Chapter 43

Innovative Strategies: Redefining E. coli control in poultry without Antibiotics

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ABSTRACT

The poultry industry has contributed a significant role in bridging the nutritional gap in many countries by producing eggs and meat products that have high level of protein and vital nutrients at a lower cost. Since the ban of antibiotic growth promoters (AGPs), the natural antibiotics alternatives including prebiotics, probiotics, organic acids, symbiotics, immunostimulants, enzymes, essential oils, and phytogenic including botanicals, oleoresins, essential oils, and herbs are most commonly used as feed additives that have gained popularity in organic poultry industry. They are widely utilized across the world due to their distinct features and good influence on poultry production. They are simple to combine with other feed ingredients, leave no tissue residue behind, enhance feed intake, feed gain, feed conversion rate, boost immunity in birds, enhance digestion, increase the availability and absorbability of nutrients, have anti-microbial properties, do not alter carcass characteristics, reduce the need for antibiotics, act as antioxidants and anti-inflammatory agents, compete for stressors, and produce nutritious organic products that are safe for human consumption. Therefore, the current review focuses on a comprehensive description of different natural antibiotic growth promoters' alternatives, the mode of their action, and their impacts on poultry production.

KEYWORDS		
E. coli, antibiotics, organic additives, poultry, AMR, health	Received: 11-Jun-2024 Revised: 27-Jul-2024 Accepted: 05-Aug-2024	A Publication of Unique Scientific Publishers

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INTRODUCTION

Organic farming is one of agricultural industries with the quickest growth. Consumer demand has driven the market since the 1990s for goods produced on organic farms, including animal products. Sales of organic food had been increased from $28.4 billion in 2012 to $35 billion in the United States in 2014. During 2023, it has approached to $70 billion (Okey, 2023). US retailers imported organic foods worth billions of dollars into the American market since demand for them exceeded supply in the past few years. Poultry meat and eggs are among the organic animal products that are readily available and well-liked by customer's worldwide (Ponnampalam et al., 2019).

According to the most recent studies, the organic poultry market will rise from 9.78 billion to 10.34 billion dollars in 2022 and 2023 respectively with 5.8% CAGR. Nevertheless, the sector has yet to be able to keep up with the growing demand for organic poultry, despite having 3.5 million certified layer hens, 400,000 certified organic turkeys, and 9 million certified broilers. This confirms the Organic Trade Association's forecast of record growth for the organic poultry industry in the upcoming years. While organic chicken production has a lot of room to grow. There are uncertainties about the organic eggs and meat that may be contaminated with food-borne diseases could bound this potential (Wan et al., 2019).

Producing broiler chicks is a significant component of the global poultry industry. In order to control pathogens in organic poultry, it is necessary to act quickly to discover alternative and appropriate antimicrobial intervention techniques. To protect animals, human beings, and their surroundings, the National Organic Program forbids extensive usage of herbicides, hormones, pesticides, and antibiotics in poultry as well as agriculture practices. This could increase the sustainability of the industry (Zhang et al., 2021).

If the use of antibiotics is restricted in poultry, colibacillosis will become a more significant problem. So, there is need to search out alternatives to antibiotics usage. So that prevent and treat colibacillosis in poultry. The purpose of the following studies is to control if herbal extracts, bacteriophage, prebiotics, probiotics, and some other things can be utilized to treat *E. coli*.

E. coli Infections in Poultry

Colibacillosis is the term used to describe an infection that is characterized by enteritis, hemorrhagic septicemia, swollen head syndrome, salpingitis, peritonitis, coliform cellulitis, synovitis, orchitis, omphalitis, and colisepticemia. It is distinguished by pericarditis, air saculitis, perihepatitis, and other diseases in its subacute form and septicemia, which can be fatal in its acute form. The features include the presence of exudations, such as serum, fibrin, and inflammatory cells (pus), in the peritoneal (abdominal) cavity. Chickens have an inflammatory reaction that results in fibrin, which is visible, also cover the surface of several organs, such as intestine, liver, lungs, oviduct, and ovary.

According to observations, avian-colibacillosis is a prevalent disease that affects all age birds globally and has significant financial effect on the poultry production. Economic losses occur from the mortality and also due to reduced productivity of infected birds, primarily during the peak period of egg production and also during the late laying period (Linden, 2015). Avian Pathogenic *Escherichia coli* (APEC) infection is also occurred with infectious bursal disease (IBD) virus, mycoplasma, New Castle disease (ND) virus, infectious bronchitis (IB) virus, and environmental influences such as ammonia, humidity, temperature and dust. Additionally, when an egg is contaminated with feces, *E. coli* can enter the shell and disseminate to offsprings during hatching, leading to early chick death and also yolk sac infection. Birds' digestive tracts naturally contain *E. coli* bacteria, and most strains do not spread illness. Yolk sac infection and colisepticemia in early chick occurs. Coli-granuloma and peritonitis of eggs in adults occur (Masud et al., 2020).

Etiology

Escherichia coli is facultative, Gram negative, anaerobic, nonacid fast, non-spore forming, bacillus that is typically 3 × 0.6 µm in size and belongs to the family Enterobacteriaceae. It is usually present in GIT tract of human beings, animals, and poultry. Generally, *E. coli* strains are not harmful to people, but some strains can potentially infect human beings and even commercially raised poultry (Ievy et al., 2022). Enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), entero-invasive (EIEC), and enterotoxigenic *E. coli* (ETEC) are some of the strains that cause food poisoning. Hosts with weakened immune systems are more susceptible to virulent *E. coli* (Lim et al., 2020).

Epidemiology

E. coli is widespread and naturally found in GIT of animals and poultry at conc. of about 10^6 per gram. At the same time, the dust in chicken houses can have concentrations of up to 10^5–10^6 per gram. Both healthy birds and their eggs are susceptible to contamination from garbage and excrement. Newborn chicks may contract the disease through the ovaries. The pathogenic *E. coli* sources include food, rodent waste, and tainted well water. While infective *E. coli* isolates in poultry often have few serogroups, especially O1, O2, and O78. But O15 and O55 can be occasionally identified in small amounts as well (Parin and Simsek, 2023).

Transmission

The gastrointestinal system of chickens and other avian species frequently contains the bacterium *E. coli* as normal bio-flora. It is possible for the same or different poultry species to transmit bacteria by oral-fecal route. It can return to the environment through infected birds' droppings. The most likely places for *E. coli* strains to be detected are in the chickens' intestines and surrounding. The most common way for poultry to get *E. coli* infection is by the inhalation of *E. coli* infected dust particles. The flies, beetle, insects, rats, mites, and wild birds are only a few vectors that can spread *E. coli* to new environments. *E. coli* transmission can take place directly, indirectly, or both horizontally and vertically. *E. coli* can be transmitted vertically and transfer to progeny (Olawuwo et al., 2022).

Clinical Signs and Lesions

Typically, broilers that are around five weeks old can be affected mostly. Due to predisposing circumstances, resistance may be compromised then chicks of any age may be infected. If their resistance has been compromised, chicks under ten days old are particularly vulnerable. In these circumstances, the infection can be moderate or persistent, without any clinical symptoms. Coli-septicemia can cause birds to lose their appetite and stop drinking and eating. The reduced water consumption may be a sign of the disease's severity. Birds who are chronically afflicted display evidence of retarded growth rate and wastefulness.

E. coli infection occurs in two forms: **i)** localized **ii)** systemic.

Infected chicken has swollen appearance of face. Skin with Inflammatory exudate that builds up due to microorganisms, mostly *E. coli*, following the upper respiratory tract (URT) viral infection, as infectious bronchitis virus (IBV), avian metapneumovirus, etc. Loose connective tissue Inflammation beneath the skin is known as cellulitis. In subcutaneous tissues, caseous and serosanguinous exudates are frequently found in the belly or between midline and thigh (Struthers, 2024).

Affected birds show following signs and symptoms:

- Swollen abdomen, depression, and huddling
- Birds are often dehydrated, stunted, vent pasting as well as enlarged gall-bladder
- Hemorrhages on the surface of the intestine and peritonitis
- Distended yolk sac with anomaly in color, smell, and consistency
- Un-absorbed yolk sac

Each type has unique pathogenic factors that influence the signs and symptoms of enteric illness. Oviduct inflammation is called salpingitis caused by *E. coli* infection. It reduces production of eggs, resulting in the mortality of breeders and laying hens. Infection is caused by the cloaca, vent pecking, infected air sacs, and prolapse (Linden, 2015). *E. coli* is most often the cause of egg peritonitis. Egg peritonitis is peritoneal inflammation induced by a cracked egg in the abdomen. Random mortality in laying or breeding hens is caused by it commonly (Rosales, 2019).

Coligranuloma, also known as Hjarre's disease that affects hens, quails, and turkeys. It is a rare type of colibacillosis. Several granulomas are seen in ventricle, pro-ventriculus, liver, small intestine, mesentery, and cecum. The pathogenic *E. coli* in blood of birds is classified as coli-septicemia. Different phases of colisepticemia are: i) sub-acute polyserositis ii) acute septicemia iii) chronic granulomatous inflammation. Air sacculitis of various degrees results in respiratory symptoms such as rales, sneezing, and coughing. Colisepticemia can cause osteomyelitis, arthritis, tenosynovitis, and spondylitis. Air sacs become opaque, enlarge, and may carry caseous exudate on PM inspection. Infected birds seem normal and are frequently discovered dead with a full culture (Panth, 2019).

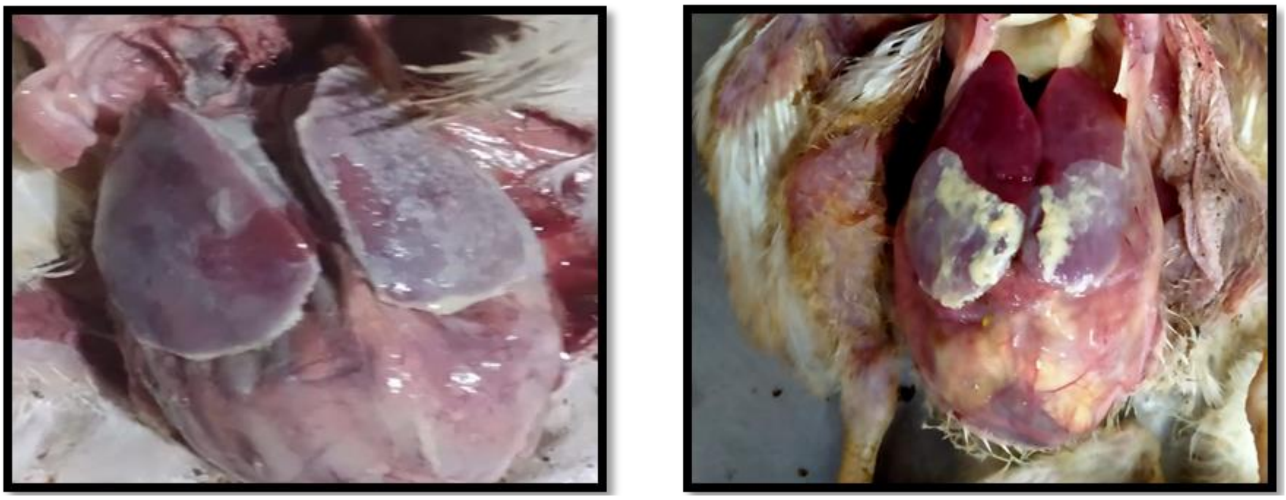

Fig. 1(a, b): Prominent sign of *E. coli* caesious layer on Liver, perihepatitis.

Antibiotic Treatment for *E. coli* in Poultry

Some *E. coli* strains have been effectively treated using antibiotics such as tetracyclines, ampicillin, sulfas, and streptomycin. Early treatment is recommended, with a follow-up that takes antibiotic sensitivity testing, considering the particular isolate. Antibiotic therapy becomes less effective when the organism becomes sequestered or encapsulated in caseous exudate; consequently, chronic phases of infection are less likely to be treated appropriately. The great majority of clinical isolates, however, are tetracycline resistant, with most APEC isolates resistant to 5 or more drugs. Fluoroquinolones are currently prohibited in several nations, including the United States (Dixit et al., 2024).

The spread of AMR is the most challenging topic in human, animal, and environmental health in the following century. AMR emerged as one of the main obstacles to economic growth (Akter et al., 2022). Antibiotic overuse and misuse are significant contributors in formation and spread of *E. coli* with antibiotic-resistant which can transfer to human beings via direct contact with ill animals or with contaminated food. There is antibiotics extensively usage in poultry to treat infectious disorders and as growth promoters (Roy et al., 2022). AMR is unavoidable due to the extensive antibiotic usage in clinical as well as non-clinical settings in countries with limited resources (Hoque et al., 2020). Bacteria such as *E. coli* have evolved multi-drug resistance (MDR) as a result of haphazard antibiotic usage (Islam et al., 2021). The rise of MDR strains resistant to antimicrobial medicines may result in increased morbidity, mortality, and healthcare costs (Dadgostar, 2019).

Between 2010 and 2021, antimicrobial resistance genes have been described in 58.8% (10/17) of the published articles. In poultry samples, resistance genes for tetracyclines, sulfa medicines, fluoroquinolones, beta-lactams, aminoglycosides, polymyxins, and phenicols etc. are found in *E. coli*. All of the resistance-genes have a more significant proportion (about 100%), suggesting a severe problem in the health structure. Furthermore, presence of transposons linked genes and natural plasmids in *E. coli* isolates obtained from poultry and poultry habitats (different bla genes, tet A, B, C, etc.) suggests the presence of many genetic mobile elements (Khruengsai et al., 2023). According to a comprehensive research, *E. coli* from poultry and its environment were resistant to fourteen anti-microbial drugs and forty-five distinct antimicrobial agents. A current study found that isolates of *E. coli* from poultry flesh was resistant to thirteen antibiotic classes, which concerns the medical community. *E. coli* isolates are also resistant to penicillin (Parvin et al., 2020).

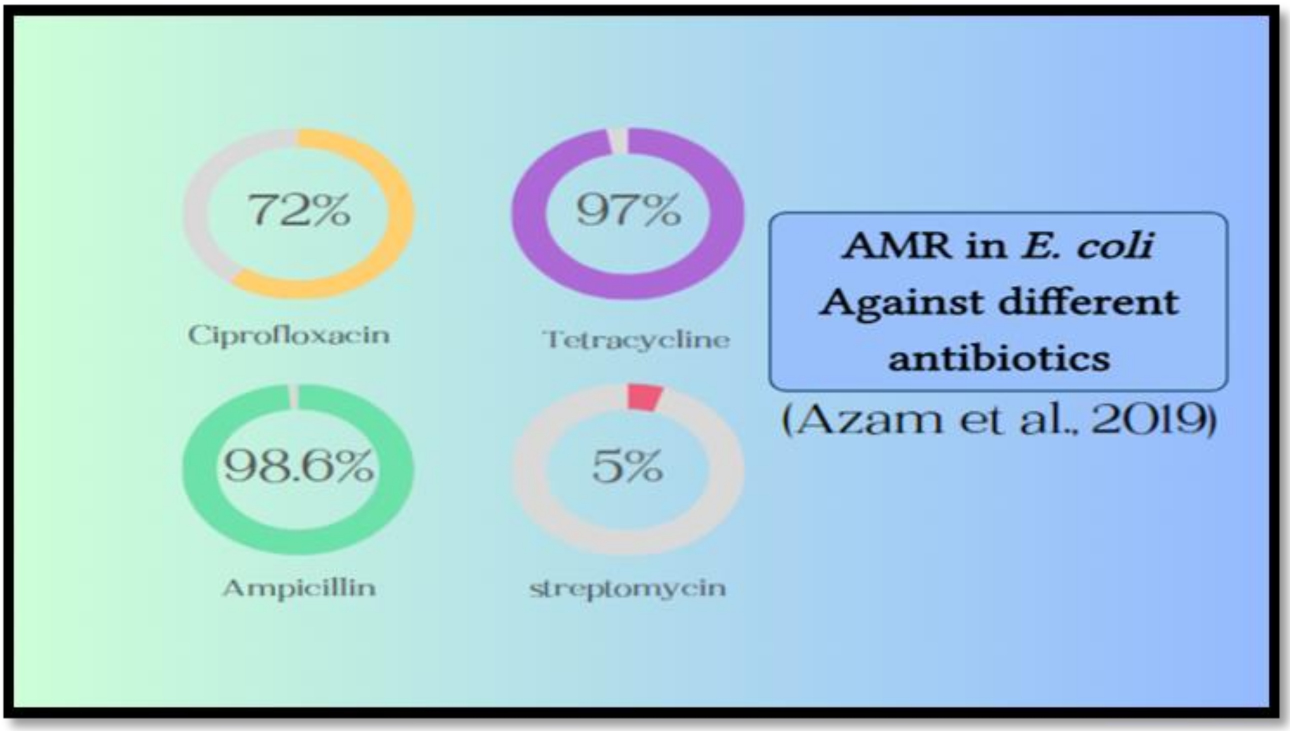

Fig. 2: AMR values against different antibiotics in *E. coli*

Alternative Treatments of *E. coli*

A. Probiotics

Antibiotics have been utilized as growth promoters or feed additives since the 1940s as it was discovered that feeding *Streptomyces aureofaciens* with chlortetracycline residues to birds or animals increased their growth rate. European Union (EU) has prohibited the use of antibiotics as food additives or growth stimulants. The reason for this was the rise of microbial resistance to antibiotics since 2006, which has been used to treat infections in animals and poultry. Furthermore, antibiotics create other problems, as beneficial bacteria in chicken gut being killed (Uzabaci and Yibar, 2023).

Due to the biohazards of antibiotic usage, such as their lingering effects on meat and food products, nutritionists have recently concentrated their efforts on developing innovative and alternatives to therapeutic supplements and growth promotors to avoid illnesses and boost avian immunity (Yadav et al., 2016). In this regard, bacteriophages, avian egg antibodies, cytokines, toll-like receptors, probiotics, and other substances has been investigated for their potential benefits for protecting animals from infections and enhancing production performance. Probiotics protective effects and advantageous uses are evident in a number of ways. Probiotics have the potential to improve growth performance, the quality of eggs, nutrient absorption, and digestibility, thereby increasing production and protecting the health of poultry. The most popular probiotic strains include Streptococcus, Lactobacillus, Bacillus subtilis, and Bifidobacterium strains. These strains not only promote growth but also have the ability to reduce harmful bacteria like *Salmonella typhimurium, Escherichia coli, Clostridium perfringens, Staphylococcus aureus,* and others. Because of its potential to limit pathogen development, the proper selection of probiotic strains might reduce the adverse effects of antibiotic treatment and has several valuable uses (Sugiharto, 2023).

Live bacteria like Lactobacillus, Bacillus subtilis, Bifidobacterium, and Streptococcus are just a few sources of probiotics. Other sources include yeasts like *Saccharomyces boulardii, Saccharomyces cerevisiae*, Candida, and fungus like Aspergillus. Probiotics can work in several different ways to inhibit pathogens, including by producing organic acids and antibiotics like hydrogen peroxide, bacteriocins, and defensins. They can also influence the host immune system's regulatory T cells, effector B and T cells, antigen-presenting cells, and enterocytes. Probiotics can also modulate the function and phenotype of dendritic cells, the production of anti- and pro-inflammatory cytokines, the stimulation of antibody (sIgA) production, the enhancement of macrophages (NK) and natural killer cells activity, the release of nitric oxide, the modulation of apoptosis and many other biological processes (Abun et al., 2024).

Probiotic supplementation resulted in lower counts of *Escherichia coli*, total coliforms and higher lactobacilli counts in the gut of chickens. Additionally, the probiotic combination increases the population of helpful bacteria while reducing *Escherichia coli* in the cecal contents, improving the bacterial count. Dietary *Enterococcus faecium* probiotic supplementation improves growth performance and decreases death rate of the broiler chickens. It boosts the humoral immune response, regulating the release of inflammatory cytokines, increasing the expression of tight junction proteins (TJ proteins), and maintaining the intestinal barrier against *E. coli* O78 infection. *E. faecium* has the power to preserve intestinal integrity and reduce the excessive intestinal permeability brought on by *E. coli* infection (Huang et al., 2019).

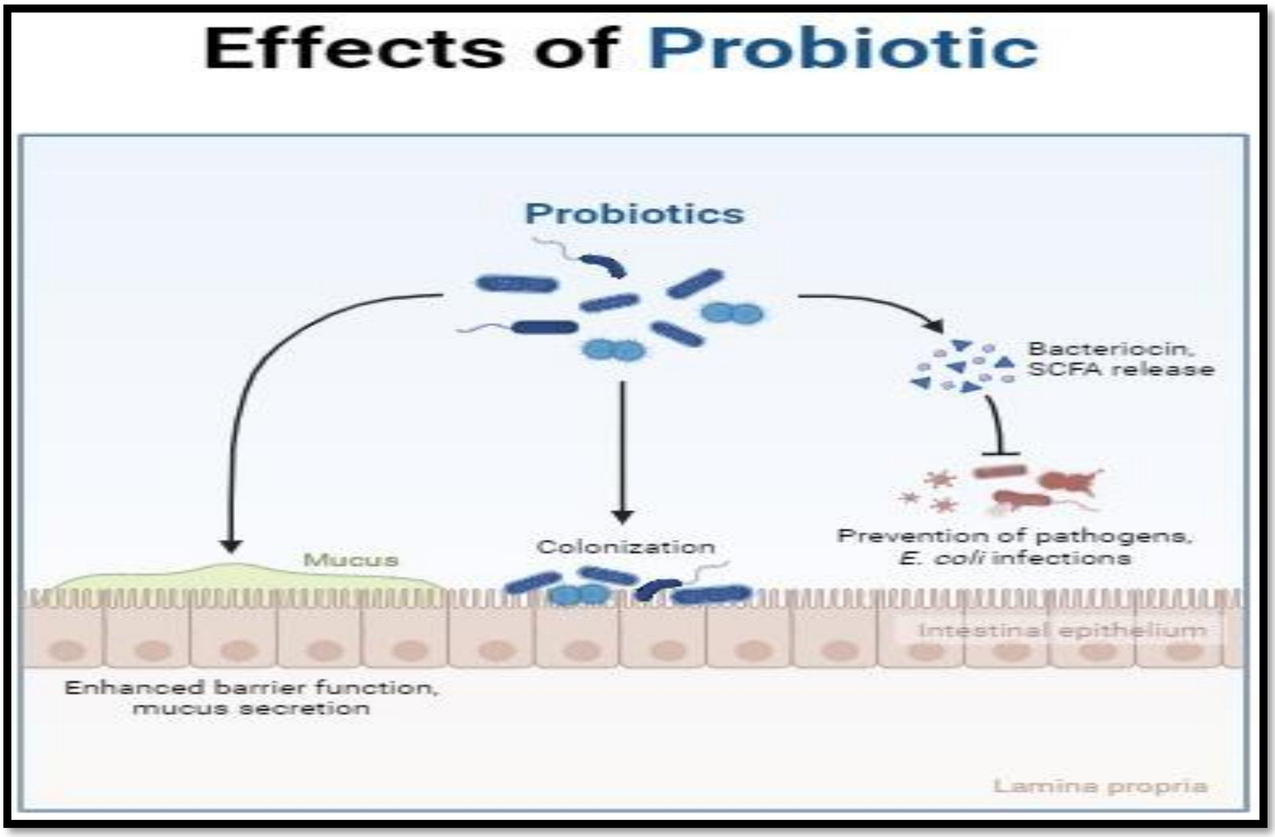

Fig. 3: Probiotic effect on intestinal villi and their mode of action.

Prebiotics

Traditionally, prebiotics were represented by a small range of carbohydrates and related chemicals. Galacto-oligosaccharides (GOS), mannano-oligosaccharides (MOS), and fructo-oligosaccharides (FOS) are among the more often used in animal and poultry research. Fundamentally, the host animal or person ingesting these substances does not make use of them, but some bacteria, such as bifidobacteria and lactic acid bacteria, can use them as substrates. Given that FOS and comparable prebiotics are thought to be largely fermentable and less likely to persist intact in the GIT for extended periods of time, prebiotic variations in pathogen and host responses may also be connected to the chemical composition of the prebiotic. In contrast, the yeast-derived MOS can directly reduce GIT infections by attaching to the flagella of microbes like *Escherichia coli* and Salmonella, reducing their colonization of the GIT by interfering with their attachment to GIT epithelial cells. By attaching to macrophages and dendritic antigen-presenting cells that carry the C-type lectins of the mannose receptor, yeast mannans have also been demonstrated to function as immunological adjuvants and directly induce immune responses (Fomentini et al., 2016).

Isolated alginate from marine brown algae called polymannuronate is thought to be a promising prebiotic. It is known to preferentially colonize helpful microorganisms while excluding pathogenic and dangerous microbes. According to new research, *E. coli* levels were found to decrease and lactic acid bacteria to increase when dietary polymannuronate levels were raised. Furthermore, a higher level of acetic acid was discovered in the broilers. Thus, the synthesis of lactic acid, acetic acid, and volatile fatty acids (VFA) might reduce intestinal pH and produce an environment that prevents the development of dangerous bacteria. In recent studies, species-specific quantitative PCR has been performed, and the results show that FOS (2.5 g/kg feed) enhanced the population of Lactobacillus while limiting the growth of *E. coli* and *C. perfringens* in broilers (Zhu et al., 2015).

Herbal Extracts and Essential oils

The large variety of functionally important secondary metabolites (phytochemicals) that plants generate; has a wide range of therapeutic benefits. Most of these chemicals are employed by plants as a kind of protection against other microbes, herbivores, and rivals. Essential oils (EOs), polypeptides, alkaloids, lectins, phenolic compounds, and polyacetylenes are the prominent phytochemicals found in plants. The complex blend of natural, volatile, and aromatic molecules known as essential oils (EOs) is produced by aromatic plants, many of which have been employed in traditional medicine. It has been noted that EOs work by disrupting cell walls and cytoplasmic membranes, which causes lysis and the leaking of intracellular chemicals. The attachment of cytoplasmic membrane to outer membrane (MO) in Gram-negative bacteria did not result in an increase in resistance to the constituents of EOs. Several substances have now proven they may disrupt the OM by releasing LPS.

Additionally, several phenolic components of EOs (such as carvacrol and thymol) have shown the capacity to interact with OM and exhibit bactericidal action (Chen et al., 2023).

Currently, administering antibiotics to treat infections causes harmful damage to the organs, tissues, and cells of the host. Herbs can prevent, treat, or combat the detrimental effects of antimicrobial agents. Certain plants have beneficial antibacterial and antifungal characteristics that can be used in medicinal settings. Clinical tests on infected birds revealed that *Cassia auriculata* plants possess vigorous microbicidal activity (Alem, 2024).

There is, however, a need for more information on how well EOs improve sanitary conditions. These natural chemical vapors may be used to control undesirable agents, according to data on the efficiency of air-dispersed EOs in lowering fungal and bacterial load in nosocomial environments. Relevant anti-*E. coli* activity is present in *L. cubeba*. Neral (32.5%) and geranial (36.4%) aldehydes are primarily responsible for the hole formation on membranes of *E. coli* cells. *M. piperita* EO has good reactivity against *E. coli* during different tests. Bacteria were tested with *M. piperita* EO; *E. coli* showed the highest level of antibacterial activity. *P. graveolens* and *O. basilicum* EOs demonstrated moderate antibacterial activity, supporting findings from prior investigations that these substances more effectively combated Gram-positive bacteria than Gram-negative bacteria. The combination of *S. aromaticum* and *C. zeylanimcum* may be an effective alternate therapy for *E. coli*. *A. fumigatus* and *E. coli* may both be eliminated by *C. citratus* when applied alone (Ebani et al., 2018).

Cloves' whole essential oil inhibits the growth of *E. coli*. It has been demonstrated in vitro that oregano essential oil is a potent inhibitor of *E. coli*. Carvacrol, a key ingredient of oregano essential oil, was discovered to have less antibacterial activity than the whole oil. Additionally, cinnamon oil has been demonstrated to have an antibacterial effect against *E. coli* in vitro. Another plant with antibacterial capabilities demonstrated in vitro is garlic (*Allium sativum*). Two strains of *E. coli* were effectively inhibited by the aqueous extract of garlic and were susceptible to its effects (Abd El-Ghany, 2024).

Modes of Action

PFAs (phytobiotic feed additives) improve health of gut and also improves its performance by different mechanisms: antimicrobial properties, antioxidative properties, growth promotion, improved digestion, improved palatability, and improved health of gut. Researches on palatability are indecisive, but PFAs can improve quality of feed by its anti-oxidative properties and slow fungal and bacterial growth. EOs breaks cell wall and cell membrane of infectious agents and also increase permeability of membranes of cell which results in release of cell contents including genome (Abd El-Hack et al., 2022).

D. Bacteriophages

The emergence of bacteria with various antibiotic resistance has increased the need to find an alternative of antibiotics to treat bacterial infections. A class of viruses known as bacteriophages is found all across nature and is exclusively linked to the life cycle of bacteria. We refer to them as parasitic bacteria. Bacteriophages are viruses that infect and have the ability to kill bacteria. Twort (1915) and d'Herelle (1917) separately discovered these viruses. Both the intramuscular and aerosol routes can be used to administer bacteriophage. Recent studies have shown that while bacteriophages administered by aerosol method do show some results, they do not yield positive outcomes against *E. coli*. Chickens that are administered bacteriophage aerosol can be protected against *E. coli* infection for up to three days. Additionally, it has been discovered that bacteriophages injected intramuscularly produce excellent and productive outcomes. The idea that bacteriophage can be used as a successful substitute for antibiotics in animal production to prevent and treat bacterial diseases is supported by the fact that bacteriophage can be therapeutic if given with high enough titers (Roth et al., 2019).

Phage treatment is safer and more effective than antibiotics in part because bacteriophages are unique to specific bacteria, meaning they can infect only one strain, species, or serotype. This mode of action does not harm the commensal gut flora. Because bacteriophages replicate themselves during treatment, applying them again is unnecessary. Another benefit of phages is that they cannot destroy eukaryotic cells. This results in drop in their titers and a significant decrease in the quantity of dangerous bacteria which cause infection. Because most phages are mostly made of proteins and nucleic acids, they have the equally significant benefit of not being poisonous (LA and Waturangi, 2023).

Phage therapy has several benefits, but its use is severely restricted, in part due to the fact that individual bacteriophages are not effective against infections with a wide range of symptoms. Complex etiological agent identification and characterization are frequently required. Furthermore, some bacterial viruses particularly lysogenic phages, which encode the genes of bacterial toxins and turn benign bacteria into dangerous ones, do not fit the criteria to be used in therapy. Additionally, they might be implicated in the spread of drug-resistant genes among bacteria. Phage therapy can also have unfavorable effects, such as phages being eliminated by the reticuloendothelial system, which shortens the phages' half-lives in the body and decreases therapeutic efficacy (Lee et al., 2024).

Phage therapy is also helpful in curing bacterial infections in various animal species. Additionally effective in treating poultry infections are bacteriophages. Evaluating the viability of bacterial viruses to control infections that significantly affect the productivity and health of animals is one of the goals of phage therapy. Treatment with phages has successfully treated chicken colibacillosis and avoided infections.

Organic Acids

Chemicals with an acidic pH are called organic acids. The most common kinds are carboxylic acids, which include butyric acid, lactic acid, sorbic acid, acetic acid, formic acid, citric acid, uric acid, oxalic acid, and propionic acid. Organic acids can support the intestinal health of chickens by enhancing feed conversion ratios, livability, weight gain, live weight, and immune responses. This is done only when combined with good management, good nutrition, and biosecurity practices (Saeed et al., 2017).

The impact of organic acids on intestinal health and broiler performance reduces pH, which makes organic acids to inhibit proton donors in aqueous solution and results in weak acids. This activity is associated with organic acids' antibacterial action. Since the dissociation of weak acids is pH-dependent, lower pH values result in increased antibacterial activity (Jadhao et al., 2019). When organic acids are not dissociated, they are lipid-soluble and can be readily incorporated into bacterial cells through both carrier-mediated and passive mechanisms. When there is alkaline environment, organic acids produce protons intracellularly. Then pH reduces to a point where it disrupts microbial metabolism by impeding vital enzyme activity. This forces the bacterial cell to expend energy exporting excess protons, which ultimately leads to starvation and death. Protons H^+ have the ability to denature DNA and proteins that are sensitive to acid in bacteria. Because unlike other bacteria as *E. coli*, Lactic acid bacteria can grow at low pH level. Lactic acid bacteria are more resistant to organic acids. Due to their high internal potassium content, gram-positive bacteria like Lactobacilli are shielded from acid anions (Araujo et al., 2019).

Organic acids lower the quantity of harmful microorganisms because of their antibacterial activity. This leads to decrease level of toxic bacterial metabolites, less bacterial competition for nutrients, better protein digestibility, and enhanced avian growth (Hassan et al., 2014). The histological structure of the gastrointestinal tract is altered by the addition of organic acids, which increases the length of the villus and enhances the intestinal mucosa's capacity to absorb nutrients. As a result, better nutrient absorption, and enhanced growth performance are achieved. pH low level in stomach promotes good bacterial growth while inhibiting harmful micro-organisms. Additionally, these acid anions combine zinc, phosphorus, magnesium, and calcium to increase their digestibility. The decreased pH in the stomach causes an increase in pepsin activity. Peptides generated by the proteolysis of pepsin trigger the release of hormones that control the digestion and absorption of proteins, including cholecystokinin and gastrin (Padmini et al., 2017).

There are many commercially available organic acid products which are micro-granulated feed acidifiers that are based on lactic acid, fumaric acid, formic acid, and ammonium format. These are considered as unprotected organic acid mixtures which are active in foregut and neutralized by bile. 49.0% benzoic acid and 3.0% calcium used in organic acid products. Due to its dissociation in alkaline pH of jejunum, organic acid combinations active in midgut to protect it. Spices and herbal extracts are essential for enhancing the health and productivity of bird. The benefits of plant extracts or active ingredients in bird feed may include:

- Boosting feed intake and appetite.
- Improving the synthesis of endogenous digestive enzymes.
- Boosting immunity.
- Having antioxidant, antiviral, antibacterial, and anthelminthic properties.

Flavonoids, glucosinolates, isoprene derivatives, and other herbal metabolites may have an impact on the gut's physiological and molecular processes in birds. Nutrient metabolism may be intermediate, along with the stabilizing effect on the gut microbiome. Numerous infections are hazardous to livestock and poultry production, causing significant financial losses (El-Saadony et al., 2021).

The herbal feed additives usage is now more popular in the production of chickens because of the restrictions on the use of various antibiotics, their harmful side effects, and their affordability. There are several uses of spices, herbs, and their extracts. These encourage feed intake, boost the immune system, function as antioxidants, and have antibacterial, coccidiostat, and anthelmintic qualities. The herbal feed additives which are mostly used in poultry feed are cinnamon, nutmeg, cloves, coriander, cardamom, cumin, anise, parsley, celery, fenugreek, pepper, capsicum, ginger, garlic, horseradish, mustard, onion, mint, shatavari, rosemary, thyme, shatavari, jivanti, and turmeric (Abou-Kassem et al., 2021).

Enzymes

Chemical reactions can be accelerated by biological catalysts known as enzymes. Enzymes are protein molecules that have significant effects on how stable they are during the digestive tract's passage and the formation of high-temperature meals (pelleting). To break down food, all creatures need enzymes. Adding specific enzymes to the feed increases the nutritional content of the food and speeds up the process of digestion. Ultimately, by lowering the amount of manure produced and the amount of nitrogen and phosphorus expelled. Enzymes in feed are utilized to increase the efficiency of feed, lower feed costs, and enhance environment (Barletta, 2010).

Enzymes are classified into different groups. They can be classified based on the substrate they act on and their origin. Enzymes are categorized based on the substrates they break down into three groups:

- Phytases, which break down phytate.
- Proteases, which break down fiber and starch.
- Proteases which are made up of enzymes that break down proteins.

The sources of enzyme are exogenous and endogenous. Since the 1920s, studies on the role of enzymes in poultry diets have been conducted. Protozyme, an enzyme product derived from *Aspergillus oryzae*, was the first documented application of this product in poultry diets. An un-pelleted poultry meal including wheat and rye at different inclusion levels, increase significantly body weight gain when enzyme cocktails containing ß-glucanase and xylanase was added (Mak et al., 2022).

Exogenous enzyme supplementation has emerged as the gold standard for enhancing nutrient utilization efficiency and digestibility. All creatures require enzymes, which are either produced by the animal itself or by microorganisms in their digestive system, in order to digest food. Enzyme supplementation aids in lowering nutrient excretion, which might incur additional costs for the farmer, feed supplier, and the environment if left unchecked (Ali and Abdelaziz, 2018).

Reduced feed costs are contingent upon the presence of exogenous enzymes. Enzymes can lower production costs by inadequately absorbing other nutrients since feed makes up the majority of production costs. Utilizing enzymes in feed becomes more financially appealing and offers a more noteworthy return on investment as the price of wheat, fat, corn, and inorganic P rises. Inositol and inorganic phosphate are the products of the hydrolysis of phytate by the enzyme phytase (AKDAĞ and KIYMA, 2023). More specifically, phytase is also referred to as myo-inositol hexa-bisphosphate phosphohydrolase. For growth and maintenance, poultry require phosphorus (P) in their diet. As a result, the diet needs to contain a certain quantity. Even with an adequate supply of total P in the diet, chickens cannot digest some of the total P derived from cereal grains. Because it is linked to phytate, roughly 60% of P is inaccessible to non-ruminant animals. Phytate causes significant decreases in nutritional availability by binding to numerous dietary cations, including fat, protein, vitamins, Ca, Mg, Fe, Cu, Zn, and Mn (Bashir and Kumar, 2021).

According to market trends, hydrolytic enzymes have become more popular as feed supplements to help with the digestion and absorption of minerals that are not readily available, including dietary phytate. Because of their low microbial population in the upper part of the digestive tract and the absence of strong endogenous phytase activity, non-ruminant animals can only digest phytate. In poultry, the digestive tract (crop, proventriculus, and gizzard) is the primary location of phytate breakdown by phytases; the distal gastrointestinal system has less degradation in this context (Barletta, 2010).

Conclusion

Large-scale use of the natural antibiotic growth promoter alternatives is advised because they are safe, healthy, and have a positive immune-modulating effect. They also digestion, improve productivity, absorbability, availability of nutrients, intestinal health, and the production of organic chicken meat that is useful, safe, and nutritious for human consumption. These all are beneficial against *E. coli* treatment as alternative of antibiotics to combat AMR.

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