



Research and Application Progress of Antibiotic Alternative Products in Animal Production

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Abstract

The widespread misuse of antibiotics in intensive animal farming has led to severe global challenges, including antimicrobial resistance, drug residues, and environmental pollution, prompting a critical shift towards antibiotic reduction and prohibition. This review systematically examines the research and application progress of key antibiotic alternative products, including microecological preparations, natural plant extracts, antimicrobial peptides, and antibacterial vaccines. It details their distinct mechanisms of action, such as modulating gut microbiota, inhibiting pathogens, and enhancing host immunity. Current applications in various livestock and poultry sectors demonstrate their potential to support growth and health. However, evidence suggests that a single product cannot fully replicate the functions of antibiotics. Therefore, the future lies in developing integrated antibiotic replacement strategies that synergistically combine these alternatives with refined management practices. Ultimately, fostering innovation coupled with robust regulatory frameworks is essential for driving the green and sustainable transformation of animal husbandry.

Since Alexander Fleming's discovery of penicillin in 1929, humanity entered a new era in the fight against microbes. The widespread use of antibiotics profoundly transformed medicine and agriculture. However, in the livestock sector, the intensive farming models that emerged from the 1940s onwards to meet the growing demand for animal products gave rise to significant animal health issues [1]. Antibiotics were once hailed as the solution, delivering short-term benefits in promoting growth and preventing diseases. Their indiscriminate use, however, has triggered a global public health crisis: the spread of bacterial resistance (notably "superbugs"), drug residues and resistance genes in animal products threatening human health through the food chain, and ecological pollution of soil and water bodies by discharged antibiotics. These problems not only undermine the sustainable development of the livestock industry itself but also create a vicious cycle of dependency [2].

To address this severe challenge, the international community has taken action. In 1986, Sweden pioneered legislation banning the use of antibiotics as feed additives, a move followed by many other countries and regions [3]. Against this backdrop, the research, development, and promotion of safe, efficient, and sustainable antibiotic alternative products have become a global research hotspot and an imperative choice for animal husbandry [4,5]. This article aims to systematically review the main categories, mechanisms of action, and current application status of antibiotic alternative products in animal production, and to provide an outlook on future development prospects. The goal is to offer theoretical reference and practical insights for the green transformation and sustainable development of animal husbandry.

Types of Antibiotic Alternative Products and their Mechanisms of Action

Currently, mainstream antibiotic alternatives primarily include microecological preparations, natural plant extracts, Antimicrobial Peptides (AMPs), and antibacterial vaccines, among others. Their core value lies not in simply replacing a specific antibiotic but in serving as components of a comprehensive antibiotic replacement

strategy. They work synergistically with refined management practices, environmental control, and biosecurity systems to jointly establish a new paradigm for healthy farming.

Microecological Preparations

Microecological preparations, also known as live microbial agents or Direct-Fed Microbials (DFM), refer to viable

microorganisms or their cultures that exert beneficial effects by improving the host's intestinal microecological balance. Main categories include probiotics (e.g., *Lactobacillus*, *Bacillus*, yeast), prebiotics (e.g., oligosaccharides), and their combination—synbiotics [6].

In response to the global crisis of antibiotic resistance in animal husbandry, veterinary microecological preparations as green, safe, and harmless novel feed additives are rapidly becoming a crucial solution in the post-antibiotic era. Their core mechanism lies in ecological regulation: by supplementing exogenous beneficial bacteria, they competitively colonize the gut, inhibiting pathogen growth [7]; they metabolically produce substances like organic acids and bacteriocins that directly suppress harmful bacteria [8]; simultaneously, they stimulate the development of the host's immune system, enhancing overall immunity [9], and improve the digestibility and absorption of feed nutrients, thereby comprehensively enhancing animal health and production performance. It is essential to emphasize that the effective application of such products does not rely on a single strain but should be viewed as the cornerstone of an integrated antibiotic replacement strategy.

Natural Plant Extracts

Plant Extracts

Plant extracts are substances isolated and purified from plant seeds, roots, stems, leaves, etc., through physical, chemical, and biological methods. They possess functions such as antibacterial, antioxidant, and immune-enhancing properties. Plant extracts offer advantages including wide availability, high medicinal value, low production cost, minimal residues, low risk of inducing resistance, and few side effects, making them considered ideal alternatives for growth-promoting feed additives in livestock and poultry. Plants contain numerous active components with pharmacological effects, therefore, the mechanisms of action of plant extracts require further detailed elucidation. Integration of metagenomics, transcriptomics, proteomics, etc., may help clarify the characteristics and mechanisms of plant extracts, providing a scientific basis for developing plant-derived alternatives to antibiotic feed additives [10].

Plant extracts are rich in active components such as phenolics, polysaccharides, flavonoids, saponins, terpenoids, and alkaloids, exhibiting broad pharmacological activities. They can modulate the immune system functions of livestock and poultry by regulating immunoglobulin and cytokine secretion, enhancing lymphocyte expression, and promoting the release of interferon-gamma (IFN- γ). Studies have found that plant crude extracts containing lectins can induce strong humoral immune responses and neutralizing antibody reactions, protecting vaccinated chicks from H5N1 virus infection [11]. Drinking water supplemented with ginger extract, propolis extract, alone or in combination, can enhance humoral and cellular immune functions in broilers [12]. Feeding lactating goats, a

compound plant extract rich in polyphenols and saponins improved lymphocyte activity and cell-mediated immune responses [13].

Plant extracts can also inhibit the growth of pathogens in the gut, regulate intestinal microbiota structure, thereby reducing antibiotic use in animal production. Research indicates that quercetin can inhibit the growth of pathogenic bacteria in broiler intestines, such as *Salmonella Typhi*, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* [14]. Compared to groups without plant extracts, piglets supplemented with eugenol, thymol, piperine, and benzoic acid had lower concentrations of *Campylobacter* and *E. coli* in the cecum [15]. *Phellodendron amurense* extract can inhibit *E. coli* adhesion to cells by downregulating the expression of *E. coli* pilus genes, thereby preventing *E. coli* colonization in porcine intestinal epithelial cells [16]. Besides antibacterial activity, some plant extracts also possess antiviral properties. Studies found that oleander extract can inhibit the activity of Bovine Viral Diarrhea Virus, Bovine Respiratory Syncytial Virus, and Bovine Coronavirus, reducing the infectivity of progeny viruses [17]. Aloe vera water extract and licorice extract have been shown to effectively inhibit the replication of Porcine Epidemic Diarrhea Virus (PEDV) and improve intestinal function [18,19]. Additionally, plant extracts exhibit good inhibitory effects on viruses like influenza virus and herpes virus [20].

Plant Essential Oils

Plant essential oils are volatile secondary metabolites with aromatic odors, mostly extracted from plant buds, flowers, leaves, branches, fruits, seeds, or bark [4,21]. They are characterized by a rich chemical composition, wide distribution, numerous varieties, and are natural, residue-free, safe, and efficient. The active components of essential oils are mainly divided into two categories: phenylpropene derivatives (e.g., eugenol, cinnamaldehyde) and terpene derivatives (e.g., thymol, carvacrol, and aliphatic compounds). Currently, over 50 types of phenylpropene derivatives and over 4000 types of terpene derivatives have been identified.

Essential oils primarily function by scavenging oxygen free radicals, chelating metal ions, inhibiting lipid peroxidation, and modulating oxidative enzymes. Furthermore, due to the lipophilic nature of their active components, essential oils can alter the structure of cell membranes and phospholipid bilayers, inhibit membrane protein expression and biofilm formation, ultimately suppressing and killing bacteria, fungi, etc. [22-24].

Antimicrobial Peptides (AMPs)

Antimicrobial Peptides, also known as Host Defense Peptides (HDPs), are small molecular active polypeptides composed of 12 to 50 amino acid residues. They possess biological activity, are often induced in vivo, and have a molecular weight ranging from 2000 to 7000 Da [25]. Most of these cationic antimicrobial peptides exhibit good thermal stability, broad-spectrum antimicrobial activity, and are strongly basic [26].

Based on their source, AMPs can be classified into two categories: First, natural AMPs is the primary source, widely distributed in animals (e.g., cecropins in insects, magainins in amphibians, defensins and cathelicidins in mammals), plants (e.g., plant defensins), microorganisms (e.g., bacteriocins), and fungi. Statistics show that among 3099 identified and isolated natural AMPs, 76.12% originate from animals. Second, synthetic AMPs are novel AMPs designed and modified using chemical synthesis or genetic engineering techniques based on the structural features and functional properties of natural AMPs. The aim is to enhance antimicrobial activity, improve stability, or reduce toxicity [27].

Most cationic AMPs achieve bactericidal effects by selectively disrupting microbial cell membranes. This mechanism relies on significant differences in the charge composition of microbial versus mammalian cell membranes. Microbial membranes are typically rich in negatively charged phospholipids and lipopolysaccharides, whereas mammalian cell membrane surfaces are mainly composed of electrically neutral phospholipids. This charge distribution difference allows AMPs to preferentially recognize and bind to negatively charged microbial membranes, enabling targeted bactericidal action. Some AMPs act on intracellular bacterial targets without significantly disrupting the membrane or after penetrating it, inhibiting cell wall synthesis [28]. For instance, some AMPs can target and bind to Lipid II, a precursor for cell wall synthesis, thereby blocking peptidoglycan synthesis and compromising cell wall integrity—a mechanism particularly crucial against Gram-positive bacteria. Additionally, AMPs can inhibit nucleic acid replication and transcription. Upon entering cells, they can bind to DNA or RNA, interfering with replication and transcription, thereby inhibiting protein synthesis [29,30]. Notably, many AMPs also possess important immunomodulatory functions, mobilizing and enhancing the host's immune defense [31]. Specific functions include: chemotactically recruiting immune cells to infection sites; regulating cytokine production—inducing pro-inflammatory factors to clear pathogens while also suppressing excessive inflammatory responses to reduce tissue damage.

Antibacterial Vaccines

Traditional bacterial vaccines, such as BCG, meningococcal polysaccharide vaccine, pneumococcal conjugate vaccine, and DTP, remain widely used. However, these traditional vaccines often struggle to effectively elicit cellular immune responses against intracellular pathogens. While live attenuated vaccines can induce such cellular immunity, their application carries potential risks, such as virulence reversion in susceptible hosts or the induction of other related diseases [32,33]. With advancements in biotechnology, various novel antibacterial vaccines have become a current research hotspot.

Inactivated Vaccines use whole bacteria inactivated by heat, radiation, or chemical methods as antigens. Their primary role is activating B cells to produce antibodies and stimulating CD4⁺ T cell responses. These vaccines contain a rich array of antigenic

components, potentially offering broad immune protection. However, they have a short persistence in vivo, are not easily taken up by antigen-presenting cells, and the inactivation process may damage surface antigen structures, reducing immunogenicity, which limits the further development of whole-cell vaccines [34,35].

Live Attenuated Vaccines are produced by treating pathogens to reduce virulence while retaining immunogenicity. Their antigens are more readily presented via the MHC I pathway, activating CD8⁺ T cell-mediated immunity. However, safety concerns exist, such as potential reversion to virulence or genetic recombination [33].

Bacterial Polysaccharide Conjugate Vaccines consist of pathogen capsular polysaccharides covalently linked to carrier proteins. The polysaccharide component can directly bind B cells via B cell receptors and receive antigenic signals from follicular dendritic cells; the protein component can be degraded into peptides, presented via MHC II molecules, activating follicular helper T cells (Tfh). Tfh secrete cytokines, promoting full B cell activation, proliferation, and differentiation into plasma cells and memory B cells, establishing long-term immune memory. Although the conjugation strategy enhances polysaccharide immunogenicity, these vaccines face challenges like variable protective efficacy across populations and potential interference from the carrier protein [36].

Subunit Vaccines are made from isolated, immunologically active protein fragments of bacteria. Due to poor immunogenicity, they require adjuvants to enhance vaccine potency and induce lasting immunity. In subunit vaccines, adjuvants should not exhibit intrinsic immunogenicity but only boost the immune response to the delivered antigen. Adjuvant effects can also vary significantly depending on the administration route (e.g., mucosal vs. injection) [37].

DNA Vaccines deliver antigen-encoding gene sequences into host cells via plasmid vectors. The antigen protein is expressed intracellularly after transcription, translation, and processing, inducing both humoral and cellular immune responses [38].

mRNA Vaccines utilize delivery systems like lipid nanoparticles to transport antigen-encoding mRNA into the cytoplasm, where the antigen protein is synthesized by ribosomes. This protein can be degraded by proteasomes into antigenic peptides presented via MHC I to activate CD8⁺ T cells; simultaneously, antigen secreted extracellularly can be recognized by B cells or taken up by antigen-presenting cells and presented via MHC II to activate CD4⁺ T cells, which then help B cells produce IgG antibodies and form immune memory [39].

Application of Antibiotic Alternatives in Animals

In recent years, Antimicrobial Peptides (AMPs) have become a prominent research focus and garnered significant attention as antibiotic alternatives. In livestock and poultry farming, AMPs

demonstrate numerous advantages, not only efficiently promoting animal growth and development but also offering disease prevention and control capabilities. As a novel green feed additive, their core strengths lie in being non-toxic, having no chemical residues, and a low propensity to induce bacterial resistance, fully aligning with the concept of green farming. Liu, et al., evaluated the efficacy of a composite AMP (containing recombinant porcine defensin and drosomycin) as a feed additive for young goats. Results showed a significant increase in beneficial genera like *Fibrobacter* and substantial weight gain in the AMP-treated group [40]. Research by Gyan, et al., found that dietary supplementation with AMPs improved the growth performance, antioxidant capacity, and innate immunity of Pacific white leg shrimp, while enhancing disease resistance. After challenge with *Vibrio harveyi* at 2.7×10^8 CFU/mL, the group supplemented with 0.4% AMP showed a significantly lower mortality rate compared to the control group [41].

The rumen and hindgut of ruminants host a diverse microbiota including bacteria, fungi, and protozoa. The rumen functions as a highly efficient anaerobic fermentation system. Through the synergistic action of rumen microbes, various nutrients in feed are decomposed, transformed, and utilized, with fiber-degrading bacteria playing a particularly crucial role in breaking down roughage—a unique advantage of ruminants not replicable in monogastric animals. Adding antibiotic alternatives like AMPs, probiotics, enzymes, and plant extracts to ruminant diets can optimize the rumen environment, promote anaerobic fermentation processes, and, through the regulatory effects of probiotics, maintain the stability of the rumen microbial ecosystem. This, in turn, improves the digestion and absorption efficiency of nutrients in the abomasum and small intestine [42].

Antibacterial vaccines hold significant potential for reducing antimicrobial use and preventing the emergence of resistant bacteria. Novel antibacterial vaccines have made progress in combating bacterial infections; for example, outer membrane vesicle-based vaccines have been successfully used to prevent *Neisseria meningitidis* infection, and mRNA vaccine technology against emerging pathogens has also seen breakthroughs in antibacterial infection research. Currently, antibacterial vaccines are primarily applied in human medicine, with no mature products yet on the market in the animal vaccine sector.

Summary and Outlook

Since the widespread misuse of antibiotics in the 20th century led to bacterial resistance, environmental pollution, and public health crises, the global livestock industry has entered a transition phase centered on reducing and prohibiting antibiotics. Various antibiotic alternatives—including microecological preparations, natural plant extracts, antimicrobial peptides, and antibacterial vaccines—have demonstrated potential in promoting animal growth and improving health through different mechanisms such as modulating gut microbiota, inhibiting pathogens, and enhancing immunity. Practice indicates that no single product can fully replace

antibiotics, therefore, a comprehensive antibiotic replacement strategy integrating refined management, environmental control, and other measures is necessary. Certain application successes have been achieved in swine, poultry, ruminant, and aquaculture.

Looking ahead, the research and application of antibiotic alternatives still face numerous challenges: inconsistent product efficacy, mechanisms requiring further elucidation, relatively high costs, and lack of unified evaluation standards. Future development should focus on the following aspects: First, strengthening research on the synergistic combinations of alternative products to build precise and efficient formulation strategies. Second, harnessing integrated multi-omics technologies (genomics, metagenomics, metabolomics), Artificial Intelligence (AI), and complementary computational tools to enable data-driven precision management of animal nutrition and health. Third, accelerating the development and industrialization of cutting-edge products like novel antibacterial vaccines and genetically engineered antimicrobial peptides. Fourth, establishing a whole-chain regulatory system and promoting the development of industry standards and norms. Only through the combination of technological innovation and systematic management can the animal husbandry industry truly move towards green, sustainable, and high-quality development, ensuring animal health, food safety, and ecological balance.

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Conflict of Interest

None.

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