cytoplasm and interacts with intracellular substances, such as inhibiting DNA, RNA and protein synthesis, inhibiting protein folding, inhibiting enzyme activity and cell wall synthesis, and promoting release of lyases to destroy cell structures. It also enhances macrophage phagocytosis ability against *Clostridium*. Cecropin II is thermostable at boiling temperature (remains active under pasteurization temperature), resistant to acid and alkaline pH and enzymes action in gut. Thus serves as a potent non-antibiotic growth promoter by promoting intestinal health and performance.

In conclusion, non-antibiotic growth promoters play a crucial role in modern poultry production by offering a safe and effective way to enhance bird health, improve production efficiency, and reduce the reliance on antibiotics. They offer a promising path towards sustainable and antibiotic-free poultry farming.

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